Role of Low-Dose Human Chorionic Gonadotropin Following Clomiphene Citrate in Folliculogenesis and Ovulation in Infertile Women

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Abstract:

Background:Ovulation disorders, usually presents as menstrual disturbance, are the cause of infertility in around 25% of couples who have difficulty to conceive. The drug most commonly prescribed for the induction of ovulation is clomiphene citrate (CC). If the patient does not ovulate after the use of CC, the choice was to add gonadotropins. Gonadotropin therapy is very expensive with significant risks of high order multiple gestations and ovarian hyperstimulation syndrome. Human chorionic gonadotropin hCG can function as a surrogate for LH and occupies LH receptors for more than 24 hours allowing prolonged stimulation also it has longer half-life and greater affinity for the LH receptor.

Objective: The purpose of this study was to compare the effectiveness of low-dose human chorionic gonadotropin (hCG) in the late follicular phase to induce ovulation in clomifene citrate resistant patients who had previously failed to ovulate on clomiphene citrate (CC) alone.

Subjects,Materials and Methods: A total of 71 patients who attend infertility clinic in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, where prospectively randomly assigned into two groups. hCG Group received 100 mg dose of CC from day 5 of menstrual cycle for 5 days, then each patient received 200 IU hCG (DICLAIR®) subcutaneous injection daily when the largest follicle mean diameter was 12 mm or larger starting on day twelve of menstrual cycle. Non-hCG group received 150 mg dose of CC from day 5 of menstrual cycle for 5 days, and both groups were monitored with transvaginal ultrasound. Ultrasound measurements of follicle number and growth, ovulation, endometrial thickness and pattern were recorded and compared between the two groups. Student t test and fisher exact test were used for statistical comparison between the two groups.

Results: The low-dose hCG group had significantly higher percentage of ovulatory cycles (80% vs 44.4% P value 0.006). There was no significant difference in the endometrial thickness between the groups but it appears higher in hCG group (10.55±1.82 vs. 9.62 ± 1.87 in non-hCG group, p value 0.056). Non significant but higher incidence of echogenic (luteinized) endometrium 48 hour post ovulation in hCG group (36/40 (90%) vs. 20/25 (80%) for non-hCG group p-value = 0.288).

Conclusions: The use of low-dose hCG after CC in the late follicular phase results in continued follicle growth, higher ovulation rate. This treatment offers an efficient and cost-effective alternative before gonadotropin therapy for this type of patients.

Keywords:low dose hCG,Clomiphene Citrate,infertile women.

Introduction:

Infertility is a complex disorder with significant medical, psychosocial, and economic problems. It is defined as the inability of a couple to conceive following 12–24 month of regular un protected sexual relation if maternal age is ≤35 years old and following 6 months of regular unprotected sexual relation when maternal age more than 35 years old (1).

An ovulation as a cause of infertility is very common problem and account for about to 25% of infertility in women and usually presents as oligomenorrhea or amenorrhea. Polycystic ovary syndrome is the commonest cause (70%-80%) of medically treatable an ovulatory infertility (2).

Many ovulation induction drugs have been proposed for treatment of an ovulation but the first line oral treatment is clomifene citrate, Clomiphene citrate has been introduced in 1956. That is the first-line method of ovulation induction in women with anovulatory infertility. It is estrogen receptor modulator exert partial agonist and antagonist effects according to the tissue estrogen receptor content and estrogen level. Clomiphene is inexpensive, well-tolerated, safe, and effective (3).

Treatment should be initiated at 50 mg daily on cycle days 2 to 5 (follicular phase) and continued for 5 consecutive days with increases of 50 mg in subsequent cycles if anovulation persists. The Food and Drug Administration advise 100 mg or less, but up to a maximum dose of 250 mg is used in some specialty practices (4). Structurally, CC is related to diethylstilbesterol, potent synthetic estrogen. Chemically related to chlorotrianisene, which is a weak estrogen. Clomiphene includes an unequal mixture of two isomers as their citrate salts, enclomiphene and zuclomiphene. Zuclomiphene is the more powerful for induction of ovulation, accounts for 38% of the total drug content of one tablet and has a longer half-life than enclomiphene, being measurable in plasma one month following its administration (5). The principal mechanism of CC action is a decrease in the negative feedback of endogenous estrogens due to continued exhaustion of hypothalamic and pituitary ER (Estrogen Receptor). This action leads to an upsurge in the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus into the hypothalamic-pituitary portal circulation, provoking an increase in the release of pituitary gonadotropins (6).

