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Original Article

The impact of HCG in IVF Treatment: Does it depend on age or on protocol?

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ABSTRACT

Objective: to evaluate the effect of the addition of low dose human chorionic gonadotropin (hCG) to human menopausal gonadotropin (HMG) throughout the early follicular phase in controlled ovarian stimulation (COS) conducted with two difference regimens. Gonadotropin-releasing hormone (GnRH) antagonist and short GnRH-agonist protocol were applied in two in vitro fertilization (IVF) clinics.
Methods: Clinical study conducted during the period 2014–2016 in two IVF clinics in a cohort of 240 women. In the first group 1 (124 women), a GnRH antagonist protocol with HMG and addition of low dose (100IU/day) h CG was applied. The other group 2 consisted of 116 women who underwent a short GnRH-agonist protocol with HMG and addition of low dose (100 IU/day) h CG.
Results: Multiple logistic regression analysis was performed. The group 2 found to be associated with greater number of follicles and oocytes. The pregnancy rates were 12.1% and 26.7% in group 1 and group 2, respectively (p = 0.004). For patients over 40 years, the number of follicles and oocytes retrieved were significant higher in group 2.The pregnancy rate in group 2 was higher than in group 1 (21, 6% vs 5%, p = 0.017).
Conclusions: Advanced age women are likely to achievepregnancy using the GnRH Short than GnRH antagonist, when HMG/hCG is used, while HMG–hCG gonadotropins have the same potentialas Recombinant follicle stimulating hormone (rFSH)–hCG used in GnRH short protocol.

Introduction

Luteinizing hormone (LH) and human Chorionic Gonadotropin (hCG) have similar molecular structures, binding to a common receptor. Thanks to technological advances, including the development of highly purified and recombinant gonadotropins, there is a notion that these hormones are not as exchangeable as it was thought. Although they bind to a common receptor, emerging evidence suggests that LH and hCG have different effects on the molecular biological pathway. Recently, attention was drawn to the effect of hCG on target cells during transplantation and the participation of this hormone to a series of immune mechanisms [1,2]. However, this peculiar variety of hormones are of use in clinical practice, during ovulation induction regimes.

Gomaa et al., demonstrated that the addition of hCG to recombinant FSH throughout the stimulation in a long GnRH IVF protocol had a beneficial effect on women over 40 years of age [3]. Also, the use of recombinant LH is associated with significantly higher pregnancy rates (PR) when administered during the early follicular phase in a long GnRH protocol [4]. Thuesen et al., using a long GnRH protocol, proposed that the optimal dose of hCG was 100–150 IU. Moreover the supplementation with hCG from the first

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day of stimulation increased the number of top-quality embryos per patient while daily doses of hCG up to 150 IU was compatible with high live birth rates [5].

In a later study, they demonstrated that when the environment of the follicular fluid (ff) was estrogenic and contained high levels of androgens, top quality embryos were presented. Thus, hCG supplementation induced a marked stimulatory effect on the intrafollicular androgen and estradiol levels, with a shift toward a more androgenic milieu [6].

Also, significantly higher intrafollicular concentrations of estrogen and progesterone were associated with good embryo quality. The estrogen to androgen ratio being highest in the large follicles with oocytes giving rise to good-quality embryos confirms the importance of the follicle's capacity for aromatization to produce high-quality oocytes. Finally, the authors concluded on the base of biochemical evidence and embryological observations, that in terms of clinical efficacy and safety, introduction of hCG supplementation up to a daily dose of 100 IU could be proposed [6].

Our group showed that the addition of hCG to rFSH in a short GnRH-agonist protocol, throughout the early follicular phase, has beneficial effect in women of the age group 35-40. We recorded significantly higher pregnancy rates, although other parameters of ovarian stimulation, such as number of oocytes retrieved and fertilization rates were in favor of rFSH alone, especially in younger population. Furthermore, hCG was associated with better quality embryos. The significance of these findings was accentuated by the fact that women, who received hCG were significantly older and with higher basal FSH levels, thereby with expectant poorer ovarian reserve [7].

Dinopoulou et al., demonstrated that in the process of in vitro maturation (IVM) in mouse denuded oocytes, the addition of hCG instead of rLH, significantly increased the nuclear maturation rate of these oocytes in vitro. The absence of any supporting cumulus cells suggests the presence of LH/hCG-Receptor (LHCGR) in the oocytes at this developmental stage in mice. Moreover, the differences of the half-life of LH and hCG could offer an explanation about the improved maturation rates in the hCG group. With the use of PCR, the authors demonstrated that LHCGR was expressed during all stages of in vitro matured mouse oocytes and early embryonic development [8].

hCG has stronger LHCGR binding affinity, probably due to differences in the carbohydrate moiety [9]. Daily doses of 50–200 IU of hCG have been used to supplement FSH in COH or even substitute FSH in the late follicular phase [10–13], whereas a single dose of 1250 IU of hCG has been used in a GnRH-antagonist protocol in combination with aromatase inhibitor in early-follicular phase [14].

