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Effect of low-dose Human chorionic gonadotropin following clomiphene citrate, on pregnancy rate and Endocrine response of women with an ovulatory infertility.

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Background
Clomiphene citrate (CC) has been the 1st line medication to stimulate a multiple follicular development and ovulation in women with anovulatory infertility. CC act as an antiestrogen on the central nervous system, so increases the pulse frequency of FSH and LH and gives a moderate gonadotropin stimulus to the ovary, thus overcoming ovulatory disturbances.
Low dose hCG can mimic the LH actions on follicular growth and oocyte maturation. Longer half life and greater affinity to LH/hCG receptors, make it a more effective alternative for ovulation induction protocols.
Objective:
To compare the efficacy of low dose human chorionic gonadotropin (hCG) following CC on pregnancy rate and endocrine response in patients who had previously failed to ovulate on clomiphene citrate (CC) alone.
Methods*:*
Seventy-one infertile women who attend infertility clinic were eligible for this study. Our trial was performed in the High Institute of Infertility Diagnosis and Assisted Reproductive Technology, Al-Nahrain University. The patients were prospectively randomly assigned to two groups. hCG Group receive 100 mg dose of CC but started a 200 IU hCG (DICLAIR®) subcutaneous injection daily when the largest follicle was 12 mm or larger in mean diameter on day twelve of menstrual cycle. Non-hCG Group received a 150 mg of CC and both groups were monitored with transvaginal ultrasound and serum levels of E2, Progesterone, and testosterone. Ultrasound measurements of follicle number and growth, ovulation, endometrial thickness and pattern were recorded, and pregnancy rate are compared between the 2 groups. The main outcome measure was pregnancy rate and endocrine response. Student t test and fisher exact test were used for statistical significance.
Results:
As compared to the non-hCG group (clomifene citrate only group), patients who were given low dose hCG had significantly higher pregnancy rate. Regarding endocrine response no significant difference found between the two group. Regarding estradiol level the results was (479.71 ± 262.74 vs. 412 ± 287.9 non-hCG group p=0.347), progesterone, (1.22±1.11vs. 0.8±0.99 in non-hCG group p=0.112), but both hormones appear higher in hCG group, while regarding testosterone level no significant difference was found between the two group (0.52±0.18 vs. 0.53±0.27 in non-hCG group p=0.769).
Conclusions:
A combination of clomiphene citrate and low dose hCG improve the outcomes in CC resistant patients with improved safety and lowered cost.

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I. Introduction:

Infertility is defined as the failure to conceive after 1 year of unprotected intercourse. Ovulation disorders are defined by the failure of an ovum to be expelled because of a malfunction in the ovary and are a major cause of infertility. Polycystic ovary syndrome (PCOS) is the most common hormonal disorder in women and accounts for ~80% of women with anovulatory infertility [(1)].

CC is the first ovulation induction drug generally used in clinical practice. It is a medication used to treat infertility in women who do not ovulate this include those who have polycystic ovary syndrome [(2)]. The commonly used dosage of CC is between 50-150 mg/day. Doses higher than 150 mg rarely required because it rise the antiestrogenic effect of CC on the endometrium and cervix, which is not suitable [(3)]. The pregnancy rate

is moderately low in spite of high ovulation rate, because it has a certain influence on the endometrium and cervical mucus. Clomiphene citrate also may cause diminution of uterine blood flow, reduced placental protein 14 synthesis, Subclinical pregnancy loss. It has an effects on tubal transport, and unfavorable effect on the oocyte [(4)]. The ovulation rate ranges between 70% and 92% in clomiphene citrate treatment; however, the pregnancy rate is much lower [(5)]. Clomiphene Citrate is beneficial in infertile patient with anovulation or oligo-ovulation and used to rise success rate of other assisted reproductive technology[(6)].

During the natural ovarian cycle, different pituitary hormones are responsible for follicle recruitment and growth. It is clear that, both LH and FSH have a prominent role in normal ovulatory actions of a natural cycle and low endogenous LH concentrations in the late follicular phase might lead to poor pregnancy outcomes [(7)]. In the early follicular phase, follicle stimulating hormone (FSH) is responsible for early follicular growth and development. FSH stimulates the creation of LH receptors on granulosa cells permitting for the secretion of small amounts of progesterone and 17-hydroxy-progesterone (17-OHP) which may exert a positive feedback on the pituitary gland to improve luteinizing hormone (LH) release [(8)].

Many studies established that LH has an additional role in follicular growth and this role starts at intermediate follicular phase but this role is not well understood. It is presumed that it plays a role in carrying and maintaining intra ovarian paracrine system by its action on granulosa and theca cells [(9)].