Clomiphene can induce ovulation in 80% of anovulatory women but only 40% of women became pregnant. Pregnancy rate per cycle can be 10-20% Unfortunately, 20-25% of the women are resistant to CC and fail to ovulate (7).

Many anovulatory infertile women are resistance to anti-estrogens and need another treatment. Due to the anti-estrogenic effect of this drug, endometrial proliferation may be inappropriate, which decreases the chance of embryo implantation. Moreover, this effect can also change the cervical mucus characteristics with a consequent reduction in sperm penetration. If the patient does not ovulate after the use of CC, gonadotropins for timed intercourse or ovarian drilling are the next steps to manage anovulatory infertility (8).

The second-line pharmacological treatment of infertility in anovulatory women with PCOS includes the use of gonadotropins [recombinant follicle-stimulating hormone (rFSH) or human menopausal gonadotropin (HMG)] for timed intercourse or intrauterine insemination (IUI) (9).Due to the cost of the treatment, the need for regular monitoring of the follicular development via ultrasound and the higher multiple pregnancy rates with timed intercourse, and the use of gonadotropin is not routine, instead, this medication is used in high-complexity treatments like IVF or ICSI (10).

Traditionally, the role of LH in the control of the menstrual cycle was believed to be limited to stimulating theca cell androgen production, triggering ovulation and support of the corpus luteum. However, the physiologic selection of the dominant follicle in spontaneous menstrual cycles is believed to be the result from the expression of LH receptors in the more mature ovarian follicles ≥10 mm in diameter (11).

Human chorionic gonadotropin is a hormone comprising an α-subunit and a β-subunit which are held together by non-covalent hydrophobic and ionic interactions.The β-subunit of hCG, somewhat is structurally similar to the β-subunit of LH. One variant of hCG is pituitary hCG, produced during the female menstrual cycle. Pituitary hCG functions in an LH-like manner to promote follicular maturation, stigma formation and meiosis in the primary follicle, ovulation, luteinization of the follicle, and progesterone production during the menstrual cycle (12).

Subjects,Materials and Methods:

This is a prospective cross section (comparative) study conducted in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University. The study was approved by the Local Medical Ethical Committee of the institute, written informed consent was obtained from each patient. A total of 71 patients who attend the infertility clinic in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, were randomly assigned into two groups.

The patients had to meet the following criteria: age between 18-40 years; failed to ovulate on CC at the 100-mg dose; period of infertility > 1 year; documented normal uterine cavity and patent tubes by either hysterosalpingogram or laparoscopy and hysteroscopy; normal fasting glucose and insulin levels, normal serum prolactin, thyroid function test and FSH. The male partner had to have a normal semen analysis according to World Health Organization criteria 2010.

Forty patient randomly assigned to hCG group received 100 mg CC on days 5 to 9 of period. Daily 200 IU hCG subcuteneouse injection were added when the mean diameter of the largest follicle was ≥12 mm and continued until the follicle reached a mean diameter of 20 mm or larger. Thirty-one in the Non-hCG group who received 150 mg CC on days 5 to 9 of their cycle. In cycle day 2 levels of FSH , LH, prolactin, Estradiol (E2) and testosterone were drawn in both groups. Serum levels of progesterone (P), testosterone, and E2 where measured on day 12 of the cycle and repeated on the day of cycle cancellation or day of ovulation triggering. Transvaginal ultrasound for follicular monitoring was started on day 12 of the cycle and repeated every 1 to 2 days until the mean diameter of the lead follicle was 20 mm or larger in both groups. If the follicle mean diameter failed to grow a minimum of 1 mm per day after a 14 mm mean diameter, or a 14 mm mean diameter was not achieved, the monitoring was stopped and the cycle was canceled. Human chorionic gonadotropin (10,000 IU IM) was given when the mean diameter of the lead follicle was 20 mm or larger. Follow-up ultrasound scans were performed 48hr after the hCG injection to confirm corpus luteum formation and any continued development of secondary follicles. Ultrasound ovulation criteria were the disappearance of the preovulatory follicle or follicles, the presence of fluid in the cul-de-sac and/or the formation of an echogenic cyst consistent with a corpus luteum. Follicle growth, number and endometrial development were followed by ultrasound scans and recorded for each patient. Student t test and fisher exact test were used for statistical comparison between the two groups.

