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ORIGINAL ARTICLE

Single-center and retrospective analysis of growth hormone supplementation in IVF patients of age 40 years and older

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ABSTRACT

Objective: Female age is the most important factor determining success in Assisted reproductive technologies (ART) cycles. The objective in this study was to observe how growth hormone (GH) supplementation affects ART outcome parameters in advanced age women (40 years and above).

Material and Methods: This retrospective study involved 807 patients of age 40 years and older who applied to the tertiary ART clinic between January 01, 2018, and December 31, 2021. Only the first ın vitro fertilisation cycles of the cases were taken into consideration in the study. The study group involved 56 cases which received GH supplementation and the control group involved 751 cases which received conventional ovarian stimulation. The cycles of 177 patients (seven patients in the study group and 170 patients in the control group) were canceled because of inadequate ovarian response, lack of oocytes or fertilization failure. In the GH group, patients used 4 mg recombinant Somadotropin (Saizen 12 mg/1.5 mL, Merck Germany) for 3 days starting from the 2nd or 3rd day of menstruation.

Results: Although the duration of infertility was longer in the group that received GH (6.07±4.91 vs. 4.43±4.70), the total amount of gonadotropin usage was lower than control group (4734.00±76.76 vs. 5191, International unit [IU]±743.70). The rate of cycles cancelled, total number of oocytes retrieved, total number of Metaphase II oocytes, rate of oocyte maturation, number of 2PN, rate of fertilization, embryo utilization rate, and number of transferable embryos were similar in the two groups. While the pregnancy rates were comparable, the live birth rate was observed to be higher in the GH group (5.8% vs. 18.8% p=0.013).

Conclusion: In women aged 40 years and older, GH supplementation is associated with lower amount of gonadotropins usage for ovarian stimulation and higher live birth rates.

Keywords: Assisted reproductive technologies, growth hormone, *in vitro* fertilisation, Infertility.

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INTRODUCTION

The overall success of human reproduction, both spontaneous and with assisted reproductive technologies (ART), depends largely on female age. The main causes of age-related infertility include decreased ovarian reserve and decreased oocyte/embryo sufficiency related to age. The biochemical and molecular mechanisms involved in age-related infertility and their effects on oocyte and embryo quality have not been precisely explained. So far, various cellular dysfunctions associated with infertility have been reported in older women. As the ovarian reserve progressively reduces, female aging leads to a decrease in the quality of oocytes and embryos due to defective physiological pathways such as epigenetic regulation, energy production and balance, metabolism, increased meiotic segregation, and cell cycle checkpoints.[1]

Different stimulation protocols and strategies have been proposed to improve chances of pregnancy in older women. These strategies include different regimens for pituitary suppression, high dosages of gonadotropins,[2] addition of adjuvant agents during ovarian stimulation,[3] and performing a modified natural *in vitro* fertilisation (IVF) cycle.[4] Aggressive ovarian stimulation with gonadotropins at high doses leads to an increase in cost and increased side effects. On the other hand, the use of natural cycle IVF in older women is disappointing because of the limited number of oocytes retrieved and high cancellation rates. For this reason, using adjuvant therapies in older women to improve IVF results is often debated. Supplementary treatment is defined as a complementing treatment used in addition to gonadotropin releasing hormone (GnRH) analogs and gonadotropins before or during IVF/Intracytoplasmic sperm injection (ICSI) cycles to improve pregnancy outcomes in women with advanced age, poor ovarian response, or previous unsuccessful IVF/ICSI cycles. One of these adjuvant agents that shows promising results is the growth hormone (GH). Several studies have shown improvement in ovarian response and clinical outcome with GH supplementation.[5–8]

In parallel with the western countries, the age of women entering into assisted reproduction treatment is constantly increasing.[9] This demographic shift fuels the debate over the use of adjuvant treatments. In this retrospective and cohort study, the aim was to compare the laboratory parameters and pregnancy results in women of advanced age who used GH supplementation and those who did not.

MATERIAL AND METHODS

This study was conducted retrospectively on 807 patients of age 40 years and older who applied to the Istanbul American Hospital ART clinic between January 1, 2018, and December 31, 2021. Only the first treatment cycles of the cases were included in the study. The study group consisted of 56 cases with GH supplementation and the control group consisted of 751 cases who with conventional ovarian stimulation.

The cycles of 177 patients (seven patients in the study group and 170 patients in the control group) were canceled due to inadequate ovarian response, lack of oocytes, or fertilization failure.

