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Hormonal changes in consecutive clomiphene citrate stimulation cycles and their effect on pregnancy rates

Ardışık klomifen sitrat stimülasyon sikluslarında hormonal değişiklikler ve gebelik oranlarına etkisi

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Abstract

Objective: To determine the relationship between the cumulative effect of sequential clomiphene citrate (CC) treatments in unexplained infertile women with intercycle and intracycle serum hormone changes.

Materials and Methods: Patients who received CC 50 mg in the first cycle (group I, n=34) as ovulation induction and those who received CC 50 mg in the second consecutive cycle (group II, n=18) were compared. Basal (cycle days 2-5) and trigger day (the day that recombinant human chorionic gonadotropin is given) levels of gonadotropin and steroid hormones were measured.

Results: The 17OHP increase on trigger day was found to be statistically significantly higher in group II compared to the basal day (p=0.083). The testosterone (T) response on the trigger day of the patients in group II was found to be statistically significantly higher than that in group I (p=0.023). The number of selected follicles was negatively correlated with a follicle-stimulating hormone decrease and positively correlated with an estradiol increase. Endometrial thickness was positively correlated with a luteinizing hormone increase, and cycle cancelation was positively correlated with decreased estradiol.

Conclusion: Based on this study, it was concluded that the reason for the increased efficiency rate in successive cycles of CC may be the cumulative increase in T and 17OHP levels. However, this result was found not to affect the clinical pregnancy rate.

Keywords: Clomiphene, ovulation induction, steroids

Öz

Amaç: Açıklanamayan infertil kadınlarda, ardışık klomifen sitrat (CC) tedavilerindeki kümülatif etkinin, sikluslar arası ve siklus içi serum hormon değişiklikleri ile arasındaki ilişkiyi belirlemek amaçlandı.

Gereç ve Yöntemler: Ovulasyon indüksiyonu olarak ilk sikluslarında CC 50 mg (grup I, n=34) ve ardarda ikinci siklusta CC 50 mg alan hastaların (grup II, n=18) siklusları karşılaştırıldı. Bazal (siklusun 2-5. günleri) ve ovulasyonun tetiklendiği günlerde (rekombinant insan koryonik gonadotropinin verildiği gün) gonadotropin ve steroid hormon seviyeleri ölçüldü.

Bulgular: Tetik günündeki 17OHP artışı, grup II'de bazal güne göre istatistiksel olarak anlamlı derecede yüksek bulundu (p=0.083). Grup II'deki hastaların tetikleme gününde testosteron (T) yanıtı grup I'dekilere göre istatistiksel olarak anlamlı derecede yüksek bulundu (p=0.023). Seçilen folikül sayısının, folikül uyarıcı hormon azalmasıyla negatif, östradiol artışıyla pozitif korelasyon gösterdiği bulundu. Endometriyal kalınlık ile lüteinize edici hormon artışı arasında, siklus iptali ile östradiol düşüşü arasında pozitif korelasyon bulundu.

Sonuç: Bu çalışmadan yola çıkılarak, CC'nin ardışık sikluslarındaki artan verimin nedeninin, T ve 17OHP seviyelerindeki kümülatif artıştan kaynaklanabileceği sonucuna varılmıştır. Ancak bu sonucun klinik gebelik oranını etkilemediği görüldü.

Anahtar Kelimeler: Klomifen, ovulasyon indüksiyonu, steroidler

PRECIS: We evaluated the cumulative effect of sequential clomiphene citrate treatments in unexplained infertile women, as well as inter- and intra-cycle serum hormone changes.

