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# Clomiphene citrate induced ovulation and intrauterine insemination: effect of timing of human chorionic gonadotropin injection in relation to the spontaneous LH surge on pregnancy rates

**Rajeevi Madankumar,[1,2] James Tsang,[1] Martin L. Lesser,[1] Daniel Kenigsberg,[1] and Steven Brenner[1]**

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***Purpose*:** To determine the optimal time for administration of human chorionic gonadotropin in clomiphene citrate induced intrauterine insemination cycles.
***Methods*:** A retrospective analysis of 171 consecutive cycles was performed. An increase in luteinizing hormone level >100% over the mean of the preceding two days was defined as luteinizing hormone surge. Human chorionic gonadotropin was given in preparation for intrauterine insemination based on the follicle size and estradiol level prior to surge in 85 cycles (Group A), with the spontaneous surge in 64 cycles (Group B) and not given in 22 cycles (Group C) due to high luteinizing hormone levels.
***Results*:** The overall pregnancy rate per cycle was 18.1% (31/171), 15.2% (Group A), 20.3% (Group B) and 22.7% (Group C), ($p > 0.50$).
***Conclusion*:** Although there may be physiological reasons to propose that timing the human chorionic gonadotropin to the luteinizing hormone surge will improve the success rate, they were not demonstrated.

**KEY WORDS:** CC-IUI; clomiphene citrate and intrauterine insemination; human chorionic gonadotropin; LH surge; spontaneous LH surge.

## INTRODUCTION

Ovulation induction combined with intrauterine insemination (IUI) is widely utilized for the treatment of infertility. Ovulation may be induced with clomiphene citrate (CC) or gonadotropins. Human chorionic gonadotropin (hCG) is frequently used in ovulation induction cycles to mimic the endogenous luteinizing hormone (LH) surge and to induce ovulation. This technique is used in couples with unexplained infertility, endometriosis, cervical factor infertility and oligoasthenospermia (1,2). The goal of IUI is to provide an increased concentration of motile spermatozoa in the proximity of the oocyte (2). Pregnancy rates can be maximized by timing the IUI to the time of ovulation (2). Ovulation may be specifically predicted by ultrasound evaluation of follicle size and/or measurement of estradiol and LH levels in the late follicular phase (3,4). Serum estradiol has been shown to be too variable to be of clinical value (3). Though, a wide variation in the level of LH at its peak exists, the identification of the LH surge has been found to be a reliable indicator of timing of ovulation (3). Martinez *et al.* (5), compared the pregnancy rates in IUI cycles after follicular rupture induced by hCG injection and after the spontaneous onset of LH surge detected by urine assay. Their study demonstrated that the mean diameter of the preovulatory follicles was significantly

[1] Obstetrics and Gynecology, Long Island Jewish Medical Center, Suite 1100, 270-05, 76th Avenue, New Hyde Park, NY 11040.
[2] To whom correspondence should be addressed; e-mail: rajeevim@att.net.

smaller and insemination was substantially earlier in the hCG induced cycles. Waiting for the spontaneous LH surge may help in the final process of natural follicular maturation.

Fuh *et al.* (6), demonstrated a significant increase in the pregnancy rate in gonadotropin-IUI cycles when hCG was given after the spontaneous LH surge. The current study evaluated whether administration of hCG in relation to the spontaneous LH surge resulted in a difference in pregnancy rates in women undergoing IUI after induction of ovulation with CC.

## MATERIALS AND METHODS

This retrospective study was based on data collected from subjects who had IUI cycles in a private reproductive medicine practice from January 2002 to October 2002. One hundred and seventy-one cycles of IUI in 93 patients after ovulation induction with clomiphene citrate were analyzed. The most common indications for IUI after ovulation induction with clomiphene citrate were male factor, frozen donor sperm insemination, unexplained infertility, anovulation and endometriosis.

### Sperm Preparation

Male partners were requested to abstain from ejaculation for 2 days before the day of sperm collection. Semen samples were collected by masturbation in sterile containers. After complete liquefaction at room temperature, each sample was analyzed for sperm concentration and motility using standard WHO guidelines. The sample was then mixed with sperm washing medium (Isolate, Modified sperm washing medium by Irvine Scientific, Santa Ana, California) and the mixture was centrifuged. The supernatant was discarded and the sperm-washing medium was added to the pellet to make up to 0.5 mL. The final sample was analyzed for count and motility. If the number of round cells in the post-washed specimen exceeded $8 \times 1{,}000{,}000$/mL, a swim-up technique was used to prepare the final specimen.

