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The effect of growth hormone addition protocols to poor ovarian responders in *in vitro* fertilization cycles

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Abstract. – OBJECTIVE: *In vitro* fertilization failure (IVF) is high in women with poor ovarian response or non-responder. For this reason, the addition of adjuvant treatments to IVF protocols has come to the fore. We assessed to investigate the effects of adjuvant GH therapy initiated in the mid-luteal phase on IVF success in poor ovarian response or non-responder women.

PATIENTS AND METHODS: A retrospective study was performed in 93 poor ovarian response or non-responder women from a single center. GH treatment was added (GH-plus group) in the mid-luteal phase of the previous menstrual cycle to 47 of the women who underwent controlled ovarian stimulation with the flexible antagonist protocol. 46 women, as another group, were applied to a flexible antagonist-only protocol (GH-free group). The IVF outcome results were evaluated and compared within the groups.

RESULTS: The number of retrieved oocytes was statistically significantly higher in the GH-plus group (2.28±1.975) than in the GH-free group (1.24±1.728) (p=0.01). Although statistically insignificant (p=0.55), the clinical pregnancy rate was higher in the GH-plus group [(8/47), 17%] than in the GH-free group [(5/46, 11%]. The cancellation rate was statistically significantly higher in the GH-free group (65.2%) than in the GH-plus group (44.7%) (p=0.04). No oocyte retrieved cycle rate was higher in the GH-free group (56%) than in the GH-plus group (25%) (p=0.002).

CONCLUSIONS: Adjuvant GH therapy administration to IVF protocol in the mid-luteal phase gives poor ovarian response or non-responder women a chance to have a baby.

Key Words:
Growth hormone, Poor ovarian response, Poor responder, Controlled ovarian stimulation, In vitro fertilization.

Abbreviations

COS: controlled ovarian stimulation; IVF: *in vitro* fertilization; GH: growth hormone; POR: Poor ovarian responder.

Introduction

Poor ovarian response (POR) and non-responder women are the leading causes of *in vitro* fertilization (IVF) failures today. The incidence of POR in women undergoing controlled ovarian stimulation (COS) for IVF can range from 9% to 24%[1]. Due to the contradictions in the POR terminology, the European Society of Human Reproduction and Embryology (ESHRE) published the Bologna criteria in 2011 to eliminate these contradictions regarding the definition of POR. Accordingly, at least two of these three criteria must be met: (i) advanced maternal age (≥40 years) or any other risk factor for POR; (ii) a previous POR (≤3 oocytes with a conventional stimulation protocol); (iii) an abnormal ovarian reserve test (i.e., Anti-Müllerian hormone level (AMH): 0.5-1.1 ng/mL or antral follicle count (AFC): 5-7 follicles)[2].

In order to get follicular feedback with COS from POR or non-responder women, different approaches are applied together in addition to standard IVF protocols. For POR, none of the different IVF protocols for treatment success has yet been found to be superior to the others[3-6].

According to meta-analysis data, adjuvant treatments in addition to COS protocols are very beneficial in poor responder women[7-9]. One of these adjuvant treatments, growth hormone (GH)

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has been shown to increase live birth rates in poor responder women[8-12].

The mechanism of action of GH in IVF is to stimulate follicle development and increase estrogen production and oocyte maturation by increasing the intra-ovarian production of insulin-like growth factor 1, which is thought to play an important role in ovarian function, along with increasing the sensitivity of follicles to gonadotropins[11-15]. There is no clear protocol defining neither the duration nor the dose of GH administration as an adjuvant in IVF cycles[16]. However, GH is generally administered in the follicular phase of the menstrual cycle in the dosage range of 4-24 IU[11].

This study aims to investigate the efficacy of adding adjuvant GH to standard COS protocols in the mid-luteal phase to achieve a follicular response in poor responder women with poor prognoses.