In a study conducted by our group, we have shown a potential beneficial effect of the addition of low dose hCG to rFSH during controlled ovarian stimulation for IVF/ICSI [9]. We demonstrated that the daily administration of 200 IU of hCG can be applied in addition to rFSH in patients with previous failed attempts during a short protocol in the first 5 days of superovulation. This regime seems to increase pregnancy rates [9].

This study has also determined that m-RNA for LHCGR in the lymphocytes of peripheral blood 40 h before egg retrieval and cDNA levels of the hCG receptor after ovarian stimulation were significantly higher among women receiving hCG compared to women receiving LH. These higher levels encountered among women, indicated the stronger affinity of the receptor when hCG was used [9].

In another study, we clearly demonstrated that there is a point towards a beneficial "hCG priming" effect in controlled ovarian hyperstimulation through a long GnRH-a down-regulation protocol, particularly in patients with previous ART failures where the percentage of patients with more than one top quality embryo was higher in the pre-treatment group. Results also showed a higher pregnancy rate in women treated with hCG [15].

Furthermore, the accumulating evidence of a potential beneficial effect of rFSH, with the addition of hCG in different ovulation induction protocols as GnRH agonists (short-long) or GnRH antagonist have been confirmed [16].

In addition, Chaca et al., demonstrated that hCG has the advantage of decreasing the doses of FSH. In this review, the clinical pregnancy rate in the hCG group was significantly higher than that in the standard FSH group (RR, 1.32; 95% CI, 1.06–1.64). Although a lower number of metaphase II oocytes was obtained in hCG group (MD, 0.3; 95% CI, 0.16 to 0.44), better oocyte quality could be the reason for the higher number of clinical pregnancies [17].

The aim of this study was to assess the effect of the addition of low dose hCG to HMG throughout the early follicular phase in COS conducted with two difference regimens; GnRH antagonist and short GnRH-agonist protocol on ART outcome in two IVF clinics.

Materials and methods

This clinical study was conducted on 124 women undergoing COS with GnRH antagonist protocol with HMG and the addition of low dose (100 IU/day) hCG (Athens IVF Center-Group 1). The other group consisted of 116 women undergoing IVF/ICSI through a short GnRH-agonist protocol with HMG and the addition of low dose (100 IU/day) hCG (Fertility Institute-Group 2).

Patient recruitment was accomplished using a computer-generated randomization table.

The sequences of randomization were concealed until intervention was assigned. Patients were subsequently selected from the daily routine practice. Each file was collected and stored after ET until all the files were analyzed. Thus, patients' outcome was unknown to the IVF clinics prior to data retrieval.

The study included a total of 240 women from 2014 to 2016, who underwent IVF/ICSI in Athens IVF Center and Fertility Institute. Inclusion criteria were women 32–47 years of age with no uterine or ovarian anomalies, having normal hormonal profile 9according to WHO guidelines), a regular menstrual cycle of 21–35 days and both ovaries intact. Each patient underwent a short GnRH-agonist or GnRH-antagonist protocol with HMG –hCG. The indications for fertility treatment were male factor, tubal factor, unovulatory cycles due to polycystic ovaries, other causes of infertility, complex etiology, and unexplained infertility. None of these women had been subjected to ovarian stimulation or any other hormonal treatment for at least three months before entering COS.

For all women anthropometric characteristics data such as age, and BMI were recorded. Reference values for early follicular phase FSH, LH, prolactin (PRL), AMH and TSH levels performed within the preceding 6 months were also recorded. In addition, the number of follicles during the monitoring, the number oocytes retrieved, the number of embryos, the percentage of the ratio ovum/embryos and pregnancy rates were recorded for each participant in the study.

The study protocol was approved by both review boards of Fertility Institute and Athens IVF Center. All participants provided informed consent for their medical records to be used in the study.

Ovarian stimulation, IVF/ICSI and embryo transfer

GnRH-agonist protocol

Short GnRH-agonist protocol was conducted according to the strict routine practice of Fertility Institute. On cycle day 2, a baseline ultrasound scan was performed. Unless scan findings were not indicative, serum estradiol and progesterone levels were determined. Once reassuring, daily subcutaneous injection of

GnRH-agonist (buserelin) was started on cycle day 2 at a dose of 0.5 mg and were kept until triggering of final oocyte maturation with hCG.

