Comparison of a Novel Minimal Stimulation Protocol with Clomiphene Citrate plus Recombinant Follicle-stimulating Hormone to Recombinant Follicle-stimulating Hormone Alone for Ovulation Induction: A Prospective Study

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Abstract

Objective: The aim of this study has been to compare a combined minimal stimulation protocol with clomiphene citrate (CC) plus recombinant FSH (recFSH) to the standard protocol using recFSH only.
Materials and Methods: In the study group (n=34), CC 100 mg/day, was used between the cycle days of three to seven, and recFSH was used on cycle days 5, 7, and 9 at a fixed dosage of 75 units. In the control group (n=31), 75 units of recFSH was started on cycle day 3, and dosage was adjusted to the ovarian response.
Results: Total dose per treatment cycle was 225 units of recFSH in the study group, and 805.64±515.00 units of recFSH in the control group. The cost was significantly different between the groups (p<0.001). Three clinical pregnancies (8.8%) in the study group, and 2 clinical pregnancies (6.5%) in the control group were not statistically different.
Discussion: Our study suggests that this protocol is as effective as standard recFSH protocol, and it has lower cost for patients and the clinic. However, in order to reach a final conclusion, more study is needed with higher number of patients.

Keywords: clomiphene citrate, recombinant FSH, minimal stimulation protocol, non IVF infertility treatment

Özet

Yumurtlama Başlatılması için Klomifen Sitrata Ek Olarak Rekombinant Folikül Uyarıcı Hormon ile Yeni Bir Düşük Doz Stimülasyon Protokolünün Sadece Rekombinant Folikül Uyarıcı Hormon Kullanımı ile Karşılaştırılması: Prospektif Bir Çalışma

Amaç: Bu çalışmanın amacı, klomifen sitrat (CC) ve rekombinant FSH'nin (recFSH) birlikte kullanıldığı düşük dozlu stimülasyon protokolünün tek başına recFSH'nin kullanıldığı standart protokol ile karşılaştırmaktır.
Materyal ve Metot: Çalışma grubunda (n=34), siklusun üçüncü ila yedinci günleri arasında 100 mg/gün CC kullanıldı. Buna ek olarak siklusun 5, 7 ve 9. günlerinde 75 ünite sabit dozda recFSH eklendi. Kontrol grubunda (n=31) siklusun 3. gününde 75 ünite recFSH başlanarak doz yumurtalık yanıtına göre ayarlandı.
Sonuçlar: Çalışma grubunda recFSH dozu tedavi siklusu başına 225 ünite iken, kontrol grubunda bu doz 805.64±515.00 ünite idi. Tedavi maliyeti, gruplar arasında anlamlı şekilde farklıydı (p<0.001). Çalışma grubunda 3 (%8.8) klinik gebelik oluşurken, kontrol grubunda 2 (%6.5) gebelik oluştu. Gebelik oranları istatistiksel olarak farklı değildi.
Tartışma: Çalışmamız, bu protokolün standart recFSH protokolü kadar etkin olduğunu düşündürmektedir. Ayrıca, hasta ve klinik açısından bu protokolün daha düşük maliyeti vardır. Ancak, bu konuda kesin karara varmak için, daha fazla sayıda hasta ile yapılan çalışmalara ihtiyaç vardır.

Anahtar sözcükler: klomifen sitrat, rekombinant FSH, düşük dozlu tedaviler, IVF dışı kısırlık tedavisi

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Introduction

Clomiphene citrate (CC) in conjunction with FSH or HMG has been used for ovarian stimulation for two decades (1). In a trial that included CC, followed by FSH later in the treatment cycle was termed as a minimal stimulation (2). For the purpose of reducing the cost, milder stimulation regimes for ovulation induction in both IVF and non-IVF cycles have been reported in the literature (3-5).