For GH group patients who were included in the study, 4 mg recombinant Somadotropin (Saizen 12 mg/1.5 mL, Merck, Germany) were used for 3 days from the 2nd or 3rd day of menstruation.

Table 1: Basal and IVF cycle characteristics of total study population (n=807)

Female age	42.4±2.1 (40–47)
Duration of infertility (years)	4.6±4.7 (1–15)
Stimulation time (days)	10.1±2.7 (5–18)
Gonadotropin dosage (IU/day)	368.3±77.0 (300–450)
Total number of oocytes	4.8±3.8 (0–11)
Number of MII oocytes	3.6±3.0 (1–9)
Number of 2PN	2.6±2.6 (0–8)
Maturation rate (%)	74.9
Fertilization rate (%)	72.1
Number of usable embryos	2.3±2.6 (0–4)
Embryo utilization rate (%)	90.0

Values are given as the mean±2SD (range). MII: Metaphase-II; 2PN: Two pronuclei; IU: International unit.

Gonadotropins were started on the 2nd or 3rd day of the menstrual cycle of women after eliminating pathologies originating from the ovaries or the endometrium by transvaginal ultrasound. The initial dose was 300 IU/day or 450 IU/day (Meriofert, IBSA Turkey, or Gonal-F, Merck, Germany) depending on the woman's ovarian reserve estimate and body mass index. On day 5 or 6 of the stimulation, the ovarian response was evaluated by transvaginal ultrasonograhy. In cases where the follicle size was 12 mm or more, 25 mg of GnRH-a (Cetrotide, Merck, Germany) was added. When two or more follicles reached a size of 17 mm or more, ovulation triggering was performed with 500 mcg (13000 IU) rhCG (Ovitrel 250 micrograms/0.5 mL, Merck, Germany), and transvaginal oocyte retrieval was performed 35–37 h later. ICSI was performed in all cases. Luteal phase support was provided with 600 mg of vaginal micronized progesterone (Progestan, Koçak Türkiye) and 25 mg of subcutaneous progesterone (Prolutex 25 mg, IBSA, Türkiye). Luteal phase support was continued until the 10th gestational week when where pregnancy was achieved.

Clinical pregnancy was defined as seeing fetal heartbeat by ultrasound in the 6th or 7th week of pregnancy. The maturity rate is the ratio of Metaphase II (MII) oocytes that underwent ICSI to the total number of oocytes collected. The fertilization rate is the ratio of the number of 2PN embryos to the number of injected MII oocytes. Embryo utilization rate is defined as the ratio of the number of embryos available for transfer or cryopreservation per 2PN embryos.

Primary outcome parameters were the oocyte maturation and embryo utilization rates. Secondary outcome parameters were the pregnancy and live birth rates.

Continuous variables were defined by mean (±2 standard deviation) and categorical variables were defined by number and percentage. The control and study groups were compared in terms of continuous variables using the independent t-test. The groups were compared using Fisher's exact test for categorical variables. In the context of two-way hypothesis evaluation, $p<0.05$ was considered statistically significant.

Table 2: Cycle characteristics and laboratory outcome parameters of women in GH and conventional stimulation groups

	Conventional stimulation group (n=751)	Growth hormone group (n=56)	p	Mean difference	95% CI
Duration of stimulation (days)	10.1±2.7	10.1±2.2	0.456	-0.04	-0.78– -0.69
Total gonadotropin dosage	5191.44±743.70	4734.00±1064.268	0.002*	457.44	145.99,768.89– -6.84
Total oocytes	5.4±3.3	5.1±4.4	0.158	-0.52	1.55–0.50
Number of MII oocytes	4.0±2.7	4.7±3.6	0.056	-0.68	1.51–0.16
Maturity rate (%)	76.5	81.7	0.061	-5.17	-11.7– -1.38
Number of 2PN	3.1±2.2	3.7±3.1	0.051	-0.57	-1.26– -0.11
Fertilization rate (%)	83.4	86.4	0.511	-2.94	-10.8– -4.87
Number of useable embryos	3.2±2.3	3.7±2.9	0.077	-0.57	-1.21– -0.19
Embryo utilization rate (%)	93.8	92.7	0.301	1.14	-3.16– -5.45
Number of embryos transferred	1.6	1.7	0.190	-0.07	-0.24– -0.09

*: P<0.05; Statistically significant; GH: Growth hormone; MII: Metaphase-II; 2PN: 2-pronuclei; IU: International unit; CI: Confidence interval.

