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What is the best initial cycle IVF protocol for patients over 35 years old?

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Summary

Purpose: The aim of this study was to find the most effective ovarian hyperstimulation protocol for patients over 35 years old. *Materials and Methods:* This is a retrospective study of 390 first IVF cycles of patients older than 35 years, that had serum follicle stimulating hormone < 10 IU/L, and that had no co-existing endocrine disorders. Long (n=181), antagonist (n=71), and micro-dose flare-up (n=138) protocols were evaluated. *Results:* Clinical pregnancy and live birth rates were highest in long protocol group and lowest in micro-dose protocol group. The difference between long and micro-dose protocol groups was statistically significant ($p < 0.05$). In multivariate logistic regression analysis, picked-up oocyte count ($p = 0.005$), endometrium thickness at hCG day ($p = 0.006$), age ($p = 0.006$), and antral follicle numbers ($p = 0.013$) were found to be predictive for obtaining clinical pregnancy. Treatment protocols were not found to be predictive for obtaining clinical pregnancy ($p > 0.05$). *Conclusion:* Treatment protocols were not found to be predictive for obtaining clinical pregnancy. Patient's age, antral follicle number, endometrial thickness at hCG day, and picked-up oocyte counts directly effect the pregnancy rates. Long protocol affects these factors positively can be preferred in younger patients with higher antral follicle numbers.

Key words: IVF hyperstimulation protocol; IVF outcome; Advanced age.

Introduction

Approximately fifteen percent of couples cannot have children despite they want and this poses a problem [1]. Today women generally wait to have children until they have a better social level. When fertility capacity change with women age is analysed, it showed a decrease of 31% in 35-39 compared to 20-24 years of age. It was found to decrease in higher rates in older women [2]. Rapidly increased loss of follicles, decreased oocyte quality, and reproductive aging after 35 years of age show low responses to assisted reproductive techniques [3].

The aim of this study was to find the most effective ovarian hyperstimulation protocol without knowing the responses at first admittance in patients older than 35 years of age, but follicle stimulating hormone levels did not increase in high amounts in whom an anxiety was present about the treatment response.

Materials and Methods

This is a retrospective study of 390 patients followed between 2004 and 2012 in Gazi University In Vitro fertilization Unit. All of the patients were given IVF treatment for any of the infertility causes (tuboperitoneal or male factor, endometriosis, anovulation, unexplained infertility), older than 35 years, serum follicle stimulating hormone level < 10 IU/L, and with no co-existing endocrine disorders (diabetes mellitus, thyroid disorders, and adrenal and pituitary gland diseases). Patients were divided into three groups according to ovarian hyperstimulation protocols used: patients given long protocol (n=181), antagonist protocol (n=71), and micro dose flare-up (n=138) protocols. Effects of the protocols used on clinical pregnancy and live birth rates were analysed. Approval of the local ethics committee from "Gazi University Clinical Research Ethics Committee" was taken before the study was begun.

GnRH agonist long protocol: one mg daily leuprolide (1 mg) was given for at least 14 days from subcutaneous route. Gonadotropin treatment was begun if the serum estradiol level was lower than 50 pg/ml at the mid-luteal phase (on the 21st day) of the cycle prior to gonadotropin was begun and menstrual bleeding occurred. Leuprolide acetate treatment was decreased to a dose of 0.5 mg/day and continued with the same dose during the gonadotropin treatment.

Micro-dose flare-up protocol: oral contraceptive treatment consisting ethinyl estradiol 0.03 mg + levonorgestrel 0.150 mg daily was given between days 1 and 21 of the prior cycle of gonadotropin treatment. Leuprolide acetate 40 micrograms bid from subcutaneous route was begun after two days from oral contraceptive drug was stopped. Gonadotropin stimulation was begun at a suitable dose after the day leuprolide acetate was begun. Pituitary suppression was continued with the same dose during gonadotropin stimulation until the day of hCG.