In Turkey, since January 2005, the cost of treatment with gonadotropins for ovulation induction met by the governmental insurance organization is limited to 1500 units per cycle, and maximally for 3 cycles. The same rule is applied for IVF cycles.

The aim of this study has been to assess whether the minimal stimulation protocol using CC and recombinant FSH (recFSH) has comparable efficacy and is cost-effective when compared to standard protocol using recFSH only. With only a small number of studies available on this topic, we decided to perform additional analyses of the data with the following original protocol as: clomiphene citrate, 100 mg/day, used between the cycle days of three to seven, and recFSH used on cycle days 5, 7, and 9 at a fixed dosage of 75 units. We wished to report our experience, using recFSH and the described protocol above that has reduced the cost of the ovarian stimulation.

Materials and Methods

Patients

The protocol for this prospective, single center study included 65 infertile women aged 18-38 years (median, 29 years) for whom ovulation induction was indicated.

The patients were diagnosed as having subfertility if no abnormality was found during an extensive infertility examination, including basal hormonal evaluation, a hysterosalpingography, and at least one normal semen analysis. Ages of the couple, duration of marriage, duration of infertility, types of infertility were recorded. Blood samples were taken for the measurement of FSH, LH, and E_2 from all women, on cycle day 3. Their husbands' smoking habits, sperm analyses and sperm morphologies were recorded. Both groups were similar with respect to these parameters.

This was a prospective cohort study. All participants had received at least three cycles of CC for ovulation induction within the last year before the study had begun, but they had not conceived by 50-100 mg CC treatment. Sixty-five consecutively seen infertile women were randomized on an alternation basis to undergo either minimal stimulation protocol with CC and recFSH (34 patients and cycles) or with the recFSH low dose stimulation protocol (31 patients and cycles).

Couples were excluded, if there were untreated endometriosis or bilaterally occluded tubes, or if a semen analysis yielded less than one million progressively motile spermatozoa, or if women had any endocrinopathy. Women with a body mass index (BMI) above 30 kilograms per square-meter were also excluded.

All participants in the study provided written informed consent for the study, and the local ethic comittee gave its approval for the study protocol.

Ovulation induction

Treatment in the study group was started on cycle day 3 with CC (Klomen®, Koçak Farma, Istanbul, Turkey) 100 mg per day and continued for 5 days. We preferred this dosage, since all participants had received at least three cycles of CC treatment for ovulation induction within the previous year. Then recFSH (Gonal F®, Serono, Basel, Switzerland) were applied on cycle days 5, 7, and 9 with 75 units, giving a total of 225 units.

Treatment in the control group was started on cycle day 3 with 75 units of recFSH.

Both groups were followed by transvaginal ultrasonography started on cycle day 8, and then repeated daily or every other day according to development of follicles. Dosage was then adjusted according to ultrasound evaluation, in the control group. Unresponsiveness to ovulation induction is defined as no follicles greater than 10 mm in diameter, and E_2 level below 50 pg/ml, on cycle day 12.

When 1 to 3 follicles reached the diameter greater than 18 mm, 10 000 units of human chorionic gonadotropin (hCG) was scheduled for injection. Blood samples were taken for determination of FSH, LH, and E_2 levels, and the endometrial thickness on the same day was recorded. Whether intrauterine insemination (IUI) was done or not; and if IUI was done, the day of the cycle, were recorded. Only single IUI was applied after 36 hours from hCG injection. Progesterone and β-hCG levels were measured on cycle day 22. Whether ovarian hyperstimulation syndrome (OHSS) developed or not was recorded. If it has developed, then it was classified as mild, moderate, or severe based on the combination of ovarian enlargement and the acute fluid shift to the extravascular space.

A diagnosis of clinical pregnancy was confirmed by serum β-hCG concentration and visualization of the gestational sac on subsequent ultrasound examination. Biochemical pregnancies and early pregnancy losses were considered abnormal pregnancy outcome.