Low dose hCG has a longer half-life and lower cost compared with recombinant FSH or LH [(10)]. Many researchers noted that adding LH activity, through administration of low-dose hCG in the late follicular phase resulted in follicles development and oocytes maturation, avoidance of premature luteinization, improved clinical pregnancies, lowering the rFSH requirements and reducing COH costs. Conversely, some other authors failed to confirm these findings [(11)].

II. Materials And Methods:

This prospective, randomized clinical trial was performed at the High Institute of Infertility Diagnosis and Assisted Reproductive Technology, Al-Nahrain University in the period from July 2017 to May 2018. The study was approved by the Local Medical Ethical Committee of the institute, and written informed consent was obtained from each patient. Seventy-one patient with anovulatory infertility who met all of the following inclusion criteria were randomly assigned into two treatment groups.

Eligibility criteria for inclusion were

a) Age of women between 18 and 40years.
b) Period of infertility > 1year.
c) Basal serum follicle stimulating hormone level (FSH) ≤12 mIU/mL in the early follicular phase.
d) had at least one patent tube proved by hysterosalpingography or laparoscopy.
e) Male partner has satisfied the normal parameters of semen analysis according to the modified WHO criteria (2010).
f) Good physical and mental health.
g) No response to clomiphene citrate, or failure of ovulation on cc.

Forty patient assigned to hCG Group received 100 mg CC on days 5 to 9 of that cycle, then received daily 200 IU hCG subcutaneous injection when the mean diameter of the largest follicle was12 mm or larger and continued until the follicle reached a mean diameter of 20 mm or larger. Thirty-one women in Non-hCG Group received 150 mg CC on days 5 to 9 of their cycle. Day 2 levels of FSH, LH, prolactin, Estradiol(E2), TSH and testosterone) were assessed 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 in both groups. Transvaginal ultrasound follicular monitoring was started on day 12 of the treatment 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 was achieved or a 14 mm mean diameter was not achieved, the monitoring was stopped and the cycle was canceled in both groups. Human chorionic gonadotropin (10,000 IU IM) was given when the mean diameter of the lead follicle was 20 mm or larger. Couples were to have intercourse. Follow-up ultrasound scans were performed 2 days after hCG injection to confirm ovulation criteria which are 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. All patients had a blood-based pregnancy test at 2 weeks after ovulation, follow-up for those with positive pregnancy test by trans-vaginal ultrasound three weeks later to confirm clinical pregnancy (gestational sac with positive fetal heart rate). Student t test and fisher exact test for paired groups were used for statistical significance between the groups and for hormonal comparisons.

III. Resultes

The demographic data of all patients whom participate in the study, regarding the age and body mass index (BMI) showed no significant difference between the two groups. The mean age of hCG group when compared to mean age of non hCG group is of no significant difference (*p* value 0.193), while the *p* value of the BMI when compared between the two group is (0.287) as table (1) illustrates.

Regarding the type and duration of infertility, no significant differences were found between the two groups *p* value (0.466, 0.612 respectively) as shown in table (1).

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

Parameter		**hCG group (Clomid+hCG) No.=40 Mean±SD**	**Non-hCG group (Clomid) No.=31 Mean±SD**	**P value**
Age (year)		26.18±5.47	27.71±3.98	0.193*
BMI (kg/m^2)		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

Hormonal level

1. Hormone profile on day two of the cycle

Concerning the hormonal status of the two groups on the second day of menstrual cycle, no significant differences were found in the values of all hormones including (FSH, LH, Prolactin, Estradiol, Testosterone, and TSH), *p* value (0.671, 0.450, 0.476, 0.145, 0.654, 0.361 respectively) as shown in table (2).

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).

2. Hormone profile on day twelve of the cycle

With regard to hormone profile on day twelve of menstrual cycle for both hCG group and non-hCG group table (3) shows that there was no significant difference in the level of estradiol, progesterone, and testosterone with *p* value (0.868, 0.265, 0.059 respectively).

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

Hormone	**hCG group No.=40 Mean±SD**	**Non-Hcg group No.=25 Mean±SD**	**P value**
Estradiol (E2) (pg\ml)	282.23±139.1	292.15±273.89	0.868
Progesterone (ng\ml)	0.31±0.29	0.41±0.4	0.265
Testosterone (ng\ml)	0.3±0.21	0.42±0.24	0.059

3. Hormone profile on day of trigger

No significant difference between the two study groups in estradiol, progesterone, and testosterone levels on the day of trigger as in table (4), *p* value (0.347, 0.112, 0.769, respectively).