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Introduction

Ovulation is a physiological process defined by the ruptured release of the dominant follicle capable of fertilization from the ovary into the fallopian tube[1]. The paracrine and autocrine effects of hormones contribute to the regulation of this process[2]. Clomiphene citrate (CC) is a selective estrogen receptor modulator that reduces negative feedback to the hypothalamus by competing with estrogen for binding to the hypothalamic estrogen receptors. It is the first-line agent of choice to support ovulation for treating infertility in unexplained infertile women. It stimulates the secretion of hypothalamic gonadotropin-releasing hormone and then activates ovarian stimulation by increasing the release of gonadotropins[3,4]. It seems possible that CC stimulation can alter the endocrine environment both systemically and locally in the ovary. However, most women will achieve pregnancy by going through more than one cycle of CC stimulation. Because the cumulative effect of CC is mentioned.
It is not exactly known how the serum hormone levels change in the next cycle following a CC stimulation cycle that does not result in pregnancy even though there is follicle development. Until date, many studies have been conducted to examine the effects of gonadotropins and steroid hormones on folliculogenesis in infertility treatments. However, most of the studies were performed either in patients with polycystic ovary syndrome (PCOS) or in assisted reproductive technology (ART) treatments.
This study aims to compare the first cycle of CC used for ovulation stimulation and the second consecutive CC cycle (immediately after) in unexplained infertile women, and to examine the effect on endogenous hormones, through which hormones the cumulative activity develops and whether this affects pregnancy rates.

Materials and Methods

This study was conducted between August 2019 and March 2020 in a tertiary referral hospital infertility outpatient clinic as a prospective case-control study. Ethical approval was obtained from the Local Ethics Committee of University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital (approval number: 2019.08.1.04.061) for the study of "hormonal changes in incremental CC stimulation doses, and their effect on pregnancy rates". This study was conducted as a subgroup analysis of the other. The study was conducted following the Declaration of Helsinki and its later amendments. All participants were included in the study after obtaining informed consent.
Fifty-two CC cycles of 34 unexplained infertile women were included in the study. The patients given CC 50 mg first cycle were divided into group I (n=34), and the patients given CC 50 mg for the second consecutive month as group II (n=18). Of the 34 patients in group I, 5 conceived. CC 100 mg was given in the next cycle because six patients did not develop follicles with CC 50 mg. Five patients wanted to interrupt the treatment. Eighteen patients who ovulated with CC 50 mg were taken into the second cycle. Women between the ages of 20 and 35 who were hormonally eugonadotropic and had no Müllerian anomaly or bilateral tubal obstruction on hysterosalpingography were included in the study. Serum Anti-Müllerian hormone (AMH) levels, body mass index (BMI), homeostatic model assessment for insulin resistance (HOMA-IR) and the type of sterility (primary/secondary) were recorded. Couples with normal spermiograms or mild male factor infertility (i.e. male partners with only one of the following abnormalities: Sperm counts <20 million/mL, normal morphology <4% or sperm motility <40% and post-wash total motile sperm counts ≥5 million/mL) of their male partners were included in the study. The following exclusion criteria were applied: severe male factor, recurrent pregnancy loss, use of co-medication (myoinositol, metformin, cortisone/prednisolone), tubal obstruction, additional endocrine (such as PCOS) or medical disorders, women with AMH <1.1 ng/mL, previous pelvic surgery, ovarian cysts, endometriosis, those with a BMI >30 kg/m^2. Those who became pregnant with the first cycle CC or those whose follicle development could not be achieved with CC 50 mg were excluded from the second cycle CC 50 mg group.
Folliculometry with transvaginal sonography and pre-stimulation hormonal testing [follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone (P_4), estradiol (E_2), prolactin (PRL), thyroid-stimulating hormone (TSH), AMH, androstenedione (A_4), total testosterone (T), dehydroepiandrosterone sulfate (DHEA-S), 17 hydroxyprogesterone (17OHP)] were performed on the second to fifth day (basal day). Blood sampling from the antecubital vein was performed after an overnight fasting between 8:00-10:00 am. CC (Klomen 50 mg; Kocak Farma-Turkey) stimulation was begun with an initial dose of 50 mg/day from the fifth day to the ninth day of the menstrual cycle having early cycle blood P_4 <0.5 ng/mL and E_2 <50 pg/mL levels. Beginning with the 11th day, patients underwent every other day transvaginal sonografic monitoring of endometrial thickening and follicular growth. When the leading follicle reached a mean diameter of 18-20 mm, ovulation was induced by subcutan application of choriogonadotropin alfa (rHCG, Ovitrelle 250 μgr, Merck Serono-Turkey) (trigger day). All hormonal tests (FSH, LH, E_2, P_4, A_4, T, DHEA-S, 17OHP) were performed again before rHCG application.
Intrauterine insemination or timing intercourse was performed within the 36th to 40th hours. Blood betaHCG levels were measured on the 15th- to 20th-day postinsemination. If the beta-HCG blood level was higher than 20 mIU/mL, the conception was confirmed. Cycle cancellations were due to no ovarian response for 21 days follow up or excessive ovarian responses (blood E_2 level >1500 pg/mL or more than two selected follicles ≥16 mm, on the trigger day). Patients who ovulated with CC 50 mg but did not become pregnant were given CC 50 mg in the same manner for the second consecutive cycle with the next menstrual cycle. For women who could not achieve ovulation