Statistical analysis

With a power of 80% (β=0.20), the sample size was calculated by taking the type 1 error as 0.05 (α=0.05), and significant difference in the dose of recFSH as 400 units. The standard deviation of 784.5 units had been calculated based on the results of our previous 183 gonadotropin cycles (unpublished data). With these figures, the best number of

patients needed in each arm (in the study and in the control group) was calculated as 31.

Groups were compared by duration of treatment, numbers of mature follicles on hCG day, numbers of follicles >10 mm in diameter on cycle day 12, peak E_2 levels on hCG day, cycle day of IUI, and total doses of gonadotropins used, and costs of treatment after one cycle of stimulation. Statistical analyses were done by using Statistical Package for Social Sciences (SPSS) 10.0 for Windows, software. χ^2, and ANOVA tests were used where appropriate. A p value of <0.05 was considered statistically significant.

Results

The two groups were comparable with respect to their demographic characteristics and their gynecologic and obstetric histories (Table 1). Twenty six (76.5%) women in the study group were primary infertile, 8 (23.5%) women were secondary infertile. In the control group these figures were 21 (67.7%), and 10 (32.3%), respectively. There were no statistically significant difference between the groups regarding the types of infertility (χ^2 test, p=0.30).

In the study group, 20 (58.82%) were anovulatory, 6 (17.65%) had unilateral tubal factor infertility, 4 (11.76%) had unexplained infertility, and 4 (11.76%) had subfertility related to both male and female. In the control group, 22 (70.96%) were anovulatory, 3 (9.67%) had unilateral tubal factor infertility, 4 (12.90%) had subfertility related to both male and female, and 1 (3.22%) had unexplained infertility. Groups were not different in this respect (χ^2, p=0.43).

The comparison between the minimal stimulation and recFSH protocol was analyzed by age of women, age of men, duration of marriage, duration of infertility, body mass indices (BMI), FSH, LH, E_2 levels on cycle day 3, numbers of cigarettes smoked per day by men, and sperm parameters. There were no statistically significant differences between the groups in all the above mentioned parameters except sperm concentration (ANOVA, p>0.05) (Table 1) which is higher in the recFSH group than in the study group.

Groups were further analyzed by duration of treatment, numbers of mature follicles on hCG injection day, numbers of follicles >10 mm in diameter on cycle day 12, peak E_2 levels on hCG injection day, cycle day of IUI, and total doses of gonadotropins used, and costs of treatment (Table 2).

The duration of treatment in study group was 6 days, whereas it was 10.00±4.86 days (4-30) in the control group. This difference was statistically significant (ANOVA, p<0.001) (Table 2).

IUI was applied to 28 (82.35%) patients in the study group, and to 24 (77.42%) patients in the control group. The usage of IUI was not different between the groups (χ^2, p=0.61). And the cycle day of IUI were not different between the groups (ANOVA, p=0.20).

Total doses of gonadotropins used were 225 units of recFSH in the study group, whereas they were 805.64±515.00 units of recFSH (300-2587.5) in the control group and the

Table 1. Comparison of groups by medical history and clinical parameters

Parameters	Minimal stimulation n=34 (Mean ±SD)	recFSH protocol n=31 (Mean ±SD)	*p*
Age of women (years)	28.64±5.83	29.90±4.01	0.32
Duration of marriage (years)	6.61±4.31	6.80±2.63	0.83
Duration of infertility (years)	5.32±3.47	5.77±2.74	0.56
Age of men (years)	33.41±4.68	35.06±4.61	0.15
Cigarettes smoked by men (numbers/day)	10.88±8.56	10.00±8.94	0.68
Body mass index (BMI) (kg/m^2)	25.20±4.69	24.15±2.29	0.26
Sperm parameters			
Concentration (million/ml)	45.17±13.97	70.16±54.65	0.01*
Motility A (%)	15.60±7.32	19.46±14.11	0.18
Motility B (%)	35.46±8.36	34.96±10.29	0.83
Motility C (%)	14.14±3.39	14.85±2.49	0.92
Motility D (%)	34.42±4.50	30.20±15.20	0.34
Total progressively motile sperm concentrations (Tpms) (million/ml)	15.28±8.01	13.45±8.00	0.51
Morphology of sperm (Kruger) (%)	8.69±3.58	8.26±5.06	0.73
Day 3 FSH (U/L)	6.76±2.31	6.03±2.34	0.20
Day 3 LH (U/L)	6.55±4.94	6.00±3.54	0.73
Day 3 E_2 (pg/ml)	56.01±39.96	51.80±30.22	0.63

