Failure of Flexible Dose of Recombinant FSH as an Adjuvant to Clomiphene in Consecutive Intrauterine Insemination Cycles

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Abstract

Objective. *The aim of this study was to investigate efficiency of flexible dose of recombinant FSH as an adjuvant to clomiphene citrate and to investigate factors that effect pregnancy rates in couples undergoing consecutive intrauterine insemination (IUI) cycles.* ***Material and methods.*** *Three hundred and seven couples who underwent 580 IUI cycles with ovarian stimulation by clomiphene citrate and/or gonadotropins were analyzed from November 2004 to September 2009, retrospectively.* ***Results.*** *The overall and the clinical pregnancy rates per cycle were 9.8% and 8.3% respectively. There was no pregnancy in 48 cycles which induced with recombinant FSH only as an adjuvant to clomiphene. The only statistically different parameter was the sperm motility between pregnant and non-pregnant groups, but discriminative analysis revealed that this sperm parameter could not be used in the prediction of pregnancy alone.* ***Conclusion.*** *Although combined use of clomiphene citrate and human menopausal gonadotropins are effective in IUI and ART cycles, to support mono follicular maturation furthermore, adjuvant low dose recombinant FSH does not seem to be helpful in IUI cycles alone.*
Keywords: *intrauterine insemination, recombinant gonadotropin, pregnancy rate, clomiphene*

Introduction

Intrauterine insemination (IUI) is one of the most common methods used in infertility treatment before offering assisted reproductive techniques (ART) in the world as it can be performed effectively with low costs and simple technologic methods. It is mainly used in non-severe male and unexplained infertility treatment. Cervical factor, anovulation and endometriosis are the other indications for using IUI in the assisted reproductive technology[1-3].

The prognostic criteria in predicting the pregnancy in IUI are not clearly defined. There are many studies about semen characteristics including motility, count or morphology before or after sperm processing in predicting the successful pregnancy with IUI treatment[4-7].

In the literature, other factors are also reported influencing the pregnancy rate in this treatment; e.g. the woman's age, the length and the type of infertility, the total dosage of gonadotropin, and the estradiol level on the day of hCG administration[8].

Gonadotropins can be used alone or in combination with clomiphene to reduce the cost and risk of ovarian hyperstimulation syndrome, especially in IVF and GIFT cycles. Similarly gonadotopins can be administrated to support follicular maturation as an adjuvant therapy to clomiphene induced cycles in IUI cycles. The term minimal stimulation (MS) protocol has been used for an ovulation induction protocol in which clomiphene citrate and human menopausal gonadotropin combined sequentially. This protocol has been used as a reasonable option that is less expensive, requires minimal monitoring, easy to administer, and minimizes gonadotropin dosage and patient discomfort in IVF cycles[9]. As originally described standard protocol consists of 5 days of clomiphene citrate (100mg/day) followed by a single dose of menopausal gonadotropin (150 IU) on cycle day 9. It has been reported that minimal stimulation was as effective as the hMG alone protocol[10].

In this study, we aimed to investigate the efficacy of flexible dose of recombinant FSH administration as an adjuvant to clomiphene in minimal stimulation protocols and to investigate prognostic parameters that could be used as a discriminative factor in prediction of

the successful outcome in our consecutive intrauterine insemination cycles.

Material and methods

A total of 307 couples undergoing 580 IUI cycles performed in a private clinic and a university hospital were analyzed retrospectively. Indications for the treatment with IUI were as follows: infertility due to male factor, unexplained infertility, endometriosis, anovulation due to Polycystic Ovarian Syndrome (PCOS) or hyperprolactinemia. Before the IUI cycle, the basal hormone levels were measured in all women. The tubal patency was evaluated in all couples with sonography, hystero-salpingography and/or laparoscopy and IUI was performed only if at least one tube was permeable. PCOS were diagnosed according to the 2003 Rotterdam criteria. Endometriosis was suspected in the presence of ovarian endometrioma in sonographic evaluation or diagnosed with laparoscopy. The woman who failed to become pregnant after one year of unprotected regular intercourse was defined as primary infertile woman. Secondary infertility is defined as the inability to become pregnant, or to carry a pregnancy to term, following the birth of one or more biological children. The women having primary or secondary infertility with FSH level below the 15 IU/L and at least one tube patency were included in the study.