with CC 50 mg, CC 100 mg treatment was started in the next cycle. However, this group was excluded in the study.

Primary outcome measure: Changes in the endogenous blood levels of FSH, LH, E_2, P_4, A_4, T, DHEA-S, and 17OHP in CC cycles.

Secondary outcome measure: Clinical pregnancy rate (CPR), live birth rate (LBR).

All hormones were measured in the biochemistry laboratory of our hospital using the UniCel DxI 800 immunochemistry analyzer (Beckman Coulter Inc., USA) according to the manufacturer's assay instructions and requirements. Access FSH, LH, PRL and TSH values were assayed using a two-site immunoenzymatic (sandwich) method. Access E_2, T, P_4, A_4, DHEA-S, and 17OHP values were assayed using the competitive immunoenzymatic method. Basal AMH concentrations were assayed following a highly specific enzyme-linked immunosorbent method. The coefficients of variation intraassay and interassay tests of these hormones are as follows [mean ± standard deviation (SD)]: 7.33±1.54 mIU/mL for FSH, 5.89±2.84 mIU/mL for LH, 40.1±16.5 pg/mL for E_2, 0.59±0.34 ng/mL for P_4, 1.04±0.44 ng/mL for A_4, 200.76±74.5 μg/dL for DHEA-S, 0.48±0.18 ng/mL for T, 1.84±0.59 ng/mL for 17OHP, 4.86±3.35 ng/mL for AMH.

Statistical Analysis

Mean SD, median, minimum, and maximum values are given in the descriptive statistics for continuous data. Number percentages are given for discrete data. The Shapiro-Wilk test and Stem-and-Leaf Plot test were used to examine the compatibility of continuous data to the normal distribution. In the comparison of continuous variables between independent groups, Student's t-test was used for normally distributed data and Mann-Whitney U test was used for data not normally distributed. In dependent groups, the t-test (paired sample t-test) was used for data conforming to the normal distribution. The Wilcoxon test was used for the data that did not show a normal distribution. Fisher's Exact and chi-square tests were used in group comparisons (cross tables) of nominal variables. The analyzes to be used were decided by testing the data for normal distribution, which is the most important assumption in the analyses. The IBM SPSS Statistics 20 program was used for evaluations and a p-value <0.05 was accepted to be statistically significant.

Results

The mean age of the women included in the study was 26.62±3.54 (20-33). Twenty-four women (70%) were primary infertile, and 10 women (30%) were secondary infertile (total n=34). Demographic characteristics of the groups are given in Table 1. The cycles were divided into 2 groups as the first cycle CC (n=34) (group I) and consecutive second cycle CC (n=18) (group II). Women in both groups were similar in terms of mean age, AMH (1.1-17.1), HOMA-IR (0.66-5.03), BMI (17.1-38.6), partner's spermiogram values, number of follicles selected after treatment (0-4) and cycle cancelation rates (p>0.05).

The comparison of basal day and trigger day hormonal values between groups is given in Table 2 and the variation of the differences in hormone values is given in Table 3. The correlation between the change (difference) hormones and the number of follicles selected is given in Table 4.