Table (4): Comparison of hormone profile at day of trigger between the two study groups, by unpaired T-test

Hormone	hCG group No.=40 Mean±SD	Non-hCG Group No.=25 Mean±SD	P value
Estradiol (E2) (pg/ml)	479.71±262.74	412.24±287.9	0.347
Progesterone (ng/ml)	1.22±1.11	0.8±0.99	0.112
Testosterone (ng/ml)	0.52±0.18	0.53±0.27	0.769

4. Pregnancy rate:

Significantly higher pregnancy rate in the hCG group 14 out of 40 (35%) compared with non-hCG group 3 out of 25 (12%) p value (0.047) as shown in Figure (1).

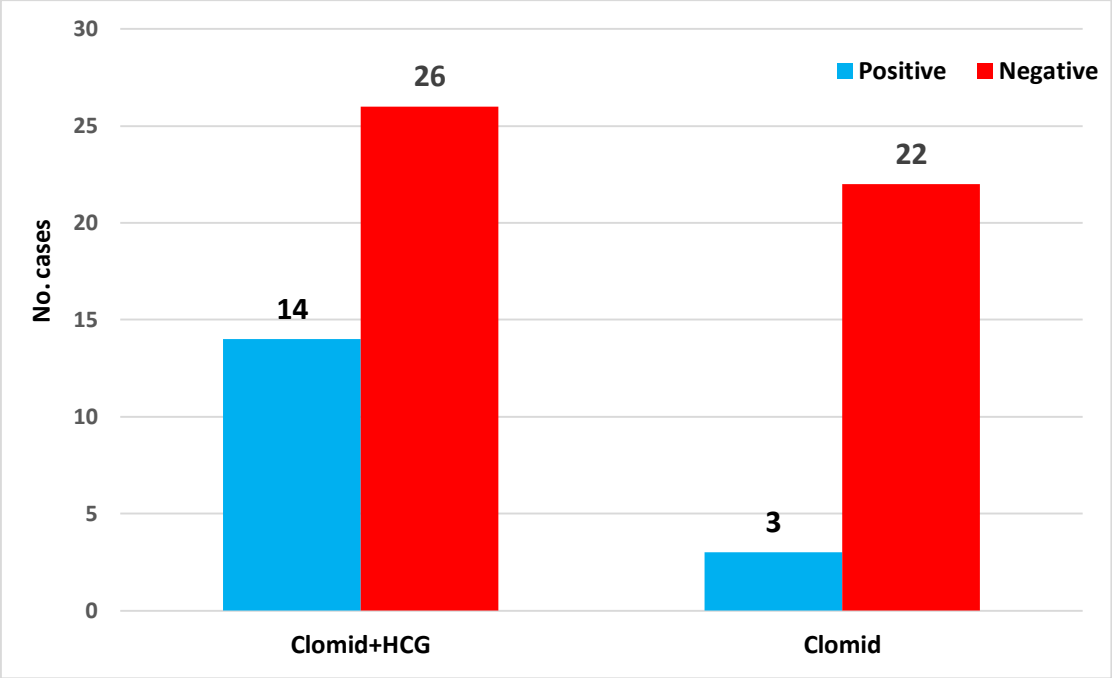

Figure (1): Pregnancy rate between the two groups.

IV. Discussion:

1. Effect of hCG on progesterone:

A mild elevation in progesterone level on day of trigger in hCG group was found. hCG is at least 6 times more potent than LH there was a concern that hCG might result in premature luteinization of the follicle [(12)]. There was no evidence of premature luteinization due to the preovulatory low-dose hCG as progesterone levels remaining ≤2.0 ng/ml as shown in table (4) and this goes with the results of Branigan et. al where they also found mild elevation in progesterone level with the low dose hCG [(13)].

Over the past few years, many different cut-off levels for elevated progesterone in stimulated cycles have been proposed, ranging from 0.8 to 2.0 ng/ml [(14)]. In current study there were no preovulatory progesterone levels ≥ 2.0 ng/ ml, and this level is not significantly differ between the two groups.

FSH stimulates the production of progesterone by driving cholesterol conversion into the steroid pathway. Progesterone biosynthesis requires two enzymatic steps: first, the conversion of cholesterol to pregnenolone (P5), catalyzed by the enzyme cytochrome P450scc (side-chain cleavage), and second its subsequent conversion to progesterone, which is catalyzed by 3β-hydroxy-steroid-dehydrogenase (3βHSD). Progesterone is further metabolized to androgens by the action of CYP17 in the thecal cells under the influence of LH. This step only takes place in the thecal cell compartment [(15)].