Couples with male factor infertility were included in the study if the total motile sperm count was higher than 5 million/mL. Kruger strict criteria were also evaluated in male factor.

Demographic characteristics of the patients like the woman's age, duration and cause of infertility; sperm parameters, estradiol level at the time of hCG administration, the number and outcome of each IUI cycle were recorded from the patient's files.

Semen Analyses and Sperm Preparation

All semen samples were obtained by masturbation after sexual abstinence of 3-5 days. The semen samples stayed 30 minutes at room temperature for liquefaction. After that, samples were studied for volume, pH, motility, sperm concentration. Sperm morphology was determined after staining fresh semen with a motile sperm. To obtain motile sperm, standard Swim-up technique or density gradient system were used as sperm processing.

Ovarian Stimulation and Insemination

Ovulation induction was performed with clomiphen citrate, gonadotropin or clomiphene plus gonadotropin in all of the IUI cycles regardless of the cause of infertility. Clomiphen citrate (Gonaphene; Schering-Plough, TR or Klomen, Koçak, TR) was started at 3rd-5th day of cycle and given 5 days with the dose of 50-150mg. Treatment with gonadotropins were performed by Recombinant FSH (Gonal-F, Serano, TR; Puregon, Schering-Plough, TR), Human Menopausal Gonadotropine (Menagon, Ferring GmbH, Kiel, Germany) with the starting dose of 50-150 IU on day 2 or 3. The first and repeated dosages and the duration of ovulation induction were differentiated for each woman according to the age, ovarian reserve, cause of infertility and ovarian response.

Recombinant FSH treatment as an adjuvant to clomiphene therapy was administered in 48 patients in whom two previous clomiphene cycles had failed or if there is no leading follicle (≥14mm) at the end of the clomiphene therapy. In this protocol, clomiphene citrate was administered on cycle day 3rd-4th day of cycle and given 5 days with the dose of 50-150 mg. On the day following the fifth day of clomiphene citrate administration, r-FSH therapy was administated for 1- 6 days with individualized dose and duration ranging from a total dose of 75 IU- 675 IU. Follicular development was followed with plasma estradiol (E2) levels and transvaginal sonography starting at the eighth day of cycle and repeating the follow up according to the maturation rate of follicles. When there was one follicle with a diameter of 17mm, ovulation was triggered with the 10000 IU of hCG (Pregnyl; Organon, Spain). IUI was performed 34-36 hours after the hCG administration with a soft catheter. Serum ß-hCG was measured 12 days after insemination. Clinical pregnancy was defined as presence of intrauterine gestational sac in transvaginal sonography.

Statistical Analyses

SPSS data software package 16.0 was used for statistical analysis. Data analyses were performed with Student t and paired t tests, life table analysis and Pearson correlation.

Results

The mean age of women was 29.3 ± 5.1 (Mean ± SD) and the mean duration of infertility was 5.1 ± 3.9 years. The distribution of reason for infertility were as follows; male factor: 39.7% (n = 122), anovulation: 45.6% (n = 140), endometriosis: 3.2% (n = 10) and unexplained infertility: 11. 4% (n = 35) were the indications for IUI.

A comparison of sperm parameters regarding to volume, count, motility, progressive motility and morphology before and after processing between women getting pregnant or not in IUI cycles were shown in Table 1. Sperm volume, and morphology pre or post processing total sperm concentration were not different between two groups ($p>0.05$). However, there was a statistically significant difference in post processing sperm motility between pregnant and non-pregnant groups ($p<0.05$). But discriminative analyses with ROC curve and life table analysis could not supported this parameter as a clinical significant factor in predicting pregnancy for our IUI cycles.

A total of 57 pregnancies were provided with 580 IUI cycles. The overall pregnancy rate was 9.8% (pregnancy/cycle). Forty-seven clinical pregnancies were occurred in all IUI cycles with rate of 8.1%. The clinical pregnancy rate per couple was 15.3%. The outcome of the pregnancies was as follows; 8 biochemical, 2 ectopic, 34 live-birth, 1 stillbirth and 12 abortion. There were 2 cases of twin pregnancies, in both clomiphene and gonadotropin therapy and both of them were ended with miscarriage. One woman had an intrauterine death following severe pre-eclampsia at 29 weeks' gestation.