There were 5/34 (14.7%) and 0/18 (0%) conceptions in groups I and II, respectively. In other words, clinical pregnancy occurred in 5 of 52 cycles (CPR: 9.6%). One of them ended in miscarriage at 6 weeks. Four had live births at term (LBR: 7.7%). A comparison of hormonal change values of women with and without clinical pregnancy is given in Table 5.

Discussion

In this study, it was found that only the increase in T levels in consecutive cycles of CC was statistically significantly higher, but this did not affect the CPR. Several markers have been introduced for the cycle outcome prediction. However, most studies have been conducted either CC in women with PCOS or with gonadotropins in ART cycles. No study could be found in the literature regarding the clinical significance of hormonal change created by consecutive CC cycles in patients with normal ovarian reserves.

Table 1. Demographic characteristics of the groups

	Group I (n=34) Mean ± SD Median (min-max)	Group II (n=18) Mean ± SD Median (min-max)	Test statistics	p-value
Age (year)	27.18±3.65 26 (20-33)	25.56±3.17 25 (20-33)	t=1.593	[a]0.117
AMH (ng/mL)	4.51±2.80 3.46 (1.12-11.42)	4.93±3.80 3.36 (1.46-17.10)	U=300.5	[b]0.916
HOMA-IR	1.94±0.92 1.74 (0.72-5.03)	1.92±0.99 1.60 (0.66-4.22)	U=294.5	[b]0.825
BMI (kg/m^2)	26.40±5.04 26.28 (17.1-38.6)	25.67±4.60 25.28 (17.1-36)	t=0.509	[a]0.613

[a]: Student's t-test, [b]: Mann-Whitney U test, SD: Standard deviation, Min: Minimum, Max: Maximum, AMH: Anti-Müllerian hormone, HOMA-IR: Homeostatic model assessment for insulin resistance, BMI: Body mass index

Table 2. Comparison of basal day and trigger day hormone values of the groups

		Basal day Mean ± SD Median (min-max)	Trigger day Mean ± SD Median (min-max)	Test statistics	p-value
FSH (mIU/mL)	Group I	7.57±1.87 7.24 (4.17-12.58)	6.46±2.97 5.8 (3.49-16.68)	Z=-2.269	[c]**0.023***
	Group II	6.79±1.53 7.01 (4.15-9.12)	6.12±2.01 5.48 (3.51-11.50)	Z=-1.241	[c]0.215
	Test statistics	t=1.500	U=284.5		
	p-value	[a]0.140	[b]0.805		
LH (mIU/mL)	Group I	6.16±3.22 5.43 (2.22-13.63)	16.53±13.59 10.42 (4.33-55.61)	Z=-4.976	[c]**0.000***
	Group II	5.87±3.26 4.99 (0.70-11.99)	17.92±16.46 10.80 (5.10-60.98)	Z=-3.550	[c]**0.000***
	Test statistics	U=284.5	U=296.0		
	p-value	[b]0.805	[b]0.984		
E_2 (pg/mL)	Group I	39.62±15,73 36 (19.10-84.0)	371.82±322.42 320 (40.8-1166.0)	Z=-4.994	[c]**0.000***
	Group II	38.39±14.58 37.3 (4.3-60.7)	370.64±293.35 267.2 (37.3-1024.0)	Z=-3.680	[c]**0.000***
	Test statistics	U=281.0	U=284.0		
	p-value	[b]0.752	[b]0.798		
P_4 (ng/mL)	Group I	0.62±0.44 0.58 (0.05-1.82)	1.25±0.87 1.04 (0.27-3.70)	Z=-3.895	[c]**0.000***
	Group II	0.69±0.48 0.53 (0.16-1.91)	1.33±1.05 0.91 (0.22-4.60)	Z=-2.853	[c]**0.004***
	Test statistics	U=270.0	U=295.5		
	p-value	[b]0.595	[b]0.976		
A_4 (ng/mL)	Group I	1.02±0.48 0.92 (0.39-2.11)	1.67±0.54 1.55 (0.82-2.88)	t=-6.324	[a]**0.000***
	Group II	1.07±0.42 1.08 (0.46-2.11)	1.76±0.74 1.71 (0.51-3.36)	t=-4.499	[a]**0.000***
	Test statistics	t=-0.383	t=-0.908		
	p-value	[a]0.704	[a]0.369		
DHEA-S (µ g/dL)	Group I	190.12±66.06 175.8 (110-361.2)	212.14±79.45 204.8 (130.5-445.8)	Z=-2.486	[c]**0.013***
	Group II	215.78±85.03 192.1 (91.2-357.6)	255.82±114.65 230.5 (143.8-600.5)	Z=-2.107	[c]**0.035***
	Test statistics	U=182.5	U=162.0		
	p-value	[b]0.442	[b]0.104		
T (ng/mL)	Group I	0.46±0.19 0.42 (0.13-0.98)	0.55±0.21 0.52 (0.32-1.10)	t=-2.422	[a]**0.024***
	Group II	0.51±0.19 0.45 (0.23-0.88)	0.71±0.25 0.62 (0.32-1.14)	t=-4.749	[a]**0.000***
	Test statistics	t=-0.896	t=-2.361		
	p-value	[a]0.376	[a]**0.023***		