### Ovarian Stimulation and Intrauterine Insemination

Patients were given CC, in doses ranging from 50 to 150 mg orally for 5 days in the early follicular phase. Patients were monitored by pelvic sonogram for the follicle size, estradiol ($E_2$) and LH levels beginning in the late follicular phase. One hundred and seventy-one consecutive CC-IUI cycles were included in the study and the results were analyzed in a retrospective fashion. The spontaneous LH surge was defined as an increase in the LH levels $\geq$100% over the mean of the preceding 2 days. The blood draw was performed between 7:00 and 9:30 A.M. and the results were available in the afternoon of the same day. When given, hCG was administered at a dose of 10,000 IU intramuscularly. In 85 cycles (Group A) hCG was administered with the follicle size of 18 mm or more and $E_2$ level 200 pg/mL or more per dominant follicle. Human chorionic gonadotropin was administered prior to the LH surge in Group A. A spontaneous LH surge was observed in 86 cycles. In 64 (Group B) of these 86 cycles, hCG was administered on the day of the LH surge (12.7–122 mIU/mL). In 22 (Group C) of the 86 cycles with a spontaneous LH surge (LH levels ranging from 26–131 mIU/mL), no hCG was administered. Insemination (IUI) was performed the next day after the hCG administration or the spontaneous LH surge. In 54 cycles, IUI was performed once, 117 cycles received IUI on 2 consecutive days and 2 cycles received IUI on 3 consecutive days. A quantitative beta hCG was performed 14 days after IUI if no menses occurred.

### Data Collection and Analysis

Data was collected retrospectively from a chart review of CC-IUI cycles completed between January and October 2002. An application was submitted to the IRB, which was subsequently approved. A spontaneous LH surge was diagnosed by an increase in LH level more than 100% over the mean of the preceding 2 days. Statistical analyses were performed using the program "SAS" (Cary, North Carolina). The pregnancy rates per cycle were compared using the Fisher's Exact Test, considering a $p$ value of $<0.05$ be significant. All results were analyzed based on cycles ($n = 171$), not on subjects ($n = 93$). We used multiple logistic regression to compare the pregnancy rates in three treatment groups while adjusting for age and etiology.

## RESULTS

Between January to October 2002, 93 patients underwent 171 cycles of IUI following induction of ovulation with clomiphene citrate. (Patients were from

**Table I.** Comparability of Treatment Groups with Respect to Age

| Age group (years) | Group A (hCG by follicle size) $n = 85$ | Group B (hCG by serum LH surge) $n = 64$ | Group C (No hCG) $n = 22$ |
|---|---|---|---|
| <30 | 21 (24.7%) | 13 (20.3%) | 2 (9.1%) |
| 31–35 | 40 (47.1%) | 32 (50%) | 12 (54.5%) |
| 36–40 | 18 (21.2%) | 16 (25%) | 5 (22.7%) |
| >41 | 6 (7.06%) | 3 (4.7%) | 3 (13.6%) |

$p$ value − 0.87 (Fisher Exact test).

25 to 41 year old age group and 44.1% of patients were between 31 to 35 years of age.)

In 85 cycles (Group A), hCG was administered based on the follicle size and estradiol level, before the occurrence of LH surge. In 64 cycles (Group B) hCG was administered after a spontaneous LH surge. In 22 cycles (Group C), hCG was not given in the presence of spontaneous endogenous LH surge. The final analysis was performed on 171 cycles. The pregnancy rate in Group A was 15.2% (13/85). The pregnancy rate in Group B was 20.3% (13/64) and the pregnancy rate in Group C was 22.7% (5/22). There was no significant difference in pregnancy rates between different groups, $p > 0.50$.

There was no significant difference in the distribution of treatment cycles with respect to age or etiology (as shown in Tables I and II). Even though no associations between age, etiology and treatment groups was observed, a multivariate analysis was nevertheless performed to adjust for these two potentially confounding variables. No significant differences in pregnancy rates across the treatment groups were observed when adjusted for age and etiology (as shown in Tables III and IV).