Table 1 Comparison of the clinical and sperm parameters between pregnant and non-pregnant patients (mean ± SD).

		Pregnant	Non-pregnant
Age (year)		28.5±4.8	29.6±4.8
Infertility duration (year)		3.2±1.9	3.8±2.1*
Pre-processing sperm	Count (Mil./ml)	39.1±18.7	39.3±23.4
	Normal morphology (Kruger)	5.2±2.5	4.3±3.3
	Motility (%)	67.2±14.3	64.6±15.6
	progressive motility (%)	21.9±15.8	19.1±13.8
Post processing sperm	Count (Mil./ml)	32.7 ±21.2	30.8 ±19.8
	Motility (%)	92.7±4.8	89.4±12.4*
	progressive motility (%)	59.8±19.3	55.8±21.3

** Statistical significant different between groups*

Twenty-three clinical pregnancies were achieved with gonadotropin therapy in 311 cycles (7.3%). The pregnancy rate (24 pregnancies) was 11.3% in 221 cycles in which clomiphene used. There was no significant difference in pregnancy rates between gonadotropin and clomiphene therapy (P<0.05). After previous assessment revealed that pregnancy had not been achieved in consecutive 48 cycles in which combined clomiphene with recombinant FSH alone used, this treatment modality was discontinued (Table 2).

There were 34 live births and real cumulative live birth rates were 11.1% for a single attempt. Cumulative real live birth rates for two, three and four treatment cycles were 13%, 16.3%, and 22.2%, respectively. Expected pregnancy rates for the 1st, 2nd, 3rd and 4th treatment cycles were 11.1%, 13.3%, 19.7% and 26.4%, respectively (Table 3). There was no statistically significant difference between women achieving pregnancy or not with the IUI treatment in regard to cause of infertility.

Forty-one clinical pregnancies were achieved in 487 cycles (8.4%) below 35 years old and the rate of miscarriage was 19.5% (8/32). There were 6 clinical pregnancies in 93 cycles (6.5%) after 35 years old and 4 of them lasted with miscarriages (66%). Although there was no statistical difference in clinical pregnancy rates, miscarriage rate was statistically different between these age categories (P<0.05). There was no pregnancy after the 40 years of age.

Discussion

Intrauterine insemination has been used widely in infertility treatment with many indications and with a wide range of success rates. In the present study, we analyzed the pregnancy rates of 580 consecutive cycles and factors that affect the successful outcome with IUI treatment, retrospectively. Also we evaluated the role of flexible dose of recombinant FSH treatment starting cycle day 9, as an adjuvant to clomiphene citrate. The results showed that the overall pregnancy rate was 9.8% per cycle which is in agreement with previous studies with pregnancy rates ranging between 8% to 28%[2,3,12,13].

Ovulation induction with Clomiphene citrate is the most commonly used regimen because of its low cost and ease of administration. In patients who are anovulatory or fail to conceive with clomiphene citrate, gonadotropin is the alternative treatment. Sequential regimens using clomiphene citrate and human menopausal gonadotropin may also be used, as the com-

Table 2 Outcomes of different treatment modalities

	Gonadotropin	Clomiphene Citrate (CC)	CC with gonadotropin
	n:311	n:211	n:48
Non-clinical pregnancy	2	6	-
Live birth	17	17	-
Miscarriage	5	7	-
Stillbirth	1	0	-
Ectopic pregnancy	1	1	-

Table 3 Outcomes and cumulative pregnancy rates of patients

	Attempt number						
	1	2	3	4	5	6	7
Negative β-HCG	132	69	36	22	1	1	1
Non-clinical pregnancy	1	1	1	0	0	0	0
Live Birth	17	10	4	2	0	0	0
Miscarriage	2	2	0	3	1	0	0
Ectopic pregnancy	1	1	0	0	0	0	0
Real cumulative ongoing and live birth rate (%)	11.1	13.0	16.3	22.2	NA	NA	NA
Expected cumulative ongoing and live birth rate (%)	11.1	13.3	19.7	26.4	NA	NA	NA

NA: Non applicable
Cumulative pregnancy rates calculated for up the four cycles where because of statistical significance.

bination of both drugs decreases the total amount of gonadotropin necessary in an ovarian stimulation cycle resulting in reduced cost and a decreased incidence of ovarian hyperstimulation.

