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Trial of support treatment with human chorionic gonadotrophin in the luteal phase after treatment with buserelin and human menopausal gonadotrophin in women taking part in an in vitro fertilisation programme

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Abstract

Objective—To evaluate the effect of support with human chorionic gonadotrophin in the luteal phase in women taking part in an in vitro fertilisation programme after buserelin and human menopausal gonadotrophin were used to hyperstimulate their ovaries.

Design—Controlled group comparison.

Setting—Outpatient department of a private hospital.

Patients—115 Women with indications for in vitro fertilisation, all of whom had at least one embryo transferred.

Interventions—After suppression of the pituitary with buserelin the ovaries of all the women were stimulated with human menopausal gonadotrophin on day 4 of the luteal phase. Human chorionic gonadotrophin (10 000 IU) was given to induce ovulation, and oocytes were recovered 34 hours later. Embryos were transferred 46 to 48 hours after insemination. Women who had received the 10 000 IU of human chorionic gonadotrophin on a date that was an uneven number (n=61) were allocated to receive support doses of 2500 IU human chorionic gonadotrophin three and six days after that date. The remaining 54 women did not receive hormonal support.

End point—Determination of the rates of pregnancy.

Measurements and main results—Support with human chorionic gonadotrophin did not significantly alter the progesterone or oestradiol concentrations in the early or mid-luteal phase. The mean (range) progesterone concentrations in the late luteal phase in women who did not become pregnant were, however, significantly higher in those who received support (16 (9-110) nmol/l v 8 (4-46) nmol/l), and the luteal phase was significantly longer in this group (14 days v 12 days). The rate of pregnancy was significantly higher in the women who received support than in those who did not (25/61 v 8/54).

Conclusions—When buserelin and human menopausal gonadotrophin are used to hyperstimulate ovaries support with human chorionic gonadotrophin

in the luteal phase has a beneficial effect on in vitro fertilisation.

Introduction

The value of providing hormonal support in the luteal phase after hyperstimulation of the ovaries and aspiration of follicles for in vitro fertilisation has been much debated. Adequate production of progesterone by the corpus luteum is necessary for implantation of a zygote and early maintenance of pregnancy.¹ This may not be achieved either because of follicular aspiration, which by removing granulosa cells during recovery of oocytes may reduce the production of progesterone in the luteal phase or, alternatively, because the dose of human chorionic gonadotrophin given to induce ovulation may not adequately stimulate the corpus luteum. Varygas *et al*, however, showed that follicular aspiration does not lead to lower progesterone concentrations²; indeed, because of the presence of multiple corpus luteum progesterone concentrations after in vitro fertilisation and transfer of an embryo are higher than in a natural cycle.³

Cycles in which conception occurs are associated with higher progesterone concentrations in the luteal phase,⁴ and in vitro fertilisation regimens often include empirical hormonal support during the luteal phase in an attempt to mimic this hormonal profile. Hyperstimulation regimens consisting of human menopausal gonadotrophin alone or in combination with clomiphene citrate have been followed by supplementation during the luteal phase with progesterone^{5,6} or human chorionic gonadotrophin,^{7,8} or both.⁹ Rates of pregnancy showed no significant improvement with these regimens, although in two studies human chorionic gonadotrophin prolonged the luteal phase,^{10,11} which was thought to exert a beneficial effect as poor rates of pregnancy have been associated with regimens that lead to short luteal phases.¹² Acceptable rates of pregnancy are achieved without hormonal supplementation in the luteal phase.^{13,14} Thus there is no clear evidence for a beneficial effect of supplementation.

Treatment with both gonadotrophin releasing hormone agonists and gonadotrophins is an effective

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and more controlled hyperstimulation regimen.¹⁵ Gonadotrophin releasing hormone agonists induce hypogonadotrophism during induction of follicular growth with exogenous gonadotrophins, thus preventing premature luteinisation.¹⁶ This reversible suppression of the pituitary may influence the adequacy of the luteal phase in establishing a suitable uterine environment for nidation. Some programmes using this form of treatment have given support in the luteal phase either with human chorionic gonadotrophin^{17,18} or with progesterone,^{15,19} but none have used a control population. We assessed the value of support with human chorionic gonadotrophin in the luteal phase in an in vitro fertilisation programme in which buserelin and human menopausal gonadotrophin were used to hyperstimulate the ovaries.