Patients and Methods

In this retrospective case-control study, the data of women who consulted the same IVF specialist in Kocaeli Konak Hospital IVF Center between January 2014 and August 2017 were evaluated.

IVF cycles of POR women who met the two criteria of the Bologna criteria, excluding the age, were included in the study (Table I). In these cycles, AFC and AMH levels were reassessed according to the nomograms of infertile women[17,18]. The maximum value of the 3rd percentile of the youngest age in the study in the nomograms of infertile women was chosen as the criterion for AFC and AMH levels (AFC $\leq$ 4.9, AMH level $\leq$ 0.31 ng/mL). The women with no retrieved oocytes, cycles with failed fertilization, or cycles with failed embryo cleavage in a previous IVF cycle were excluded from the study whereas the inclusion criteria had regular menstrual cycles but no amenorrhea and administrating COS with flexible antagonist protocol in all IVF cycles.

IVF cycles of 93 women, aged between 27-44 years, who met these criteria were evaluated retrospectively. In the IVF cycle of 47 women, GH was added to the cycle in the mid-luteal phase with the flexible antagonist protocol (GH-plus group). The remaining 46 women were applied to a flexible antagonist-only protocol (GH-free group).

Cases with infertility due to male factor, altered karyotype, history of recurrent spontaneous abortion, tubal adhesions or hydrosalpinx, uterine cavity abnormality, and other chronic or serious diseases (thyroid hyperfunction, diabetes mellitus, hyperprolactinemia, and adrenal cortex hyperfunction, and polycystic ovary syndrome, and endometriosis, and leiomyoma, and adenomyosis, etc.) were excluded from the study.

Adjuvant GH use for POR women in Turkey along with the COS protocol is not funded by the government. However, in this IVF center, the use of adjuvant GH is routinely recommended to all POR women.

In this center, the same COS protocols with the addition of similar adjuvant treatments are applied to POR or non-responder women. Up to the last 4 weeks before the onset of COS, women were given a combination of 25 mg/day dehydroepiandrosterone (DHEA) and a daily oral contraceptive pill for 8-12 weeks. In the menstrual cycle before COS administration, DHEA administration was stopped, but the daily oral contraceptive pill intake was continued for another 21 days. Letrozole was started on the third day of the menstrual cycle in which COS was applied for 5 days at a dose of 5 mg/day. On the third day of the menstrual cycle, stimulation of 225-400 IU of human menopausal gonadotropin (hMG) was initiated. When the leading follicle diameter was 15 mm, ganelix was added to the protocol as a gonadotropin-releasing hormone (GnRH) antagonist and used until the day of ovulation induction. In the GH plus group, GH injection was started in the mid-luteal phase (12 IU /in every 3 days) of the cycle before COS administration and continued until the antagonist administration started. Oocyte retrieval was completed 36 hours after final oocyte maturation was established with a single dose bolus of human choriogonadotropin alpha (hCG). Embryos were transferred to the women on the third day, and none of the remaining embryos were stored as cryopreserved. All women were given intravaginal progesterone in the luteal phase. Serum β-hCG level was evaluated 12 days after embryo transfer (ET).

In the study, gonadotropin dose, the number of retrieved oocytes, the number of embryos, clinical pregnancy rate, live birth rate, fertilization rate, implantation rate, cycle cancellation rate, and no oocyte retrieved cycle rate were determined as IVF outcomes. Clinical pregnancy was defined as the detection of fetal cardiac activity by transvaginal ultrasound within a healthy gestational sac. Live birth rate was considered as the number of live births reaching the 28th gestational week. The fertilization rate was calculated by

Table I. Study criteria of poor or non-responders.

Anti-Mullarian Hormone level ≤ 0.38 ng/mL and/or Follicle stimulating hormone level > 15 IU/L
Total antral follicle count ≤ 4
Previous cycle(s) with no oocyte or no embryo transfer
At least one cancelled previous IVF cycle

dividing the total number of retrieved oocytes by the total number of fertilized oocytes in each group. Implantation rate was defined as the ratio of the number of gestational sacs to the number of embryos transferred.