## DISCUSSION

Intrauterine insemination combined with induction of ovulation is an effective treatment for several types of infertility. The aim of IUI is to provide an increased concentration of motile spermatozoa in the proximity of the oocyte. The oocyte is capable of being fertilized only in the hours immediately following ovulation. Administration of hCG to facilitate the final maturation process of the follicle and oocyte release is widely used in IUI protocols.

IUI is a recommended form of treatment for women using cryopreserved donor sperm. The outcome of IUI cycles is influenced by many factors. To maximize the pregnancy rates, IUI should be closely timed with ovulation (1). Hormonal events of ovulation assist in timing the administration of hCG. Many studies have demonstrated that the LH surge is a crucial event for final maturation and ovulation (7). Martinez *et al.* demonstrated that there might be a beneficial effect in timing the hCG injection to the spontaneous onset of LH surge (5). Their analysis of mid-cycle events showed that the day of hCG injection based on the follicle size was significantly earlier in the cycle compared with the spontaneous urinary LH surge.

Testart *et al.* (7) demonstrated that the ideal time for oocyte collection in IVF cycles is 34–35 h after the initiation of LH surge. Testart and Frydman showed that the onset of LH rise was found to be a more accurate criterion than the LH peak in determining the time of ovulation (9). Testart and Frydman defined the initiation of LH surge as the time when the LH

**Table II.** Comparability of Treatment Groups with Respect to Etiology

| Etiology | Group A (hCG by follicle size) $n = 85$ | Group B (hCG by serum LH surge) $n = 64$ | Group C (No hCG) $n = 22$ | $p$ value (Fisher Exact test) |
|---|---|---|---|---|
| Unexplained | 35 (41.1%) | 23 (35.9%) | 7 (31.8%) | 0.53 |
| Male factor | 30 (35.3%) | 24 (37.5%) | 10 (45.5%) | 0.82 |
| Anovulation | 17 (20%) | 10 (15.6%) | 5 (22.7%) | 0.80 |
| Cervical factor | 8 (9.4%) | 6 (9.4%) | 0 | 0.37 |
| Endometriosis | 2 (2.4%) | 3 (4.7%) | 3 (13.6%) | 0.12 |
| Multiple factors | 9 (10.6%) | 2 (3.1%) | 2 (9.1%) | 0.22 |

*Note*. Frequencies may sum to greater than the treatment group sample size because subjects with multiple factors also appear in the individual factor groups.

**Table III.** Pregnancy Rates in Three Treatment Groups with Respect to the Age Group

| Age group (years) | Group A (hCG by follicle size) $n = 85$ | Group B (hCG by serum LH surge) $n = 64$ | Group C (No hCG) $n = 22$ |
|---|---|---|---|
| All age groups | 13/85 (15.2%) | 13/64 (20.3%) | 5/22 (22.7%) |
| <30 | 4/36 (11.1%) | 5/36 (13.9%) | 0/36 (0%) |
| 31–35 | 6/84 (7.1%) | 5/84 (5.9%) | 1/84 (1.2%) |
| 36–40 | 3/39 (7.7%) | 3/39 (7.7%) | 2/39 (5.1%) |
| >41 | 0/12 (0%) | 0/12 (0%) | 2/12 (16.7%) |

$p > 0.50$ by Fisher exact test.

level increases by 80% over the mean LH level in the previous 24 h (8). A study by Martinez *et al.* (5), suggested the LH surge is a reflection of the natural maturation process of the follicle. In this study, the pregnancy rates in CC induced cycles after IUI were 9.3% (4/43) in the group of hCG-induced ovulation compared with 20.5% (9/44) in the group of spontaneous ovulation (ovulation detected by urinary LH assay). They also found that the mean diameter of the pre-ovulatory follicle was significantly smaller and insemination was substantially earlier in hCG induced cycles.