*Statistically significant difference, ANOVA.

Table 2. Comparison of groups by characteristics of the treatment cycles

Characteristics of treatment cycle	Minimal stimulation n=34 (Mean ±SD)	recFSH protocol n=31 (Mean ±SD)	*p*
Endometrial thickness on hCG day (mm)	7.85±1.82	9.77±2.23	0.000*
Numbers of follicles on day 12	3.35±2.14	3.70±2.29	0.52
Numbers of mature follicles on hCG administration day	2.11±2.02	1.67±1.07	0.28
Peak E_2 before hCG administration (pg/ml)	1037.63±972.93	684.90±468.35	0.17
Day of IUI	14.78±1.77	14.16±3.34	0.20
Duration of treatment (days)	6.00±0.00	10.00±4.86	0.000*
Totally used gonadotropins (units)	225.00±0.00	805.64±515.00	0.000*

*Statistically significant difference, ANOVA.

difference was found statistically significant (ANOVA, $p<0.001$) (Table 2).

Since CC was very cheap and there were no difference in the number of patients having IUI, the difference in costs of treatment was mainly due to the amount of gonadotropins used. Therefore, we calculated the difference of total recFSH doses between the groups, and we expressed it as the cost of 580 units (nearly 600 units) of recFSH. We did not give any currency, because the cost of recFSH might vary from country to country.

Stimulation cycle was cancelled in one woman (2.94%) in the study group and in one woman (3.22%) in the control group. Cancellation occured when there were no follicles greater than 10 mm in diameter, on cycle day 12.

Groups were compared by the development of OHSS. There were no moderate or severe OHSS in both groups. Mild OHSS developed in 6 women (17.6%) in the study group, whereas there were 3 women with mild OHSS (9.7%) in the control group. But this difference was not statistically significant (χ^2, $p=0.28$).

There were 3 clinical pregnancies (8.8%) in the study group, while there were 2 clinical pregnancies (6.5%) in the control group. However, one of the pregnancies in the study group resulted in an abortus within the first 8 weeks of pregnancy. Pregnancy rates of study and control groups were not statistically different (χ^2, $p=0.77$). Although, endometrial thickness (Table 2) and sperm concentrations of husbands (Table 1) in study group were significantly less, these differences did not affect the clinical pregnancy rates.

Discussion

This study showed that, as a part of minimal stimulation protocol, CC followed by recFSH at a fixed dosage of 225 units, resulted in comparable ovulation and pregnancy rates when compared to stimulation by recFSH alone. This protocol caused a significant reduction in the amount of gonadotropins used with a mean difference of nearly 600 units of recFSH, and therefore reduced the cost of ovulation induction in non-IVF cycles, significantly. It also significantly decreased the duration of treatment.

In the past, a fixed dose human menopausal gonadotropin (hMG) with CC was used (6). The availability of recombinant gonadotropins allows us to design ovulation induction protocols with various FSH and LH combinations. By using recombinant hormones, the dose of FSH and LH can independently be changed. Weigert et al. compared CC plus recFSH (225 units) plus recLH (75 units) to long protocol with recFSH for IVF cycles and found similar pregnancy rates with reduced cost. They used, on average, nearly 3 ampules less recFSH than the long protocol (4).