GnRH antagonist protocol: gonadotropin stimulation was begun on the third day of the cycle with the appropriate dose if the cystic lesion was not found with ultrasonography without pituitary suppression treatment. Cetrorelix 0.25 mg/day from subcu-

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Table 1. — *Epidemiologic and stimulation characteristics.*

	Long protocol (n=181)	Antagonist protocol (n=71)	Micro-dose protocol (n=138)	*p*-value
Age (mean ± SD)	37.1±1.9[a,b]	39.5±3.2[a]	38.9±3.0[b]	<0.001
Antral follicle count	7 (0-20)[a,b]	4 (0-16)[a]	4 (0-16)[b]	<0.001
Male age (mean ± SD)	40.1±4.8	41.2±5.9	40.5±5.4	0.356
Body mass index (mean ±SD)	25.5±4.2	25.3±3.8	25.1±3.8	0.703
Duration of infertility (month)	90 (6-288)[a]	48 (6-276)[a,c]	72 (6-324)[c]	<0.001
Baseline FSH (IU/L)	6.3 (2.0-9.9)	6.5 (1.4-9.7)	6.6 (1.4-9.9)	0.122
Serum E2 on day 3 (pg/dL)	47 (10-664)	45 (10-252)	48 (7.7-854)	0.666
≥ 17 mm follicle count	3 (0-12)[a,b]	2 (0-7)[a]	2 (0-9)[b]	<0.001
Serum E2 on day of hCG (mean)	1564 (133-10210)[a,c]	1112 (100-5120)[c]	1204 (92-4844)[a]	<0.001
Duration of gonadotropin stimulation (mean)	10 (6-17)[a,b]	9 (5-16)[a,c]	11 (5-20)[b,c]	<0.001
Total dose of FSH (mean)	2387.5 (1050-6300)[b]	2125 (900-3875)[c]	3000 (900-6300)[b,c]	<0.001
hCG day endometrial thickness (mean)	11 (7-19)[a,b]	10 (6-15)[a]	10 (6-17)[b]	<0.001

a: The difference between long protocol and antagonist treatment groups was statistically significant ($p < 0.001$).
b: The difference between long protocol and micro-dose treatment groups was statistically significant ($p < 0.001$).
c: The difference between micro-dose and antagonist treatment groups was statistically significant ($p = 0.005$).

Table 2. — *Laboratory and pregnancy outcomes.*

	Long protocol (n=181)	Antagonist protocol (n=71)	Micro-dose protocol (n=138)	*p*-value
Oocytes retrieved	9 (0-47)[a,b]	4 (0-34)[a]	4 (0-42)[b]	<0.001
MII oocytes	6 (0-36)[a,b]	3 (0-29)[a]	3 (0-37)[b]	<0.001
Fertilized oocyte number	5 (0-28)[a,b]	2 (0-19)[a]	3 (0-29)[b]	<0.001
Embryos transferred	3 (0-5)[a,b]	2 (0-5)[a]	2 (0-5)[b]	0.004
Implantation rate (%)	13.5±21.5[b]	13,9±27.0	7.3±17.9[b]	0.009
Clinical pregnancy rate	59 (32.6%)[b]	19 (26.8%)	24 (17.4%)[b]	0.009
Live birth rate	40 (22.1%)[b]	12 (16.9%)	11 (8.0%)[b]	0.003
Number of cancelled cycles (%)	21.0	28.2	31.2	0.108

a: The difference between long protocol and antagonist treatment groups was statistically significant ($p < 0.05$).
b: The difference between long protocol and micro-dose treatment groups were statistically significant ($p < 0.05$).

taneous route was begun when the dominant follicle reached a 14-mm diameter and given with gonadotropins until the day of hCG. Gonadotropin treatment dose was ordered due to ovarian response. Statistical analyses were made by SPSS 11.5. Descriptive statistics were shown as mean ± standard deviation or median (minimum - maximum). Categorical variables were shown as number of cases and percentages. Difference of means between groups was evaluated with Student's *t*-test and One way variance analysis. Difference of medians were evaluated with Mann Whitney U test and Kruskal Wallis test. Factors having statistically significant effect on pregnancy at univariate analysis or thought to have effect on pregnancy were evaluated with multivariate logistic regression analysis. Statistical significance level was accepted as $p < 0.05$.