Materials and methods

We studied 115 women attending an in vitro fertilisation unit. Each had at least one embryo transferred. The ovaries of all of the women were stimulated with human menopausal gonadotrophin (Pergonal) after suppression of the pituitary with the gonadotrophin releasing hormone agonist buserelin (Suprefact). Buserelin was taken by nasal spray in three doses of 200 µg daily, starting during the luteal phase of the previous cycle and continuing throughout the treatment cycle until human chorionic gonadotrophin (Profasi) was given. Human menopausal gonadotrophin was given (300 IU in four ampoules daily) from day 4 of the luteal phase until there were at least two follicles >17 mm diameter. Final maturation of oocytes was induced with 10 000 IU human chorionic gonadotrophin, and oocytes were recovered 34 hours later.

Blood samples were taken on days 4 and 7 and daily

from day 9 after the start of menstruation for estimation of serum oestradiol concentrations. Oocytes were recovered by transvaginal aspiration guided by ultrasonography and were incubated in HEPES buffered Earle's medium (Gibco, United Kingdom) for six hours before insemination. Embryos were transferred 42 to 46 hours after insemination with a Wallace catheter (Wallace, United Kingdom).

Women were allocated to receive support with human chorionic gonadotrophin during the luteal phase depending on the date that the dose of human chorionic gonadotrophin to induce ovulation was given—if the date was an uneven number support was given, if an even number support was not given. Human chorionic gonadotrophin was given to 61 of the 115 women in a dose of 2500 IU three and six days after the dose of 10 000 IU given to induce ovulation. All of the women were informed and their consent obtained.

Blood samples were also taken one, six, and 12 days after the dose of 10 000 IU human chorionic gonadotrophin for estimation of serum oestradiol and progesterone concentrations. These were measured by commercially available radioimmunoassays (Sero Diagnostics and Amersham International, respectively).

Results

The mean (SD) age of the women who received support with human chorionic gonadotrophin was 32.2 (4.0) years compared with 31.9 (4.8) in those who did not receive support. The indications for in vitro fertilisation were similar to both groups (table I). Thirty three of the women who received support were having their first attempt at in vitro fertilisation compared with 36 of those who did not receive support.

The median serum oestradiol concentrations did not differ significantly between the two groups (Mann-Whitney test) on either day 1 or day 6 in cycles in which conception did not occur after the dose of 10 000 IU human chorionic gonadotrophin (table II). In addition, no difference was seen between the serum progesterone concentrations on day 1 or day 6 of the luteal phase (table II). There was no significant difference between the groups in the ratio of oestradiol to progesterone concentrations on either day 1 or day 6. On day 12, however, the serum progesterone concentration was significantly higher in the women who received support with human chorionic gonadotrophin (16 (9-110) nmol/l *v* 8 (4-46) nmol/l, $p < 0.001$) (table II). The luteal phase in women who did not become pregnant was significantly longer in those who were given support (14 days *v* 12 days, $p < 0.001$, $t = 4.6$).

No significant differences in oestradiol concentrations before ovulation was induced and on days 1 and 6 of the luteal phase (table II) were found between women who became pregnant and those who did not, whether or not support with human chorionic gonadotrophin was given. Progesterone concentrations were also not significantly different on days 1 and 6, and no significant difference was seen in the ratio of oestradiol to progesterone concentrations (Mann-Whitney test). Support in the luteal phase did not produce significant differences in the median (range) progesterone concentrations on day 12 in the cycles in which conception occurred (125 (23-350) nmol/l in those who received support *v* 85 (12-110) nmol/l in those who did not).

Evidence of a gestation sac on ultrasonography was necessary for pregnancy to be diagnosed—that is, pregnancies detected only by biochemical tests were excluded. The rate of pregnancy was significantly higher in the women who received support with human chorionic gonadotrophin (25/61 (41%) *v* 8/54 (15%), $p < 0.01$). The mean number of embryos transferred was similar (3.16 *v* 2.91).

TABLE I—Indications for in vitro fertilisation in women who did and did not receive human chorionic gonadotrophin (HCG) in luteal phase

Indication	Women who received HCG (n=61)	Women who did not receive HCG (n=54)
Factor in partner	16	15
Tubal disease	29	27
Other*	16	12

*Included mucus hostility, endometriosis, tubal disease and endometriosis, unexplained infertility, and anovulation with no bias.

