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Sequential clomiphene citrate and human menopausal gonadotrophin for ovulation induction: comparison to clomiphene citrate alone and human menopausal gonadotrophin alone

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The need for frequent injections and monitoring, the possibility of multiple gestations, and the higher cost compared to clomiphene citrate, prevents many clinicians from using human menopausal gonadotrophin (HMG) for ovulation induction. A sequential medication regimen, in which HMG is taken after clomiphene, overcomes these problems. We retrospectively compared per cycle fecundity and birth rates in 119 cycles of clomiphene–HMG, 524 cycles of clomiphene alone, 57 cycles of HMG alone, and 79 cycles of concurrent HMG and clomiphene in patients receiving intra-uterine insemination (IUI), who were free of endometriosis or tubal disease. Per cycle fecundity for clomiphene–HMG was 22% [95% confidence interval (CI) 12–34%], double that of clomiphene alone (11%) (95% CI 8–14%) ($P < 0.01$), and equal to HMG alone (18%) (95% CI 7–29%) or HMG and clomiphene together (19%) (95% CI 10–28%). The multiple birth rate for clomiphene–HMG (7/21) equalled that for HMG alone (3/12) and HMG and clomiphene together (3/8). The average number of ampoules of HMG required [follicle stimulating hormone (FSH) 75 mIU, luteinizing hormone (LH) 75 mIU] was decreased by 65% from 24.5 ± 1.0 for HMG or HMG and clomiphene together to 8.6 ± 0.3 for clomiphene–HMG ($P < 0.001$). Per cycle fecundity was identical when one, two or three ampoules of HMG per day were administered after clomiphene. We conclude that ovulation induction with sequential clomiphene–HMG results in fecundity double that of clomiphene alone and equal to HMG alone or concurrent with clomiphene, thereby reducing the requirement for HMG.

Key words: clomiphene citrate/fecundity/human menopausal gonadotrophin/multiple pregnancy/ovulation induction

Introduction

Human menopausal gonadotrophin (HMG) is required for ovulation induction in patients with pituitary or hypothalamic amenorrhoea. However, the most frequent reason for using HMG is to increase the number of pre-ovulatory follicles in normal ovulating women undergoing in-vitro fertilization (IVF), gamete intra-Fallopian transfer (GIFT), or intra-uterine insemination (IUI). In unselected patients, per cycle fecundity averages 13–14% when HMG is used prior to IUI (Dodson *et al.*, 1987; Dickey *et al.*, 1991), compared to 7% when clomiphene citrate is used prior to IUI (Robertson *et al.*, 1976). Per cycle fecundity after either clomiphene or HMG is reduced by 50% or more in the presence of endometriosis (Dickey *et al.*, 1991, 1992a), tubal disease (Hammond *et al.*, 1983; Dodson *et al.*, 1987; Dickey *et al.*, 1991, 1992a), abnormal sperm or mucus factors (Corson *et al.*, 1983; Hammond *et al.*, 1983; Dickey *et al.*, 1991, 1992a), and when the patient's age is ≥35 years (Dickey *et al.*, 1991, 1992a). Because of the high cost, the need for repeated injections and for frequent monitoring with ultrasound and serum hormone levels, a method of reducing the total amount of HMG utilized and the number of days of administration without losing the benefits of additional pre-ovulatory follicles and higher per cycle fecundity, would be desirable.

Sequential use of clomiphene followed by HMG was first proposed by Kistner in 1972 and later by others (Taymor *et al.*, 1973; March *et al.*, 1975), to increase pregnancy rates over those with clomiphene alone, and to reduce the amount and duration of HMG required for ovulation induction. Additional benefits anticipated for sequential clomiphene–HMG were a reduction in multiple births and hyperstimulation syndrome compared with HMG alone. Although high pregnancy rates were reported in early studies, few patients were treated and results were not reported on a per cycle basis, nor were results compared to clomiphene alone or HMG alone (Kistner, 1972; Goldfarb *et al.*, 1972; Taymor *et al.*, 1973; March *et al.*, 1975; Robertson *et al.*, 1976). There have been no studies of sequential clomiphene–HMG reported in recent years.

We have previously demonstrated that per cycle fecundity is related to the number of pre-ovulatory follicles ≥12 mm when ovulation is induced with clomiphene alone (Dickey *et al.*, 1992a) or with HMG alone or combined with clomiphene (Dickey *et al.*, 1991). We also have reported that when IUI is performed, initial semen quality does not affect fecundity unless the concentration is $<5 \times 10^6$/ml or progressive motility is <20% (Dickey *et al.*, 1991, 1992a).