Results:

The patient characteristics of the two treatment groups are shown in Table (1,2). No significant differences between the groups were observed in age, body mass index (BMI), duration, type of infertility or baseline hormone levels. A total of 71 patients were randomly assigned, four patients in the non-hCG group (150-mg CC group) were cancelled on day twelve of the cycle because they failed to achieve follicle diameter of 12mm or larger and another two patients were cancelled after day twelve of the cycle because they failed to complete their follicular growth as shown in figure (1). This resulted in 40 patients in hCG group and 25 in non-hCG group (150-mg CC) for a total of 65 patients who completed the study.

Table (1): Comparison of demographic data between two study groups

Parameter		hCG group No.=40 Mean±SD	Non-hCG group No.=31 Mean±SD	P value
Age (year)		26.18±5.47	27.71±3.98	0.193*
BMI (kg/m²)		28.10±3.85	29.13±4.10	0.287*
Duration of infertility (year)		4.11±2.64	3.64±2.45	0.466*
		No. (%)	No. (%)	
Type of infertility	Primary	21 (52.5)	11 (44.0)	0.612**
	Secondary	19 (47.5)	14 (56.0)	

BMI (body mass index)
* Unpaired t-test,** Fisher exact test.

Table (2): Comparison of hormone profile at cycle day two between the two study groups by unpaired T-test

Hormone	hCG group No.=40 Mean±SD	Non-hCG group No.=31 Mean±SD	P value
FSH (mIU\ml)	7.16±2.3	6.95±1.89	0.671
LH (mIU\ml)	5.28±2.18	5.82±3.41	0.450
Prolactin (ng\ml)	14.42±6.22	13.31±6.63	0.476
E2 (pg\ml)	47.64±21.18	56.5±24.69	0.145
Testosterone (ng\ml)	0.62±0.4	0.59±0.25	0.654
TSH (µU\ml)	2.16±0.97	2.36±0.62	0.361

FSH (follicle stimulating hormone), LH (luteinizing hormone), E2 (estradiol), TSH (thyroid stimulating hormone).

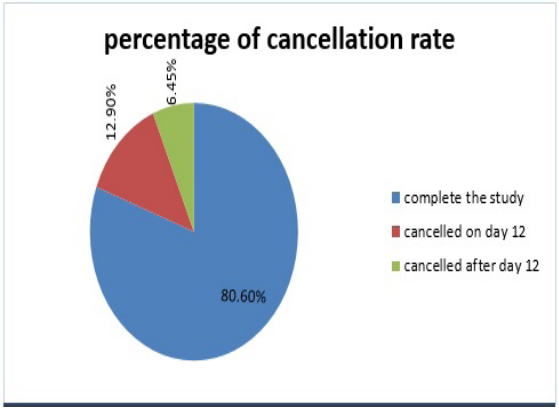


Figure (1): Cancellation Rate non-hCG group

Concerning the endometrial thickness on day twelve of the cycle, table (3) showed no significant difference was found between hCG and non-hCG group p-value (0.260). Regarding the number of follicles which is ≥12mm on day twelve of the cycle, table (3) also shows no significant difference between the two group p-value (0.264).

Table (3): Comparison of ultrasound parameter at cycle day twelve between two study groups, by unpaired T-test

Ultrasound parameter	hCG group No.=40 Mean±SD	Non-hCG group No.=25 Mean±SD	P-value
No. of follicle >12mm	2.58±1.2	2.24±1.13	0.260
Endometrial thickness (mm)	6.34±1.17	6.76±1.62	0.264

With regard to ultrasound parameters on the day of trigger which include endometrial thickness and number of follicles of ≥ 17 mm in diameter, table (4) illustrate greater thickness and higher number of follicles in hCG group although no significant difference was found between the two study group, p value (0.707), and (0.203) respectively.