Table 2. Continued

		Basal day Mean ± SD Median (min-max)	Trigger day Mean ± SD Median (min-max)	Test statistics	p-value
17OHP (ng/mL)	Group I	1.73±0.58 1.8 (0.86-2.65)	2.23±1.16 2.36 (0-3.89)	Z=-0.889	[c]0.374
	Group II	1.98±0.64 2.17 (0.89-2.88)	3.48±1.49 3.86 (1.98-6.20)	Z=-2.366	[c]0.018*
	Test statistics	U=37.5	U=21.0	-	-
	p-value	[b]0.600	[b]0.083	-	-
Number of follicles selected	Group I	-	1.50±0.99 1 (0-4)	-	-
	Group II	-	1.56±0.78 2 (0-3)	-	-
	Test statistics	-	U=283.5	-	-
	p-value	-	[b]0.646	-	-
Male factor n (%)	Group I (mild) (absent)	-	3 (8.8%) 31 (91.2%)	-	-
	Group II (mild) (absent)	-	2 (11.1%) 16 (88.9%)	-	-
	Test statistics	-	χ^2 =0.071	-	-
	p-value	-	[d]1.000	-	-
Cycle cancelation n (%)	Group I (yes) (no)	-	6 (17.6%) 28 (82.4%)	-	-
	Group II (yes) (no)	-	3 (16.7%) 15 (83.3%)	-	-
	Test statistics	-	χ^2=0.008	-	-
	p-value	-	[d]1.000	-	-

[a]: Student's t-test, [b]: Mann-Whitney U test, [c]: Wilcoxon test, [d]: Chi-square, SD: Standard deviation, Min: Minimum, Max: Maximum, *p<0.05, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E_2: Estradiol, P_4: Progesterone, A_4: Androstenedione, DHEAS: Dehydroepiandrosterone sulfate, T: Total testosterone, 17OHP: 17 hydroxyprogesterone

In CC cycles, it has been shown that FSH increases with the first dose of CC and starts to decrease in the middle of the cycle with the last dose[5]. In our study, we found that FSH decreased statistically significantly in group I on the trigger day compared with the basal day. We also found a decrease in consecutive second cycle CC patients, but the difference was not statistically significant. Perhaps the cumulative effect of CC lasting up to 6 weeks can be explained by this effect on FSH[6]. Since we did not sample in the mid-follicular phase, we could not catch the physiological FSH peak[7]. As in the natural menstrual cycle[2,5-9], in this study, all hormones (LH, E_2, P_4, A_4, DHEA-S, and T) except FSH increased significantly on the trigger day according to the basal day values in both groups. However, when the two groups were compared with each other, the FSH change values (decrease amounts), LH, E_2, A_4, P_4 DHEA-S, T, and 17OHP change values (increase amounts) were found to be similar from basal day to trigger day. This result does not support the cumulative effect of the CC.