TABLE II—Median (range) serum oestradiol and progesterone concentrations in women who did and did not receive human chorionic gonadotrophin (HCG) in luteal phase and did not become pregnant

	Women who received HCG (n=36)	Women who did not receive HCG (n=46)
Oestradiol (pmol/l): day 1	4225 (1350-24 500)	4275 (1550-17 000)
Day 6	3375 (1050-16 000)	3500 (575-12 500)
Progesterone (nmol/l): day 1	15 (6-100)	16 (7-54)
Day 6	160 (60-74)	170 (80-525)
Day 12	16 (9-110)	8* (4-46)

* $p < 0.001$.

TABLE III—Total number of embryos transferred and rate of implantation in women who did and did not receive human chorionic gonadotrophin (HCG) in luteal phase according to how many embryos were transferred to each woman

No of embryos transferred	Women who received HCG		Women who did not receive HCG	
	Total No of embryos transferred	Total No of embryos implanted	Total No of embryos transferred	Total No of embryos implanted
1	5	2	4	1
2	18	1	14	
3	54	13	78	6
4	116	20	64	4
Total	193	36	160	11

Table III shows the number of embryos that implanted in relation to the number that were transferred. The rate of implantation in the women who received support was consistently higher for one, two, three, or four embryos transferred. The same team of clinician and embryologist recovered all oocytes and transferred all embryos.

Among the women who received support treatment 24 babies were delivered, including five sets of twins and one set of triplets; one stillbirth, three first trimester abortions, and one ectopic pregnancy occurred, and the attempted selective reduction of a quadruplet pregnancy resulted in a complete abortion. Two pregnancies were continuing at the time of writing. Among those who did not receive support with human chorionic gonadotrophin three babies were delivered and three abortions (including two sets of twins) and one ectopic pregnancy occurred. One further twin pregnancy was induced at 28 weeks after the death of the second twin, the first having died at 18 weeks.

Discussion

The need for hormonal support in the luteal phase after ovarian stimulation with clomiphene citrate and human menopausal gonadotrophin has been much debated.^{2-4 14-20} Randomised trials have failed to show a significant difference in the rate of pregnancy between women who received support and those who did not.¹¹⁻²¹

We attempted to ascertain whether support with human chorionic gonadotrophin in the luteal phase was necessary when the luteinising hormone releasing hormone analogue buserelin and human menopausal gonadotrophin were used to stimulate the ovaries. A significantly increased rate of pregnancy was found when cycles were supported. There was no biochemical evidence during the early and mid-luteal phase to account for this improvement. Oestradiol and progesterone concentrations during cycles in which conception occurred were not significantly different between cycles that were supported and cycles that were not. Biochemical evidence in the late luteal phase, however, indicated the role of human chorionic gonadotrophin in supplementation of the luteal phase. Non-pregnant women who received support (in whom a valid comparison could be made) had significantly raised progesterone concentrations on day 12 of the luteal phase (table II), suggesting that it is at this point that human chorionic gonadotrophin exerts its beneficial effects.

The raised progesterone concentrations in women who received support with human chorionic gonadotrophin add weight to previous findings of inadequate production of progesterone during the late luteal phase in women stimulated with buserelin and human menopausal gonadotrophin who did not receive support. Smitz *et al* observed a dramatic decline in serum progesterone concentration from day 8 after the dose of human chorionic gonadotrophin to induce ovulation in 10 women who did not become pregnant.²² Moreover, in another study only one pregnancy resulted from 13 unsupported cycles compared with 29 pregnancies from 91 cycles when hormonal support in the luteal phase was given.¹⁴

Pregnancy after hyperstimulation with clomiphene citrate and human menopausal gonadotrophin has been associated with a lower ratio of oestradiol to progesterone concentrations in the luteal phase,²³⁻²⁴ suggesting that support during the luteal phase may relieve the contraceptive effect of the high oestradiol concentrations after ovulation. Despite showing a significant decrease in the ratio of oestradiol to progesterone concentration's with support with human

chorionic gonadotrophin Buvat *et al* did not show any improvement in the rate of pregnancy.¹¹ In contrast, our study showed no influence of support with human chorionic gonadotrophin on the ratio of oestradiol to progesterone concentrations but showed a significantly higher rate of pregnancy.

The luteal phase is extended when support with human chorionic gonadotrophin is given,¹⁰ as our study confirmed. Cycles in which conception did not occur were significantly longer when human chorionic gonadotrophin was given (mean 14 days *v* 12 days). The main result of our study was the difference produced in the rates of pregnancy. Among women receiving support with human chorionic gonadotrophin the rate of pregnancy was 41% for each embryo transfer compared with 15% among women who did not receive support.