Table 3: Success in MII-oocyte retrieval in the study and control groups

	Conventional stimulation group		Growth hormone group		Total	
	n	%	n	%	n	%
Number of MII oocyte retrieved	51	6.79	1	1.78	52	6.44
At least one MII oocyte retrieved	700	93.2	55	98.2	755	93.5
Total	751		56			

P=0.251. The fisher exact test; MII: Metaphase-II; n: Number.

RESULTS

Table 1 displays the basal and IVF cycle characteristics of total study population. The mean female age was 42.3, ranging between 40 and 47 years. The mean duration of infertility at the time of presentation was 4.6 years. Patients received ovarian stimulation using an average dose of 368 IU of gonadotropins for a mean duration of 11 days. The mean number of total and MII oocytes retrieved was 4.8 and 3.6, respectively. The fertilization rate was 72.1 and the embryo utilization rate was 90%.

Comparison of the cycle characteristics and laboratory outcomes between the GH group and the conventional stimulation group is given in Table 2. While two groups were similar in terms of female age and duration of stimulation, the mean duration of infertility was longer (6.1 vs. 4.3 years, p=0.011) and the total dose of gonadotropins was less (5191 vs 4734 [IU], p=0.002) in the GH group compared to the conventional stimulation group. The total number of oocytes retrieved was similar in both groups (5.4 vs. 5.1, p=0158). Both the number of M-II oocytes and the maturation rate were higher in the GH group compared to the control group, but the differences were marginally insignificant (4.7 vs. 4.0, p=0.056 and 81.7% vs. 76.5%, p=0.061; respectively). While the fertilization and embryo utilization rates were similar for two groups, the number of usable embryos was higher in the GH group than the control group (3.7 vs. 3.2). However, this difference lacked of statistical significance (p=0.077).

Table 3 shows the rate of success in oocyte retrieval the study and control groups. While at least one M-II oocyte was retrieved in 98.2% of women in the GH group, this rate was 93.2% in the conventional stimulation group. The difference was not statistically significant (p<0.05).

More patients have reached to embryo transfer in the GH group compared to the control groups (87.2% vs. 75.7%); however, the difference lacked statistical significance (p=0.0674) (Table 4).

The clinical outcome in each group was summarized in Table 5. The pregnancy rate was higher in the GH group than control group (20.4% vs. 11.3%); however, the difference was not significant. The live birth rate was significantly higher in the GH group (18.8%) compared to the conventional stimulation group (18.8% vs. 5.8%, p=0.013).

DISCUSSION

When we scrutinize the currently available data regarding the effect of adjuvant GH in women with advanced age and poor ovarian response, we can see that a definite conclusion has not been reached yet. The most important reason for this is that cases included in these studies and stimulation protocols are highly heterogeneous. However, there are two outcomes shared by the majority of published studies. First, no benefit of GH supplementation could have been shown

Table 4: Patients who have reached to embryo transfer in GH and conventional stimulation groups

	Conventional stimulation group		Growth hormone group		Total	
	n	%	n	%	n	%
Cycle cancellation	170	24.2	7	12.7	177	23.4
Embryo transfer	530	75.7	48	87.2	578	76.5
Total	700		55			

P=0.0674. The fisher exact test.

Table 5: Comparison of the clinical outcome between GH and conventional stimulation groups

	Conventional (n=530)		Growth hormone (n=48)		p
	n	%	n	%	
Pregnancy rate	74	11.3	10	20.4	0.158
Live birth rate	38	5.8	9	18.8	0.013

Fisher's exact test. GH: Growth hormone.

in normoresponder patients and second, the use of GH seemed to be beneficial in women with diminished ovarian reserve and poor-quality embryos. This positive effect is more evident if combined with additional factors such as old age or repeated implantation failures.[10]

Molecular studies have shown GH receptor gene expression in granulosa cells, cumulus cells, and oocytes. This suggests that GH has a direct effect on the ovary.[11] Recently, GH mRNA has been observed to be expressed in granulosa cells derived from small follicles that mature *in vitro* in bovine oocytes, suggesting that GH is also synthesized in the ovary. Such an effect is not controlled by GH-releasing hormone (GHRH) indicating the presence of paracrine and autocrine effect of GH as well as the known endocrine effect.[12] In a study on abortion materials conducted by Abir et al.,[13] simultaneous detection of GH and GHRH in fetal ovaries using both in situ hybridization and immunocytochemistry has been reported for the 1st time. In this study, proteins and mRNA transcripts for both GH and GH receptor were detected in all ovarian cellular components.