In the present study, per cycle fecundity and multiple births were retrospectively compared after sequential clomiphene–HMG, to results after clomiphene alone and HMG alone and HMG concurrent with clomiphene. The treatment parameters were studied in patients without endometriosis or tubal disease in cycles in which ovulation and insemination were controlled by human chorionic gonadotrophin (HCG) administration and IUI. In addition, we analysed the effect of different doses of HMG and the number of days of HMG administration on fecundity in cycles in which sequential clomiphene–HMG was used.

Materials and methods

All cycles in which a single IUI with washed spermatozoa was performed after HCG administration and ovulation induction with clomiphene and/or HMG between January 1983 and January 1990, were retrospectively reviewed. The reason for administering clomiphene and HMG was to increase the number of pre-ovulatory follicles in patients requiring IUI. IUI was performed either because of the failure to find five or more progressively motile spermatozoa per microscopic field at ×400 magnification on an overnight post-coital test (569 cycles) in patients with ≥1 year of infertility, or because of the need for cryopreserved donor spermatozoa (210 cycles). Patients who failed the post-coital tests had parameter values for semen quality lower than World Health Organization (WHO) standards (sperm count per ml $\geq 20 \times 10^6$; total sperm count $\geq 40 \times 10^6$; progressive motility ≥50%; normal forms ≥50%) in 322 cycles. In 247 cycles, spermatozoa were normal by WHO standards prior to wash preparation. Patients were excluded from analysis if they had endometriosis or tubal adhesions, and individual cycles were excluded if pre-wash sperm concentration was $< 5 \times 10^6$/ml or pre-wash sperm motility was <20% on the day of IUI, or if an ultrasound was not performed on the day of HCG administration. After all exclusions, 779 cycles were available for analysis.

Ovulation induction

Patients received one of the following ovulation induction regimens: clomiphene 50–150 mg/day for 5 days beginning on cycle day 3 followed by 1–3 ampoules of HMG for 3–7 days (n = 119) (clomiphene–HMG); clomiphene 50–150 mg/day for 5 days (n = 524) (clomiphene); HMG only 2–4 ampoules for 7–10 days beginning on cycle day 2 (n = 57) (HMG); or HMG, 2–3 ampoules, concurrently with clomiphene, 50–150 mg/day, from cycle day 2–7 and followed by HMG alone until HCG administration (n = 79) (HMG and clomiphene). Two patients who failed to develop at least two pre-ovulatory follicles on clomiphene alone were switched to clomiphene–HMG. When clomiphene–HMG was chosen, the dose of HMG was randomly assigned as one, two or three ampoules per day. Comparison between groups was confined to cycles 1–3 because in our experience fecundity decreases after the third cycle of HMG–IUI (Dickey *et al.*, 1991).

The methods of ovulation timing, sperm preparation, ultrasound, and serum oestradiol determinations were as previously described (Dickey *et al.*, 1991, 1992a). Briefly, an initial ultrasound was performed after 3 days of HMG for clomiphene–HMG, after 7 days of HMG or HMG and clomiphene, and on the twelfth cycle day for clomiphene only. Human chorionic gonadotrophin, 10 000 IU, was administered if one or more follicles were ≥18 mm in diameter for clomiphene alone, and if one or more follicles were ≥15 mm in diameter for cycles in which HMG was used. Ultrasound was repeated at 1–3 day intervals until the criteria for follicular maturity were met. Patients were instructed to perform a urine LH test each morning, beginning the day before the first ultrasound. Cycles in which a spontaneous LH surge occurred were excluded from this study. A single IUI was performed with washed spermatozoa 24–30 h after HCG for fresh spermatozoa and 34–40 h after HCG for cryopreserved spermatozoa.

Table I. Per cycle fecundity, births, and multiple births for four ovulation induction regimens in patients aged <35 years and ≥35 years

Regimen	No. cycles	Pregnancies no./% cycles	Births no./% cycles	No. twins	No. triplets
Age <35 years					
CC–HMG	92	20[a] 22	17[a] 19	4	2[b]
CC	409	46 11	36 9	1	0
HMG	30	9 30	8 27	3	0
HMG and CC	58	13 22	11 19	3	0
Age >35 years					
CC–HMG	27	6[c] 22	4[c] 15	1	0
CC	115	13 11	8 7	1	0
HMG	27	1 4	0 0	0	0
HMG and CC	21	2 10	1 5	0	0
All ages					
CC–HMG	119	26[d] 22	21[d] 18	5	2[e]
CC	524	59 11	44 8	2	0
HMG	57	10 18	8 14	3	0
HMG and CC	79	15 19	12 15	3	0

CC–HMG = sequential clomiphene and human menopausal gonadotrophin.
HMG and CC = concurrent HMG and clomiphene.
Chi-square test:
[a]Fecundity and births, compared to CC, $P < 0.007$.
[b]Multiple births, compared to CC, $P < 0.005$.
[c]Fecundity and births, compared to HMG, $P < 0.05$.
[d]Fecundity and births, compared to CC, $P < 0.005$.
[e]Multiple births, compared to CC, $P < 0.002$.