In *in vitro* fertilisation programmes buserelin is stopped before the dose of human chorionic gonadotrophin required to induce ovulation. Although the metabolism of buserelin from the circulation is rapid, the reserve of gonadotrophin in the pituitary has been shown to be inadequate by a highly specific assay for luteinising hormone that uses monoclonal antibodies, which showed that serum luteinising hormone was absent until 12 days after buserelin was stopped.²⁵ This finding was confirmed by Smitz *et al*, who gave boluses of luteinising hormone releasing hormone to women seven days after they stopped treatment with a gonadotrophin releasing hormone agonist; their results showed inadequate gonadotrophic responses.²²

We conclude that the more adequate late luteal phase and the significant increase in the rate of pregnancy among women given support treatment in this study suggest that support with human chorionic gonadotrophin when buserelin is used with human menopausal gonadotrophin for ovarian hyperstimulation has a beneficial effect on *in vitro* fertilisation.

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Perimenopausal risk of falling and incidence of distal forearm fracture

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Abstract

A postal survey of 2000 women and 2000 men sampled from the electoral roll in Oxford was undertaken to ascertain whether changes with age in the risk of falling might explain the stepwise increases in age specific incidence rates of distal forearm fracture which occur in women at around the age of 50. Corrected response rates were 83% for women and 72% for men. In women, but not in men, there was a rise in the risk of falling from 45 years, peaking in the 55-59 year age group, and sinking to a nadir at ages 70-74. In both sexes rates rose in extreme old age. These variations were not attributable to preferential response from people who had suffered a fracture.

It is concluded that changes in the risk of falling interact with osteoporosis to produce a perimenopausal rise in the incidence of forearm fractures and contribute to the fluctuations in incidence of these fractures in old age.

Introduction

In women the age specific incidence of fractures of the distal forearm rises steeply between the age groups 40-44 and 55-59 but thereafter shows no consistent further increase (see figure).^{1,2} The incidence in men is constant with age. This pattern is conventionally attributed entirely to perimenopausal osteoporosis in women increasing the likelihood that a fracture will result if a fall occurs, but the frequency of falls among women during their middle years has not been examined. We carried out a postal survey to examine the possibility that an increase in the frequency of falls in middle age might contribute to the steep rise in incidence of distal forearm fracture in women at this time of life.

Subjects and methods

The age and sex of all residents of Oxford city who suffered a fracture of the distal forearm during 1986 were identified from the fracture clinic records at the John Radcliffe hospital to confirm that the local epidemiological pattern was broadly similar to that found elsewhere.

A postal questionnaire designed for self completion was sent to 2000 women residents of Oxford city, selected at random from the 1986 electoral register in postcode districts OX1-4. Subjects were asked for age

at last birthday and for "yes" or "no" answers to questions used in a previous survey: "Have you had any falls in the last year?" and "If so, did you break any bones in your falls?" Local press and radio coverage was organised without disclosing the hypothesis being tested. To assess response bias 500 initial non-responders were sent a second copy of the questionnaire after four weeks. A similar study was undertaken of men identified from the 1988 electoral register.

Results

A total of 124 fractures of the distal forearm were identified in Oxford city residents age 20-74. Estimated age specific yearly incidence rates (table 1) showed the expected stepwise increase in middle aged women and not in men.

FALLS AND FRACTURES

Women—Replies were received from 1335 women. After allowing for an estimated 19.2% obsolescence in the 1986 electoral register at the time of the survey⁴ the crude response rate of 67% corrected to roughly 83%. Ten respondents gave insufficient information and 16 were under 20, leaving 1309 women; of these 286 (22%) reported falling, of whom 30 suffered a fracture. The 1141 initial responders and 168 who responded to the second mailing did not differ significantly in the

TABLE 1—Numbers and yearly incidence rates of distal forearm fracture in Oxford, 1986

Age group (years)	Women		Men	
	No of fractures	Yearly incidence (per 10 000)	No of fractures	Yearly incidence (per 10 000)
20-24	6	12.8	12	25.6
25-29	3	11.4	2	4.1
30-34	5		1	
35-39	2	9.8	4	10.0
40-44	3		1	
45-49	3	21.1	4	10.3
50-54	7		1	
55-59	15	60.2	3	6.5
60-64	16		0	
65-69	17	62.0	1	7.3
70-74	16		2	

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