GH and IGF-1 have an effect on steroidogenesis and follicle development while locally affecting granulosa cells. This fact might explain why the stimulation time as well as the amount of gonadotropin used for stimulation are less when GH is used.[14,15] In this respect, Hart et al.[16] found that the stimulation time was shorter, and the number of oocytes obtained was higher in cases where GH was used. Although the duration of infertility was longer in our study, the stimulation time was found to be shorter in the group using GH. The amount of gonadotropin used was significantly lower as well.

The first study on GH supplementation was conducted by Owen et al.,[8] in 1991 and showed that GH adjuvant therapy improved outcomes in women with poor ovarian response. Clinical studies in the following years suggested that GH supplementation during ovarian stimulation improves the laboratory and clinical outcome.[5–7] Du et al.[17] reported that serum estradiol levels and number of oocytes obtained were higher on the day of hCG in their study on normoresponder cases. Kucuk et al.[18] indicated a significant increase in the number of oocytes obtained. Likewise, many authors report increased oocyte count and quality in the GH group.[19–21] In a recent meta-analysis of poor responder patients,[22] the number of metaphase II oocytes (mean difference 1.62) and the number of useable embryos (mean difference 0.76) were significantly higher. Our study, in accordance with these studies, showed a marginally insignificant increase in the number of mature oocytes, maturation rates, number of 2PN embryos obtained, and number of useable embryos.

Recently, an increase in clinical pregnancy and live birth rates has also been observed in Randomized controlled trials (RCTs) conducted on the effect of GH on poor responder patients.[23] Yang et al.,[24] in a recent meta-analysis on the effect of GH in poor responder cases, reported that GH use improved clinical pregnancy and live birth rates. In this study of 1448 cases and 15 RCTs, live birth rates (RR, 1.74; 95%, Confidence interval [CI], 1.19–2.54), clinical pregnancy rate (RR, 1.65; 95% CI, 1.31–2.08) and retrieved oocytes number (SMD, 0.72; 95% CI, 0.28–1.16) increased, while cancelled cycle rate (RR, 0.62; 95% CI, 0.44–0.85) and amount of gonadotropin used decreased (SMD,–1.05 95% CI,–1.62––0.49) with the use of GH in poor ovarian response patients.

In our study, the clinical pregnancy rate was observed to be higher in the GH group (20.4% vs. 11.3%). However, this is not a statistically significant difference. In contrast, live birth rates are 18.8% in the GH group, which is significantly higher when compared to 5.8% in the conventional group.

While the debate on adjuvants in ART continues, many scientific societies such as the human fertilizations and embryology authority, the Royal college of obstetricians and gynecologists in the UK, and the Australia-based Victoria assisted reproductive treatment authority have issued guides and declarations urging the public to exercise caution about these treatments and question the use of such treatment methods.[25] The insufficient number of cases in the meta-analyses, the differences in the stimulation protocols used, and in patient profiles prevent reaching a definite conclusion.

The limitations of our study are its retrospective nature and small sample size, on the other hand, the strenght of study comes from single clinician experience. As a result, our study shows that the use of GH as an adjuvant to ovarian stimulation in older women increases live birth rates. The number of MII oocytes, fertilization rate, the number of 2PN embryos obtained, and the number of useable embryos appear to be increased, but this difference marginally failed to reach statistical significance. Like most of the adjuvant treatments currently used, the available evidence regarding GH supplementation is not optimal, and better-designed studies are needed to give definitive answers. Large and randomized trials in IVF are needed but they are difficult to conduct for various reasons. In addition to the clinical data, it would be useful to determine the effects of GH on oocyte and embryo quality in more detail from the perspective of the laboratory data.

CONCLUSION

During ovarian stimulation, usage of growth hormone as an adjuvant therapy can increase the live birth rates, especially in older patient population. Future studies are needed for different aged patient groups.

Statement

Ethics Committee Approval: The Koç University Clinical Research Ethics Committee granted approval for this study (date: 28.07.2022, number: 2022.254.IRB1.095).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – SA, SE, EA; Design – SA, SE, EA; Supervision – SA, SE, EA; Resource – SE; Materials – SA; Data Collection and/or Processing – SA; Analysis and/or Interpretation – SE, SA; Literature Search – SE, SA; Writing – SA, SE; Critical Reviews – SE.

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