Table (4): Comparison of ultrasound parameter at day of trigger between two study groups by unpaired T-test

Ultrasound Parameter	hCG group No.=40 Mean±SD	Non-hCGgroup No.=25 Mean±SD	P value
No. of follicle >17mm	2.0±0.68	1.92±0.91	0.707
Endometrial thickness (mm)	8.71±1.22	8.25±1.51	0.203

Concerning endometrial pattern at day of trigger, although table (5) shows no significant difference between the two-study group p-value (0.066), but hypoechoic (triple) endometrial pattern was higher in the hCG group than the non-hCG group.

Table (5): Comparison of endometrial pattern at day of trigger between two study groups by Fisher exact test

Parameter		hCG group No.=40 No. (%)	Non-hCG group No.=25 No. (%)	P value
Endometrial Pattern	Hyperechoic (lutenized)	10 (25%)	12 (45%)	0.066
	Hypoechoic (triple)	30 (75%)	13 (52%)	

With reference to the sign of ovulation (48 hour) after trigger, the hCG group had significantly higher percentage of ovulatory cycles (32/40) 80% versus (11/25) 7%, P value (0.006) compared with non-hCG group shown in figure (2), while figure (3), demonstrates no significant difference between the two group regarding endometrial thickness (10.55±1.82 vs. 9.62 ± 1.87 in non-hCG group, p value 0.056).

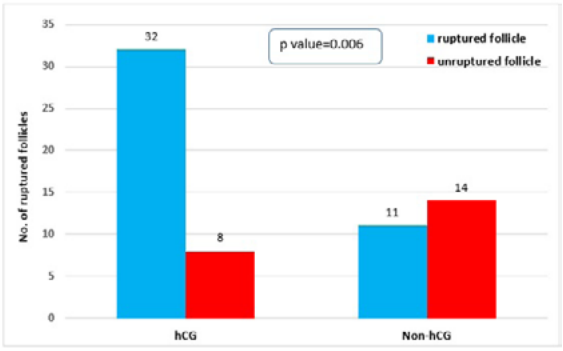
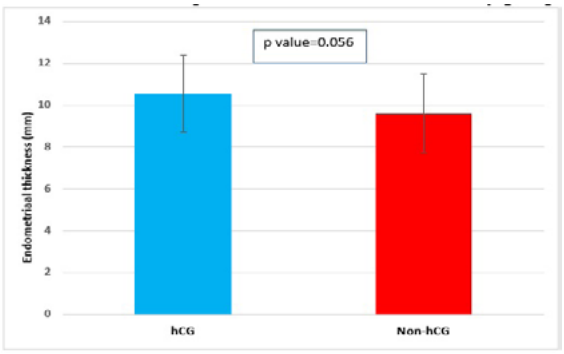


Figure (2): Number of ruptured follicles 48 hour post ovulation in the two study groups, by Fisher exact test.



Figure(3): Endometrial thickness 48 hour post ovulation in the two study groups, unpaired T-test.

With regard to endometrial pattern figure (4) illustrates no significant difference but the hyper echoic pattern is higher in hCG group where 36 out of 40 (90%) patients have hyper echoic pattern 48 hour post trigger compare to non-hCG group where 20 out of 25 (80%) patients have hyper echoic pattern 48 hour post ovulation (p-value 0.288).

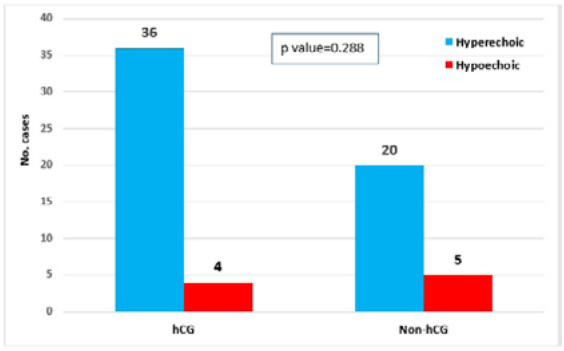


Figure (4): Endometrial pattern 48 hour post ovulation in the two study groups, by Fisher exact test.