Transvaginal sonographic estimation of the follicular diameter and detection of LH in the urine by LH kits are commonly used methods of attempting to predict ovulation (10). Monitoring by transvaginal sonography is a non-invasive office technique with a pitfall resulting from the wide variation in the pre-ovulatory follicle size. Vermesh *et al.* (4), showed the size of the pre-ovulatory follicle ranges from 18–30 mm. The time of rupture of the follicle from the time of administration of hCG is also variable. Anderson *et al.* (11), showed a range of 34–46 h for the follicle to rupture from the time of administration of hCG based on follicle size of 18 mm or more by transvaginal sonography. Urinary LH monitoring also has limitations with occurrence of false negative and positive results. Irons and Singh (10) found that some women might ovulate before LH can be detected in the urine. When LH kits alone were used, in 9% of the women the urinary LH surge was detected after the follicle rupture was demonstrated by ultrasonography (12). Home urine testing can detect LH with serum levels of 20–40 IU/L. Arici *et al.* (13), showed 12% of normal ovulatory women have LH peak values of less than 20-IU/L.

The prospective, randomized, crossover study by Zreik *et al.* (14), compared the pregnancy outcomes in hCG-timed versus urinary LH-timed IUIs in CC induced cycles. This study showed no statistically significant differences in the pregnancy rate with the use of hCG induction based on the follicle size versus urinary LH monitoring to time IUI. Awonuga *et al.* (15), combined transvaginal sonography and LH monitoring to determine the timing of IUI and did not demonstrate an increased pregnancy outcome.

The study by Fuh *et al.* (6) in gonadotropin-IUI cycles revealed a significantly higher pregnancy rates

**Table IV.** Pregnancy Rates in Three Treatment Groups with Respect to Etiology

| | Group A (hCG by follicle size) $n = 85$ | | Group B (hCG by serum LH surge) $n = 64$ | | Group C (No hCG) $n = 22$ | |
|---|---|---|---|---|---|---|
| Etiology | Etiology not present | Etiology present | Etiology not present | Etiology present | Etiology not present | Etiology present |
| Unexplained | 5/49 (10.2%) | 7/35 (20%) | 9/40 (22.5%) | 3/23 (13%) | 4/17 (23.5%) | 1/7 (14.3%) |
| Male | 11/54 (20.4%) | 1/30 (3.3%) | 7/39 (17.9%) | 5/24 (20.8%) | 4/14 (28.6%) | 1/10 (10%) |
| Anovulation | 8/67 (11.9%) | 4/17 (23.5%) | 8/53 (15.1%) | 4/10 (40%) | 2/19 (10.5%) | 3/5 (60%) |
| Cervical | 11/76 (14.5%) | 1/8 (12.5%) | 11/57 (19.3%) | 1/6 (16.7%) | 5/24 (20.8%) | 0 |
| Endometriosis | 12/82 (14.6%) | 0/2 | 11/60 (18.3%) | 1/3 (33.3%) | 4/21 (19%) | 1/3 (33.3%) |
| Multiple factors | 11/75 (14.7%) | 1/9 (11.1%) | 11/61 (18%) | 1/2 (50%) | 4/22 (18.2%) | 1/2(50%) |

*Note*. Frequencies may sum to greater than the treatment group sample size because subjects with multiple factors also appear in the individual factor groups.

in the group where hCG was given after the LH surge. In this study the initiation of the LH surge was diagnosed by serum LH levels. Due to the inaccuracy of urine LH testing, a combination of transvaginal sonography and serum LH levels may be a better predictor of ovulation for timing of IUI.

A retrospective analysis by Mitwally *et al.* (16), revealed a significantly higher pregnancy rate in CC cycles when hCG was administered with an endogenous LH surge. They defined the LH surge as an increase in LH level $\geq$100% over the mean of the preceding 2 days. In the current study, patients were monitored by transvaginal sonography and serum LH levels. The pregnancy rate was 15.2% (13/85) in cycles where hCG was given with a follicle size of 18 mm or more and 20.3% (13/64) in cycles where hCG was administered after an endogenous increase in LH levels were observed. Even though an increase in the pregnancy rate was found, the difference was not statistically significant ($p > 0.50$). The pregnancy rate was 22.7% (5/22) in cycles where there was an endogenous LH surge and hCG was not given. Groups B and C together (86), who had spontaneous increase in LH levels, the pregnancy rate was 20.9% (18/86).

In conclusion, timing of the hCG injection to the LH surge did not result in a significant increase in the pregnancy rates, however success rates trended higher. Our data showed that hCG administration did not improve outcome after an endogenous LH surge was detected. Additional studies with larger number of patients may be needed to fully answer the questions of whether or not hCG injection in relation to the LH surge could significantly alter success.

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