Experience with minimal stimulation protocols in IVF cycles showed that pregnancy rates were similar when compared to the standard long GnRH-a protocol during the "fresh" transfer cycle. Additionally, patients undergoing a minimal-stimulation protocol used significantly less medication, with a mean difference of nearly 20 gonadotropin ampules (7). However, the minimal stimulation regimen results in fewer oocytes recovered, fewer embryos available after fertilization, and fewer excess embryos available for cryopreservation (4,7). Therefore, one must weigh the benefits of cost and disadvantages of minimal stimulation in deciding about the treatment protocol for IVF cycles (7).

Houmard et al. investigated factors affecting the pregnancy rates for non-IVF cycles with CC plus FSH in a retrospective study. Clinical pregnancy rates were compromised in women over 40, those with more than 3 years of infertility, and those who produce few follicles during stimulation. They concluded that the minimal stimulation protocol did not lead to pregnancy rates higher than that reported for CC/IUI cycles. However, their study was based on a retrospective chart review from 1996 to 2000. And of the 658 cycles reviewed, FSH (most probably urinary FSH) was usually given at 150 units (n=540 cycles) on day 9, but the dose varied from 150 to 450 units (5). Therefore, their results should be interpreted with caution.

In our study, although the duration of treatment in the study group was shorter than in the control group, the cycle day of

IUI was similar in both groups. In the study group, since follicular development continues after the treatment, follicles get mature around the same days of the cycle as in the controls, and therefore the cycle day of IUI should not be different between the groups.

One of the major concerns related to the use of CC in ovulation induction cycles is the antiestrogenic effects of CC. It is well documented that although CC is successful in inducing ovulation in 90% of cases, the number of pregnancies achieved after ovulation induction with CC is much lower than expected (8). Antiestrogenic effects of CC have been found to be related to the timing of administration of CC in the ovulation induction cycle. Earlier administration, on day 1 rather than day 5, results in higher pregnancy rates (9).

In this study, we started CC treatment on cycle day 3, and with this protocol, we reached similar numbers of mature follicles, similar peak E_2 levels, and similar clinical pregnancy rates as in the control group. This can be explained partially by the earlier administration of CC in the cycle, and partially by the addition of recFSH. With this protocol, E_2 levels were higher than the control group and more mild OHSS developed than the control group, but these differences were not statistically significant. However, the higher level of E_2 can be attributed to addition of recFSH in minimal stimulation protocol. There may be two other explanations for this finding: Firstly, CC treatment usually begins on the fifth day of the cycle since earlier administration is associated with multiple follicular development (10). As we started CC treatment on the third day of the cycle one will expect more follicles to develop. However, we have found no difference in the numbers of follicles developed between the groups. Secondly, CC induces a rise in both FSH and LH like the administration of human menopausal gonadotropin (hMG) (10). A review by Hughes et al. suggests that the use of FSH preparations results in a significant reduction in OHSS compared with hMG preparations (11). Although the difference in the development of mild OHSS did not reach statistical significance, this difference can be attributed to hMG-like affect of CC.

Cost-effectiveness plays an important role when choosing between alternative treatment protocols. Cost-effectiveness comparisons are most conveniently made when the treatments compared are similar in effectiveness. Our study revealed that the effectiveness of the study protocol was similar to the control group.

Cost-effectiveness analyses are most helpful to policymakers, and insurance administrators whose goal is to maximize the net health benefit to a target population from a fixed amount of resources.

Nevertheless, increasing costs are important in patient care, and therefore, cost-effectiveness analyses of infertility treatments are valuable to aid decision making for both institutions and individuals.

Our study suggests that the proposed minimal stimulation protocol for ovulation induction in non-IVF cycles is as effective as recFSH protocol, and it seems to have lower cost for patient and clinic. However, in order to reach a decisive conclusion, more studies based on higher number of patients are necessary.

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