Statistical Analysis

Retrospective data were statistically evaluated with the Statistical Package for the Social Sciences program (version 20, SPSS Inc., Armonk, NY, USA). Data were collected as mean and SD of quantitative variables. Frequency and percentage were used to summarize the qualitative variables. The odds ratio and 95% confidence interval were calculated for the results. Comparisons between groups were performed using the Chi-square test and Student's *t*-test for qualitative variables. A *p*-value of <0.05 was considered statistically significant.

Results

The data of 93 women who were applied flexible antagonist protocol and evaluated according to the criteria of being poor or non-responder stated in Table I, were divided into two different groups according to the use of adjuvant GH (GH plus group, GH free group). In 47 of these cycles, GH was added to the flexible antagonist protocol as an adjuvant. The demographic characteristics of women in IVF cycles such as age, body mass index, basal FSH and AMH levels, duration of infertility, the number of previous cycles with poor response, the earlier cycles with no oocyte or with failed fertilization or failed embryo-cleavage were shown in Table II. When the GH plus and free groups were compared in terms of these demographic characteristics, there was no significant difference between these two groups.

The GH plus and the GH free groups were compared for IVF outcomes (Table III). The number of retrieved oocytes was statistically significantly higher in the GH-plus group (2.28±1.975) than in the GH-free group (1.24±1.728) (p=0.01). Although statistically insignificant, the clinical pregnancy rate, live births rate, fertilization rate, and implantation rate are higher in the GH-plus group than in the GH-free group. However, cancellation rate (p=0.04) and cycles with no oocyte retrieved (p=0.002) were significantly lower in the GH plus group. No side effects were observed in either treatment group.

Discussion

The Bologna criteria in the ESHRE guideline are used in many studies in poor responder patients. In this study, a modified version of the Bologna criteria was used. The maximum value of the 3rd percentile of the youngest age in the study in the nomograms of infertile women was chosen as the criterion for AFC and AMH lev-

Table II. Demographic characteristics for GH plus group and GH free group.

	GH plus group	GH free group	*p*-value
	N: 47	N: 46	
Age (mean ± SD)	37.57 ± 5.63	37.89 ± 5.54	0.26
Body mass index (kg/m^2) (mean ± SD)	27.5 ± 51	27.6 ± 6.1	0.14
Duration of infertility (year) (mean ± SD)	7 ± 3.2	7.9 ± 2.6	0.12
Number of previous cycles with poor response (mean ± SD)	3.1 ± 1.05	3.5 ± 1.4	0.19
Previous cycles with no oocyte	32 (68.1%)	32 (69.6%)	0.87
Previous cycles with failed fertilization	8 (17%)	5 (15.2%)	0.81
Previous cycles with failed embryo cleavage	7 (15%)	9 (19.6%)	0.55
Basal follicle stimulating hormone (IU/L) (mean ± SD)	18 ± 5.7	17.8 ± 5.8	0.91
Anti-Mullerian hormone (ng/mL) (mean ± SD)	0.17 ± 0.1	0.18 ± 0.1	0.95
Antral follicle count (mean ± SD)	1.3 ± 0.8	1.2 ± 0.7	0.21

Table III. Comparison between the first (March-April 2020, Group 1B) and second wave (November-January 2020/2021, Group 2B) of COVID-19 epidemic.