A urine test for pregnancy was performed 14–16 days after insemination if menses did not occur. Positive urine tests were confirmed by serum quantitative beta HCG determination. Outcome was reported as both per cycle fecundity and per cycle births. Fecundity (i.e. total pregnancies) included pre-clinical and clinical abortions, ectopic pregnancies, and births. Ectopic pregnancies and pre-clinical abortions were counted as single implantations when determining the implantation rate per pre-ovulatory follicle. Results are reported as mean ± SEM with 95% confidence intervals (CI) when indicated. Statistical analyses were performed using chi-square with Yate's correction, Pearson's correlation coefficient, and Student's t-test. Results were considered significant at $P < 0.05$.

Results

Per cycle fecundity, according to sperm source, was as follows: normal fresh spermatozoa 13% (CI 9–17%), subnormal fresh spermatozoa 13% (CI 10–17%), cryopreserved donor spermatozoa 17% (CI 12–22%). The proportion of inseminations performed for each of the three semen groups (husband normal, husband sub-standard, and cryopreserved donor) was evenly distributed across ovulation induction treatment groups. Fecundity and births per cycle and the number of multiple births arranged by medication group and age for the first three cycles of IUI are presented in Table I. For all ages, per cycle fecundity for clomiphene–HMG (22%) (CI 12–34%) was twice that of clomiphene alone (11%) (CI 8–14%) ($P < 0.01$), and was no different from HMG alone (18%) (CI 7–29%) and to HMG and clomiphene (19%) (CI 10–28%). Per cycle fecundity for patients who continued to take clomiphene in cycles 4–6 (n = 39) was

Table II. Number of follicles ≥12 mm per cycle, number of implantations per follicle, mean oestradiol level on day of human chorionic gonadotrophin (HCG) administration and oestradiol level per follicle ≥12 mm

Ovulation induction regimen	No. cycles	No. follicles per cycle ≥12 mm	No. patients	Mean oestradiol per cycle on day of HCG (pg/ml)	Mean oestradiol/follicle ≥12 mm (pg/ml)	Implantations/follicle (95% CI)
CC–HMG	119	3.2 ± 0.2[a]	106	962 ± 80	456 ± 76[b]	0.100 (0.068–0.132)[c]
CC	524	2.4 ± 0.1	62	613 ± 48	254 ± 18	0.047 (0.035–0.059)
HMG	57	4.6 ± 0.6	75	1194 ± 161	278 ± 37	0.054 (0.026–0.082)
HMG and CC	79	5.9 ± 0.5	56	1674 ± 739	329 ± 21	0.040 (0.022–0.058)

[a]Number of follicles per cycle, Student's *t*-test; compared to CC, $P < 0.001$; compared to HMG, $P < 0.001$; compared to HMG and CC, $P < 0.001$.
[b]Oestradiol, Student's *t*-test; compared to CC, $P = 0.011$; compared to HMG, $P = 0.038$.
[c]Implantations per follicle, chi-square; compared to CC, $P < 0.003$; compared to HMG, $P < 0.05$; compared to HMG and CC, $P < 0.001$.
CC–HMG = sequential clomiphene and human menopausal gonadotrophin.
HMG and CC = concurrent HMG and clomiphene.

13% (CI 2–23%). Multiple births as a percentage of total births for clomiphene–HMG (7/21, 33%) was similar to HMG alone (3/8, 38%), and HMG concurrent with clomiphene (3/12, 25%) and was higher for all three regimens utilizing HMG, than for clomiphene alone (2/44, 4%) ($P < 0.002$). One of two patients who switched from clomiphene only to clomiphene–HMG became pregnant and gave birth to triplets.

The number of follicles ≥12 mm diameter, the number of implants per follicle, oestradiol level per patient ($n = 299$), and oestradiol level per follicle ≥12 mm for the four ovulation induction regimens are presented in Table II. Ten or more follicles ≥12 mm diameter were present on the day of HCG in 1/485 (0.2%) (CI −0.2–0.4%) clomiphene cycles, 2/115 (1.7%) (CI −0.6–4.1%) clomiphene–HMG cycles, 8/79 (10.1%) (CI 3.4–16.7%) HMG and clomiphene cycles, and 4/56 (7.1%) (CI 0.4–13.8%) HMG cycles. The number of implantations per follicle ≥12 mm diameter was twice as high for clomiphene–HMG (0.100) (CI 0.068–0.132), compared with clomiphene alone (0.047) (CI 0.035–0.059) ($P = 0.0003$), HMG alone (0.054) (CI 0.026–0.082) ($P < 0.05$), or for HMG and clomiphene (0.040) (CI 0.022–0.058) ($P < 0.001$). The oestradiol level per follicle ≥12 mm diameter approximated the differences in implantation rates per follicle for the four ovulation induction regimens. The oestradiol level per follicle was nearly twice as high for clomiphene–HMG (456 ± 76 pg/ml), compared with clomiphene (254 ± 18 pg/ml) ($P = 0.011$), or HMG alone (278 ± 37 pg/ml) ($P < 0.04$). There were no cases of hyperstimulation syndrome in patients who received clomiphene alone and one case of mild hyperstimulation each in patients who took clomiphene–HMG or HMG alone.