Results

Comparison of demographics, endocrinologic variables, and stimulation characteristics of these three stimulation groups are summarized in Table 1. Baseline characteristics as body mass index, male age, and third day estradiol and FSH levels were similar among all three groups. However age was lower ($p < 0.001$), and number of antral follicles, mature follicle number ≥ 17 mm, estradiol and endometrial thickness at hCG day was higher in long protocol group at a statistically significant level ($p < 0.001$). Statistically significant difference was found between gonadotropin treatment duration and doses among groups ($p < 0.001$). The shortest stimulation duration and least gonadotropin usage were seen in antagonist protocol group.

Laboratory and pregnancy results are summarized in Table 2. Picked-up oocyte number, fertilized oocyte number, and as a result transferred embryo numbers were similar in antagonist protocol and micro-dose protocol groups, but they were higher in long protocol group at a statistically significant level ($p < 0.005$). Implantation rate was similar in long protocol and antagonist protocol groups and higher than micro-dose protocol group ($p < 0.05$). Cycle cancel rates were higher in micro-dose protocol group but the difference was not statistically significant among the three groups (21,0%, 28,2%, and 31,2% in long, antagonist, and micro-dose protocol groups, respectively; $p = 0.108$). Clinical pregnancy and live birth rates were highest in long protocol group and lowest in micro-dose protocol group. The difference between long and micro-dose protocol groups was statistically significant ($p < 0.05$).

Table 3. — *Multivariate logistic regression analysis of all probable significant factors for distinction of clinical pregnant and non-pregnant group within study group.*

Parameter	Odds ratio	95% Confidence interval		*p*-value
		Lower limit	*Upper limit*	
Age	0.848	0.755	0.953	0.006
Long protocol	1.110	0.587	2.099	0.748
Antagonist	1.783	0.828	3.840	0.139
AFC	1.094	1.019	1.175	0.013
Total oocyte count	1.062	1.018	1.108	0.005
Endometrium thickness	1.186	1.051	1.338	0.006
Sixth day estradiol	1.000	1.000	1.001	0.470

Multivariate logistic regression analysis in study population is summarized in Table 3. According to this analysis; picked-up oocyte count ($p = 0.005$), endometrium thickness at hCG day ($p = 0.006$), age ($p = 0.006$), and antral follicle numbers ($p. = 0.013$) were found to be predictive for obtaining clinical pregnancy. Treatment protocols were not found to be predictive for obtaining clinical pregnancy ($p > 0.05$).

Discussion

Today demographic data show that women postpone having children. It is a reality that natural fecundity decreases with increasing age, but no interpretation can be made about after what age it is impossible to have children.

In this study long protocol data was more successful than antagonist protocol and micro-dose protocol was the weakest one regarding clinical pregnancy, and live birth rates in controlled ovarian hyperstimulation applied patients at first admission, older than 35 years of age, and FSL level < 10 IU. Further statistical analysis showed none of the protocols had direct effect on pregnancy.

The best ovarian stimulation protocol in advanced age patients must have acceptable cycle cancel rates, highest numbers of the highest quality mature oocyte count, reasonable duration and costs, suitable endometrium for implantation, and maximum pregnancy and live birth rates. Choice of the best protocol in advanced age patients is still controversial because of the heterogeneity of the treatment protocols used and patients' clinical features.

Several GnRH agonist protocols were attempted in which dose and timing were different. It was thought that high dose gonadotropin treatment in weak responsive patients might stimulate follicular development and decrease cycle cancel rates in late 1980s [4]. However contrasting opinions were proposed after a short time [5]. It was proposed that decreasing gonadotropin requirement with decreasing GnRH agonist dose might be more rational for increasing oocyte numbers [4, 6].

Antagonist protocol showed to have a short duration, less amount of gonadotropin usage, and a lower cost in weak responsive patients in the literature [7,8]. Despite these data, Malmusi *et al.* found in their study that total gonadotropin dose used in micro-dose flare-up protocol was lower than antagonist protocol [9]. In the present study consistent with the literature, gonadotropin dose used and duration was lower in antagonist protocol than the long protocol and were highest in micro-dose flare-up protocol at the statistical significant level.