Standard minimal stimulation protocol has a favorable pregnancy rate of 20.8% in a relatively young population with a high rate of ovulatory dysfunction (40%). Although in many studies, similar pregnancy rates with sequential regimen using combination of clomiphene citrate and gonadotropin and gonadotropin alone were reported[10,13,14], differences in the pregnancy rate between these two protocols has also been demonstrated in a few studies[15,16]. However, the effect LH activity on pregnancy rates with this combination is challenging. In hyperstimulation protocols it has been reported that the combination of pure urinary FSH and clomiphene citrate (CC) does not result in sufficient stimulation[17]. Similar results were also found by other investigators[18].

FSH was usually given at 150 IU in standard protocol, but the dose varies from 150-450 IU. Therefore, in the present study, flexible doses of recombinant FSH was used, for mono follicular development according to ovarian response in the patients. However, these flexible doses of FSH administration alone were discontinued as pregnancy was not achieved in any of the 48 cycles. This might be attributed to the lack of LH effect on follicular maturation. Also, the experiences reported from other studies that stimulation of combination of pure urinary FSH alone and clomiphene citrate (CC) in hypestimulation protocols for IVF is not as effective as hMG in a way supports the failure of our flexible protocol with recombinant FSH[19].

The present data showed that the main sperm parameter affecting the IUI success rate was found to be the post-processing total motility. Although some authors reported an increase in the pregnancy rate from 17.1% to 30.4%, if the total motility was higher than 20%[20], others did not find any relation between the total motility and IUI success rate[21]. With respect to sperm morphology, our results were in agreement with previous studies that any relationship between IUI success and sperm morphology could not be found[22,23]. It has also been concluded that sperm morphology, when considered as a single parameter, did not predict the IUI success[24]. In another study, authors suggested that the number of inseminated total motile sperm count could compensate for inadequate sperm morphology[25]. But there are also many studies that report the relationship between pregnancy rate and sperm morphology[8,26]. Although the other sperm parameters like pre or post processing total motile sperm count, progressive motility was found to affect the IUI success rate in several studies[8,24,25,27], the present study showed a significant correlation only between post washing sperm motility and IUI success. But this finding has no predictive value in estimating pregnancy with discriminative analysis.

In current study, the pregnancy rate per cycle in anovulation was % 9. It was % 14, % 10 for unexplained and male factor infertility respectively. There was no pregnancy in patients with endometriosis. It has been reported that the pregnancy rates per cycle in anovulation as 19.1%, in male infertility 11%, in cases with endometriosis 9.1% and 10% in unexplained infertility[28]. The duration of infertility was another factor that affects the IUI success in our study. It was shown that duration of infertility longer than four years decreased the overall pregnancy rate in current study. In contrast to present study, some authors have not been shown the relation between duration of infertility and the success rate of IUI[8,29]. However, similar to present study, it has been reported that pregnancy rates decreases significantly with the duration of infertility longer than six years[30].

In this study, 34 (61%) pregnant women were aged <30 years. Only 6 (10%) pregnant women were ≥36 years. Moreover, the advanced age has also deleterious effect on pregnancy outcome in our study population. While the rate of spontaneous pregnancy loss in women who were ≥36 years old were 66% the rate was decreased to 30% for pregnant women who were younger than 30 years. Similarly, in a study, it has been reported spontaneous preg-

nancy loss was higher with advancing age (29.6% in 30 years to 52.6% over 40 years)[31]. Besides this, it has been reported that no pregnancy occurred in women aged >40 years[28]. There was no pregnancy after the age of 40. The effect of age on the success rate of IUI and ongoing pregnancies were clearly demonstrated with the present study and the other studies[2,8,28-31].

In conclusion, based on findings in the present study, we suggest that flexible recombinant FSH alone as an adjuvant to clomiphene citrate therapy in sequential fashion should not be administered to improve mono follicular development further from near ending FSH window, by the time if there is no leading follicle. Alternatively, earlier recombinant FSH or later recombinant FSH with recombinant LH might be a more reasonable option in achieving pregnancy. Additional studies with larger number of series should be undertaken to further strengthen the assumptions of the present study. The present study confirms that the post-processing sperm motility was a significant factor between pregnant and non pregnant women. Post processing sperm motility could not be used as a parameter to predict a pregnancy. ■

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