Previously, androgens were suspected to cause follicular atresia[10,11]. However, in subsequent studies, androgens have been shown to play an important role in follicular development[12-14]. Even today, numerous studies on androgens continue to produce conflicting results. The major androgens in the serum of normal cycling women are A_4, DHEA-S, T, and dihydrotestosterone (DHT)[15,16]. 17OHP is synthesized from P_4 with 17αhydroxylase and converted to A_4, T, E_2 by aromatase in the adrenal gland[17]. In this study, T levels were significantly increased in group II compared with group I. It was therefore concluded that this situation is one of the main responsible factors of CC in follicular development. Fanelli et al.[18] showed that the upper levels of 17OHP and T were higher in the luteal phase than in the follicular phase, but androgen levels did not change during the menstrual cycle. Studies have suggested that cumulative A_4 response[19], basal DHEA-S[20] or basal T levels[21,22] are predictors of follicle number, fertilized oocyte, mature oocyte count, embryo development in ART cycles. In contrast, Abide Yayla et al.[23] showed that basal

Table 3. Comparison of the hormone values on the trigger day of the groups according to basal day hormone values (differences)

	Group I Mean ± SD Median (min-max)	Group II Mean ± SD Median (min-max)	Test statistics	p-value
FSH change (difference) on trigger day relative to basal day	-1.10±3.77 -1.55 (-8.78-10.51)	-0.67±2.60 -1.14 (-4.38-4.0)	U=258.0	[a]0.442
LH change (difference) on trigger day relative to basal day	10.37±13.08 5.12 (-0.15-47.63)	12.05±15.21 6.03 (-2.82-50.52)	U=271.0	[a]0.608
E_2 change (difference) on trigger day relative to basal day	332.20±315.23 243.1 (-0.6-1082)	332.26±290.48 240.5 (-4.7-983.4)	U=281.0	[a]0.752
A_4 change (difference) on trigger day relative to basal day	0.62±0.47 0.64 (-0.52-1.40)	0.69±0.63 0.75 (-0.26-2.16)	U=185.5	[a]0.787
DHEA-S change (difference) on trigger day relative to basal day	22.02±36.78 18.35 (-46.2 (94.30)	40.03±76.99 34.1 (-61.4-279.60)	U=183.5	[a]0.587
T change (difference) on trigger day relative to basal day	0.09±0.18 0.08 (-0.49-0.45)	0.19±0.17 0.18 (017-0.53)	U=133.0	[a]0.060
17OHP change (difference) on trigger day relative to basal day	0.49±1.29 0.91 (-1.73-1.92)	1.49±1.22 1.31 (0.41-4.03)	U=21.0	[a]0.299

[a]: Mann-Whitney U test, SD: Standard deviation, Min: Minimum, Max: Maximum, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E_2: Estradiol, A_4: Androstenedione, DHEAS: Dehydroepiandrosterone sulfate, T: Total testosterone, 17OHP: 17 hydroxyprogesterone

Table 4. Correlation between the change (difference) in hormones and the number of follicles selected

	Number of selected follicles	
	r	p
FSH change (difference) on trigger day relative to basal day	-0.424	**0.002***
LH change (difference) on trigger day relative to basal day	-0.111	0.439
E_2 change (difference) on trigger day relative to basal day	0.642	**0.000***
A_4 change (difference) on trigger day relative to basal day	0.030	0.854
DHEA-S change (difference) on trigger day relative to basal day	-0.035	0.829
T change (difference) on trigger day relative to basal day	-0.175	0.273
17OHP change (difference) on trigger day relative to basal day	-0.244	0.362

r: Correlation coefficient, *p<0.05, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, A4: Androstenedione, DHEAS: Dehydroepiandrosterone sulfate, T: Total testosterone, 17OHP: 17 hydroxyprogesterone

serum DHEA-S and T levels have no value in predicting any cycle outcome parameter in a study of 120 women with diminished ovarian reserve. In this study, although hormonally T levels were significantly higher, and there was no difference between A_4, 17OHP, and DHEA-S levels, we could not find a relationship between them and the number of selected follicles and pregnancy outcomes.