The total number of ampoules of HMG administered was reduced by 65% from 24.5 ± 1.0 with HMG alone to 8.6 ± 0.3 with clomiphene–HMG ($P < 0.001$). The relationship of per cycle fecundity and birth rate for clomiphene–HMG, according to the number of ampoules of HMG per day and number of days HMG administration, is presented in Table III. There was no difference in per cycle fecundity or birth rate when a single ampoule per day of HMG was used compared with two or three ampoules per day. Ninety-two per cent of patients received HCG after 3–5 days of HMG. There also was no difference in the average number of days HMG was administered when one or two ampoules were used, and <1 day's difference when three ampoules were used (data not shown). The number of ampoules and the number of days HMG was administered were unrelated to either patient weight or age for the clomiphene–HMG ovulation induction regimen.

Table III. Fecundity and birth rate with sequential clomiphene citrate and human menopausal gonadotrophin (HMG) by number of ampoules per day and number of days of HMG administration

Ampoules per day	No. of cycles	Pregnancies no./% cycles	Births no./% cycles
1	35	8 23	6 17
2	58	12 21	9 16
3	26	6 23	6 23
Number days HMG			
3	13	4 31	4 31
4	39	10 26	9 23
5	57	11 19	8 14
≥6	10	1 10	0 0

Discussion

The present study demonstrates that when one ampoule per day of HMG is administered for 3 days following clomiphene, per cycle fecundity is doubled, compared to clomiphene alone, and is equal to two or more ampoules per day of HMG alone for ≥7 days. These results confirm previous findings, in which pregnancy rates with sequential clomiphene–HMG were equal to HMG alone (Kistner, 1972, 1976; Taymor *et al.*, 1973; Goldfarb *et al.*, 1972; March *et al.*, 1975; Robertson *et al.*, 1976). In the largest of those studies, Kistner (1972) reported a first cycle fecundity rate in 80 patients aged <35 years of 19% after coitus, similar to the 22% rate found in the present study, Similarly, our result of 19% live birth rate in the under 35 age group, with clomiphene–HMG and IUI, is comparable to the 22% live birth rate following GIFT and the 14% live birth rate following IVF in 1990 in the USA as reported by the Society for Assisted Reproductive Technology (Medical Research International, 1992).

The explanation for increased fecundity when HMG is administered after clomiphene, compared to clomiphene alone, is related to both increased numbers of pre-ovulatory follicles and a doubling of the implantation rate per follicle. The oestradiol level per follicle also nearly doubled for clomiphene–HMG compared to clomiphene alone. A similar relationship between

oestradiol is related to fecundity, other than the obvious relationship to follicle number, is unknown. Higher oestradiol level may improve the quality of either the oocyte or cumulus mass within the follicle, or of the tubal milieu for fertilization and preimplantation embryonic development or of the endometrium, or a combination of any or all of these factors. Ultrasound evaluation of the endometrium prior to ovulation was initiated at the end of the study period but was not analysed. In IVF and GIFT it is known that fecundity is related to endometrial thickness (Smith *et al.*, 1984; Glissant *et al.*, 1985; Gonen and Casper, 1990; Dickey *et al.*, 1992b).

The 65% reduction in the total number of ampoules of HMG required and in the number of days of HMG administration, compared to cycles in which HMG was started on cycle day 2, are important advantages for the sequential clomiphene–HMG regimen. A similar result was reported by March *et al.* (1975), who observed an average reduction in total HMG administered from 20 to eight ampoules and in the duration of therapy from 7.5 to 3.5 days, compared to HMG alone. Further reduction in the number of ampoules of HMG required may be possible since fecundity rates were similar when one, two or three ampoules of HMG were used per day. An additional advantage of sequential clomiphene–HMG was that, in many cases, only a single ultrasound scan was required for timing of HCG administration, thereby reducing the cost of cycle monitoring. One anticipated advantage of sequential clomiphene–HMG treatment was not realized. The percentage of multiple births was the same for clomiphene–HMG, HMG alone, and HMG concurrent with clomiphene.

These results indicate that sequential clomiphene–HMG treatment is an effective alternative to longer HMG regimens in non-amenorrhoeic women for whom additional pre-ovulatory follicles are desired due to advanced age, unilateral tubal obstruction, or other reasons. Advantages of sequential clomiphene–HMG treatment, over HMG alone, are a reduction in drug cost and in the frequency of cycle monitoring.

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