Malmusi *et al.* [9] found total and mature oocyte counts were higher in GnRH agonist group than GnRH antagonist group. Prapas *et al.* showed that more oocytes had been obtained with agonist protocol, but metaphase II oocyte counts were similar with antagonist protocol [10]. Craft *et al.* found higher oocyte counts and higher pregnancy rates when they compared GnRH antagonist protocol results with patients' prior GnRH agonist cycles [11]. It was also shown that lower counts of oocytes had been obtained with antagonist protocol compared to micro-dose protocol in prospective studies [9, 12]. However there was no difference of picked-up oocyte and mature oocyte counts between GnRH agonist and antagonist protocols in a meta-analysis published in 2011 [13]. In the present study it was shown that higher number of oocytes were obtained with long protocol consistent with literature data [7, 8] and showed that picked-up oocyte counts directly effects the pregnancy rates.

The GnRH agonist treatment was proposed to increase endometrial receptivity by decreasing nitric oxide synthethase levels and implantation success with micro-dose flare-up protocol was attributed to this proposal [14]. Decreased growth factor synthesis is believed to decrease estrogen levels and cause insufficient endometrial growth for implantation in GnRH antagonist treated patients [15]. Malsumi *et al.* also found higher implantation rates in micro-dose flare-up protocol supporting this knowledge [9]. There was no significant difference between implantation rates between long and antagonist protocols in the study of Prapas *et al.* [10]. Endometrial thickness was similar in antagonist and micro-dose protocols and higher in long protocol, and it was found to have direct effect on predicting pregnancy in the present study. Implantation rates were similar in long and antagonist protocol, but lower in microdose flare-up protocol group at the statistically significant level. The negative effect of high dose gonadotropin used on endometrium might cause low implantation rates but similar transferred embryo numbers and endometrial thickness in microdose flare-up and antagonist protocol groups.

Antagonist protocol was suggested to be related with increased pregnancy success in older patients [8]. On the contrary, there are some data that show that long protocol was more successful in initial cycle compared to antagonist protocol [10] and it was suggested that pregnancy rates were higher with microdose protocol [12, 13, 16]. There was no difference between protocols in several studies and Cochrane 2010 review, but pregnancy rates were fewer in

antagonist protocol [3, 8, 9, 12, 17-24].

Clinical pregnancy and live birth rates were found to be higher in long than antagonist protocols and were significantly lower in micro-dose flare-up than other protocols in first cycle of controlled ovarian hyperstimulation treatment in the present study. These results might be related with long protocol that was preferred in relatively young patients regarding the treatment duration and pregnancy rates might be higher in this group of patients. However further statistical analysis showed none of the protocols was directly predictive when adjusted for age between groups.

Cycle cancel rates were similar among all three groups. Antagonist protocol was suggested to be with a less cycle cancel rate in weak responsive patients in some prospective studies [8]; however Prapas *et al.* suggested the contrary [10]. Cycle cancel rates were found lower in antagonist protocol comparing same patient' prior GnRH agonist cycle results in two retrospective studies [11]. However two different meta-analyses published in 2006 and 2011 showed there were no differences detected in cycle cancel rates between GnRH antagonist and agonist protocols [12, 25].

Conclusion

The best stimulation protocol in advanced age patients must have acceptable cycle cancel rates, potential highest number of the best quality oocytes, acceptable costs and duration, convenient endometrium for implantation, and maximum pregnancy and live birth rates. Choice of the best treatment protocol in older patients is still controversial due to heterogeneity of the treatment protocols used and clinical features of the patients in the literature.

Treatment protocols were not found to be predictive for obtaining clinical pregnancy. It was concluded that patient age, antral follicle numbers, endometrium thickness at hCG day, and picked-up oocyte counts directly affect the pregnancy rates. Long protocol affecting these factors positively can be preferred in younger patients with higher antral follicle numbers. However antagonist protocol can be preferred in older patients with low antral follicle numbers because of the shorter stimulation time and due to less gonadotropin usage. Success rates might be increased and cycle cancel rates might be decreased with a proper treatment protocol chosen individually. Therefore controlled ovarian hyperstimulation protocol must be chosen regarding critical factors like individual features, endocrinologic factors, and age for achieving the highest success rates for each patient.

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