Discussion:

FSH is considered to be the principle driver of folliculogenesis and the role of LH in this process is not as well understood. In the normal menstrual cycle, elevation of FSH levels in the early follicular phase stimulate recruitment and growth of preantral and antral follicles (13). By mid and late follicular phase, FSH levels decline and progressive rises in LH concentrations are associated with the selection of the dominant follicle and the regression of the smaller follicles. This is thought to be related to the expression of LH receptors on the granulosa cells of the dominant follicle that allow it to be responsive to LH and less dependent on FSH (14).

Regarding the endometrial thickness which is higher in the hCG group although no significant difference was found between the two groups and this may be due to the fact that increase number of growing follicles which occur as a result of higher dose of clomiphene citrate leading to increase estrogen production which increase endometrial thickness (15). While in reference of endometrial pattern in the current study, the trilaminar endometrial pattern (hypoechoic) on the day of trigger was higher in the hCG group compared to the non-hCG group, as clarified in (table 5).

Receptive endometrium is very essential for the implantation of an embryo. Endometrial thickness, tri-laminar pattern and, Doppler assessment of blood flow toward the endometrium are essential detriments of endometrial receptivity (16).

In natural cycle little changes of the endometrium are visible 36-48 hour after LH surge. But under the influence of progesterone, the epithelial glands and vasculature continue to grow and become spiral, whereas the endometrial thickness is relatively changed, resulting in a denser endometrium. Endometrial thickness (ET) measurement is a predictor for a successful implantation following ovulation induction, with many studies reporting more success with a thickness of 9 - 10 mm (17).

Chen *et al.* performed a study in 2010 and concluded that a combined analysis of endometrial thickness and pattern on the day of HCG administration was a better predictor of the outcome of IVF/ICSI and may be more helpful for patient counseling than the separate analyses (18).

hCG might also affect endometrial function, stimulate endometrial growth and maturation and enhance the endometrial angiogenesis. These effects could extend the angiogenesis. These results could lengthen the implantation window (19). Tesarik *et al*. showed that the administration of hCG to oocyte recipients increased the endometrial thickness on the day of embryo transfer and improved the implantation rate (20).

The number of dominant follicles in both groups nearly similar as shown in table (4) and this goes with results of researcher which found that increased CC dose shows poor completion of folliculogenesis, lower estradiol, and endometrial development. This is consistent with increased FSH priming but failure to select a dominant follicle and complete folliculogenesis (15).

In this study two patients were cancelled from the study in the non-hCG group because of failure to complete follicular growth while no patient where cancelled in the hCG group as illustrated in figure (1).

Filicori *et al* showed that in the final stages of ovulation induction in controlled ovarian hyperstimulation by using purified FSH-only preparations, that after 7 days of FSH-only priming, the completion of folliculogenesis could be achieved with the administration of low-dose human chorionic gonadotropin (hCG) (19).

It seems that adding low dose hCG in the late follicular phase to the FSH regimen has a positive impact on oocyte maturation. Mahnaz Ashrafi *et al* in their study showed that replacement of rFSH with low dose hCG (100 or 200 IU, daily) stimulated follicle growth and estradiol production to levels comparable with those of patients who continued to receive rFSH alone. In other words, the combination of FSH and low dose hCG (100 IU) has similar folliculogenesis when compared with low dose hCG alone (200 IU) (21). This finding was similar to Filicori *et al*. who reported a similar number of mature oocytes that developed in both the conventional protocol and low dose hCG regimen the similar E2 level and number of large pre-ovulatory follicles could be the reasons for similar folliculogenesis in low dose hCG groups (19).

In this study low-dose hCG injections was added when a lead follicle of 12 mm mean diameter was achieved. The ovulation was much better in hCG group. This suggests that in these patients, the LH receptors are expressed that allows the hCG to complete folliculogenesis. The growth in follicles were associated with endometrial development, and regression of secondary follicles this aligned with normal ovulatory menstrual cycle.This study has shown that the selective addition of LH in the form of low-dose hCG, can in a selected population be used to complete folliculogenesis in patients who were previously resistant to CC alone. The use of CC and low-dose hCG resulted in ovulation in those patients. This, approach represents a new efficient, easy to use, and low-cost alternative for those patients before moving to gonadotropin therapy.

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