Early increased exposure to progesterone can advance the endometrium, leading to asynchrony of embryo development to endometrial development and the reduction of implantation. LH stimulates the

conversion of progesterone into androgens, which can be further aromatized to estrogens. The addition of LH may benefit the endometrium by decreasing the risk of a premature progesterone increase and therefore improve the likelihood of implantation and clinical pregnancy as receptive endometrium is vital for the implantation of an embryo [(16)]. During the early follicular phase, however, the enzymatic activity necessary to convert 17-OH-progesterone to androstenedione, is absent or very low. Therefore, this process leads to increasing concentrations of progesterone and oestradiol, as the follicular diameter increases [(17)].

2. Effect of hCG on estradiol

The E2 level was slightly higher in hCG group, this does not go with the results of Branigan et al. where they found significant difference in E2 level which is higher in the hCG group. The explanation for this is that the increased CC dose shows an increase in overall number of follicles with diameter of 12 mm or larger but poor completion of folliculogenesis, lower estradiol, and endometrial development [(13)].

3. Effect of hCG on androgen

Testosterone level has mild elevation in hCG group this is consistent with other study done by Branigan et al. who found that the growth in follicle size was associated with mild increase in androgen levels and endometrial development and regression of secondary follicles that is typical of a normal menstrual cycle, this mild elevation of androgens seen in these patients may be increased substrate for conversion to estradiol and may have caused the atresia of the secondary follicle [(13)]. Mahnaz Ashrafi et al. also found a higher pre-ovulatory serum level of testosterone in patients who received 200 IU low-dose hCG[(18)].

This increase in androgen levels in the low dose hCG groups may also be due to inability of the small follicles to aromatize androgens to estradiol. Androgen production from cholesterol and release during folliculogenesis is dependent on the stimulation of the theca cells by LH and FSH. This is universally recognized as the key driver of ovarian follicle growth and maturation. Ovarian steroidogenesis in the preovulatory follicle takes place through LH receptors on theca and FSH (possibly plus LH) receptors on granulosa cells. LH induces androgen production in the theca compartments. Then these theca-driving androgens are converted into estradiol by aromatize enzymes. The steroidogenic acute regulatory protein (StAR protein) is the primary regulator of production of androstenedione, which subsequently diffuses into granulosa cells to serve as an estrogen precursor. In the preovulatory follicle, cholesterol in theca cells arises from circulating lipoproteins [(19)].

LH administration enhances follicular androgen production followed by its aromatization to estrogen. It also controls progesterone production by granulosa cells, which is also FSH dependent [(20)].

4. Effect of hCG on pregnancy rate:

Higher pregnancy rate in patients who received low dose hCG than the second group and these results is consistent with the results of Branigan et al. who found significantly higher pregnancy rates (18% vs 0% P value 0.001) [(13)] and, Razeih Dehghani- Firouz abady et al. who also found significantly more chemical and clinical pregnancies (26% vs. 10%, p = 0.02) [(21)]. A likely explanation for the mechanism underling the lower rate of pregnancy in Clomiphene Citrate group is due to the anti-estrogen effect of Clomiphene citrate, resulting in long-lasting estrogen receptor (ER) depletion, as it accumulates in the body due to its long half-life (2 weeks), causing an adverse effect on the quality and quantity of a cervical mucus, these undesirable effects of Clomiphene citrate on the endometrium may explain the relatively poor pregnancy rate associated with Clomiphene citrate [(22)].

Luteinizing hormone and hCG are characterized by specific molecular and biochemical features; they interact with distinct binding sites of the same receptor, resulting in lower dissociation rate by hCG than LH binding. Low dose hCG has a longer half-life and lower cost compared with recombinant FSH or LH. In addition, full development of large follicles, adequate ovarian hormonal levels, oocyte maturation, avoidance of premature LH surge, and increased pregnancy rates have been demonstrated by the addition of low dose hCG in late foliculogenesis [(23)].

Drakakis*et al*, in a prospective randomized study tried to determine whether low dose hCG added to rFSH for ovarian stimulation could produce better results compared to the addition of rLH in women entering IVF-ET in a short protocol, especially in those women with previous failed IVF attempts.Results showed that, due to the use of hCG, less gonadotropin ampoules were needed for ovarian stimulation and higher fertilization and higher pregnancy rates were recorded. There was also a tendency for a better implantation rate, even in women of advanced reproductive age with higher basal FSH levels, which are often considered to have poor ovarian response to stimulation. In addition, the percentage of mature oocytes and the number and quality of embryos was comparable between rLH and hCG groups. [(24)].

V. Conclusion

A combination of Clomiphene citrate and low dose hCG improve the outcomes in CC resistant patients with improved safety and lowered cost.

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