	GH plus group	GH free group	*p*-value
	N:47	N:46	
Total gonadotropin dose (mean ± SD)	3086.70 ± 997.93	2837.50 ± 1131.90	0.78
Number of retrieved oocytes (mean ± SD)	2.28 ± 1.975	1.24 ± 1.728	0.01
Number of embryos (mean ± SD)	0.57 ± 0.715	0.43 ± 0.688	0.32
Clinical pregnancy rate	(8/47) 17%	(5/46) 11%	0.55
Live birth rate	(6/47) 13%	(4/46) 9%	0.74
Fertilization rate	43.92%	47.36%	0.79
Implantation rate	29.62%	25.00%	0.99
Cancellation rate	21 (44.7%)	30 (65.2%)	0.04
No oocyte retrieved cycles	12 (25%)	26 (56%)	0.002

els (AFC ≤ 4.9, AMH level ≤ 0.31 ng/mL). In our study, although the age criterion was not met, the ovarian reserves of women were below the values specified in the Bologna criteria. In addition, all women included in the study sought cancellation criteria from the previous IVF cycle. As such, it would be more accurate to say "worst responder" instead of defining our patient population as "poor responder".

There are several studies on the results of COS protocols in poor responder women[3]. In a retrospective study comparing four different COS protocols using GnRH agonists (long, short, and mini flare) and antagonists without the use of adjuvant GH in poor responder women, different COS protocols were found to be non-superior to each other[19]. However, numerous studies in the literature have reported that the use of GnRH antagonists in COS protocols in poor responder women has a positive effect on IVF outcomes[4,20,21]. Based on these findings, flexible antagonist protocol was applied to all women in our study.

In meta-analyses evaluating treatments using adjuvant GH in IVF cycles, it has been shown that GH increases the live birth rate and pregnancy rate in poor responder women[11]. There are different approaches for the duration and dosage of adjuvant GH use in COS cycles for POR women[22]. There are some arguments that GH administration in the follicular phase is less effective for follicular development than the administration in the mid-luteal phase due to the delay in timing[23]. However, unlike several studies on the use of GH in the follicular phase in the literature, there are limited studies on the use of GH in the mid-luteal phase and the number of patients in these studies is not sufficient[8,23].

Our study shows the importance of GH use in the mid-luteal phase on IVF success by increasing the number of retrieved oocytes in the GH plus group and decreasing the cancellation rate and the number of cycles with no oocyte retrieved. In addition, even if they are not statistically significant, we observed that critical IVF outcomes including clinical pregnancy rate, live births rate, fertilization rate, and implantation rate increased in women who were administered adjuvant GH and can be described as the worst responders. Although the use of adjuvant GH is not recommended in poor responder women; When the results of our study and previous literature data are considered together, adjuvant GH seems to be the last option in this patient group[24].

The most important limitation of our study is the small number of samples. In future prospective studies, if the large sample size is taken into account, the obtained results will be strengthened. However, studies comparing different COS protocols in addition to GH treatment and/or the use of GH at different phases of the IVF cycle are needed.

Conclusions

The pregnancy rate of poor ovarian response or non-responder women with *in vitro* fertilization is quite low. The addition of growth hormone to the flexible antagonist protocol in the mid-luteal phase remarkably increases the clinical pregnancy rate. More studies are needed on the effects of adjuvant mid-luteal GH addition on IVF success in IVF cycles.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethics Approval

The study was approved by Kocaeli University Non-invasive Clinical Research Ethics Committee with the project number of 2017/304 and the reference number of KU GAKAEK 2017/15.6. Ethical approval was waived by the Non-invasive Clinical Research Ethics Committee of Kocaeli University in view of the retrospective nature of the study and all procedures performed were part of routine care.

Informed Consent

Informed consent for the use of their medical records in scientific studies and for the publication of their data is routinely obtained from all patients admitted to this IVF Center before the IVF protocol begins.

Availability of Data and Materials

The data that support the findings of this study are available from Kocaeli Konak Hospital IVF Center, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of Kocaeli Konak Hospital IVF Center.

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Authors' Contributions

RAB, CO and EC contributions to conception and design, RAB, CO, RA, BA and EC contributions to acquisition of data, all authors contributions to analysis and interpretation of data, drafting the article or revising it critically for important intellectual content and final approval of the version to be published. All authors read and approved the final manuscript..

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