It was found that as the decrease in FSH on the trigger day and the increase in E_2 increased, the number of selected follicles increased. When the relationship of hormonal changes between the groups with cycle cancelation was examined, no relationship was found with any hormonal change, except for the E_2 response.

Regardless of the groups, no difference was found between the hormonal changes of women with or without conception. This result may have occurred because there were no patients with low ovarian reserve or PCOS in the study group. In CC cycles, pregnancy rates per cycle are reported to be 9.7-24.6% in anovulatory women[24] and 11.4-21.5% in ovulatory women[25]. Our results were consistent with this, and 5 women (9.6%) remained pregnant. However, all the pregnant women were from group I. We concluded that the main reason for this was the small number of patients in the groups.

Study Limitations

Owing to the rapid development in ART treatments, studies on first-line infertility treatments have unfortunately lost their appeal. The study strength is that it draws attention to this issue again. The small number of patients in the groups and the absence of a control group are the limitations of the study.

Table 5. Comparison of hormonal change values of women with and without clinical pregnancy

	Woman with clinical pregnancy Mean ± SD Median (min-max)	Woman without clinical pregnancy Mean ± SD Median (min-max)	Test statistics	p-value
FSH change (difference) on trigger day relative to basal day	0.70±6.4 1.06 (-8.78-7.64)	-1.13±2.99 -1.62 (-5.42-10.51)	U=75.0	[a]0.218
LH change (difference) on trigger day relative to basal day	15.45±13.75 10.57 (2.57-34.19)	10.47±13.80 5.47 (-2.82-50.52)	U=87.0	[a]0.395
E2 change (difference) on trigger day relative to basal day	229.76±148.23 243.1 (59.8-385.3)	343.36±315.11 243.9 (-4.70-1082)	U=102.0	[a]0.701
A4 change (difference) on trigger day relative to basal day	0.70±0.08 0.70 (0.64-0.77)	0.65±0.55 0.68 (-0.52-2.16)	U=35.5	[a]0.877
DHEA-S change (difference) on trigger day relative to basal day	14.75±1.48 14.75 (13.7-15.8)	30.24±58.21 21.21 (-61.6-279.6)	U=30.0	[a]0.624
T change (difference) on trigger day relative to basal day	0.05±0.05 0.05 (0.02-0.09)	0.14±0.19 0.12 (-0.49-0.53)	U=23.0	[a]0.380

[a]: Mann-Whitney U, SD: Standard deviation, Min: Minimum, Max: Maximum, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E_2: Estradiol, A_4: Androstenedione, DHEAS: Dehydroepiandrosterone sulfate, T: Total testosterone, 17OHP: 17 hydroxyprogesterone

Conclusion

To our knowledge, this is the first study to examine hormonal changes after consecutive CC stimulation cycles in unexplained infertile women with normal ovarian reserve. The findings of this clinical study suggest that CC had a cumulative effect on T. However, the observed effects of CC stimulation on the hormonal profile seemed to be of minor clinical relevance. This still needs to be further studied in covering the luteal phase and larger prospective studies.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Local Ethics Committee of University of Health Sciences Turkey, İstanbul Bağcılar Training and Research Hospital (approval number: 2019.08.1.04.061).
Informed Consent: All participants were included in the study after obtaining informed consent.
Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.E.K., Ö.K.A., T.K., Concept: E.E.K., E.Ş.Ö., Design: E.E.K., E.Ş.Ö., Data Collection or Processing: E.E.K., Ö.K.A., T.K., Analysis or Interpretation: E.E.K., E.Ş.Ö., T.K., Literature Search: E.E.K., Ö.K.A., Writing: E.E.K., E.Ş.Ö.
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