HMG was administered on day 3 at a dose of 200 IU and the dose was adjusted according to ovarian response on a daily basis, 6 days after the onset of HMG administration.

hCG was administered intramuscularly at a dose of 100 IU per day along with 200UI of HMG, starting on day 3 of cycle throughout the follicular phase, until the day of triggering of final oocyte maturation.

Serum E2 levels were measured daily starting on day 5 of ovarian stimulation with gonadotropins (day 7 of cycle) until the day of triggering final oocyte maturation with 10,000 IU of hCG given intramuscularly. Follicular tracking started on day 6 of stimulation (day 8 of cycle) and subsequent ultrasound scans were performed every day until oocyte retrieval. Follicular aspiration and oocyte retrieval took place 36 h after the administration of 10,000 IU hCG by transvaginal ultrasound-guided puncture.

Luteal phase support was provided with 200 mg of micronized progesterone administered intra-vaginally three times daily from the day after egg collection onwards and serum hCG was measured 14 days later. Clinical pregnancy was defined as the presence of a gestational sac on ultrasound at six gestational weeks.

Hormone assessments were performed in the same Lab. Ultrasound scans, oocyte retrievals and embryo-transfers were conducted by either of the two fertility specialists of the Centre. Similarly, oocyte grading, fertilization, early embryo development and embryo grading were conducted by either of the two senior embryologists of the Centre.

GnRH-antagonist protocol

Short GnRH-agonist protocol was conducted according to the strict routine practice of Athens IVF Center. On cycle day 2, a baseline ultrasound scan was performed. Unless scan findings were not indicative, serum estradiol and progesterone levels were determined. On cycle day 5, daily administration of GnRH-antagonist (ganirelix) was initiated and kept until triggering of final oocyte maturation with hCG.

HMG was administered on day 3 at a dose of 200 IU and the dose was adjusted according to ovarian response on a daily basis, 6 days after the onset of HMG administration.

hCG was administered intramuscularly at a dose of 100 IU per day along with HMG, starting on day 2 of the cycle throughout the follicular phase, until the day of triggering of final oocyte maturation.

Serum E2 levels were measured daily starting on day 5 of ovarian stimulation with gonadotropins (day 7 of cycle) until the day of triggering final oocyte maturation with 10,000 IU of hCG given intramuscularly. Follicular tracking started on day 6 of stimulation (day 8 of cycle) and subsequent ultrasound scans were performed every day until oocyte retrieval. Follicular aspiration and oocyte retrieval took place 36 h after the administration of 10,000 IU hCG by transvaginal ultrasound-guided puncture.

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Statistical analysis

Statistics Package for Social Sciences was employed to analyze the data of the study.

Quantitative variables were expressed as mean values (SD) or as median values (interquartile range = IQR). Qualitative variables are expressed as absolute and relative frequencies. For the comparisons of proportions chi-square tests tests were used. If the normality assumption was satisfied for the comparison of means between two groups, Student's *t*-test was used. Mann-Whitney test was used for the comparison of continuous variables between two groups when the distribution was not normal. Multiple linear regression analysis was used with dependent variables the IVF outcomes in order to explore differences between the two study groups after adjusting for age and body mass index (BMI) and after logarithmic transformations. Adjusted regression coefficients (β) with standard errors (SE) were computed from the results of the linear regression analyses. Multiple logistic regression analysis was also performed in order to identify differences in the likelihood of pregnancy after adjusting for age and BMI. Adjusted odds ratios with 95% confidence intervals were computed from the results of the logistic regression analysis. All reported p values are two-tailed. Statistical significance was set at $p < 0.05$ and analyses were conducted using SPSS statistical software (version 22.0).

Results

The study group consisted of 240 women (124 in Group 1 and 116 in Group 2) with mean age 40.5 years (SD = 3.5). Demographics for the two groups are presented in Table 1. Women of roup A were older while BMI levels were similar between groups. Comparison of IVF outcomes between the two groups showed that group 2 succeeded greater number of follicles and oocytes and lower

Table 1
Clinical characteristics, comparison of IVF outcome and comparison of hormone levels between the two groups.

	Group 1 N = 124 Mean	SD	Group 2 N = 116 Mean	SD	P
Age (years) range (from to)	41.4	3.4	39.5	3.3	<0.001
BMI	25.3	2.1	24.8	2.7	NS
	Mean(SD)	Median(IQR)	Mean(SD)	Median(IQR)	P
FSH (mIU/ml)	8.1(3.2)	7.4(6.1-9.6)	7.9(3.2)	7(6.1-9.3)	0.478
LH (mIU/ml)	5.8(2)	5.5(4.5-6.8)	5.8(2.3)	5.6(4.1-7.3)	0.794
PRL (ng/ml)	17(10.3)	15.6 (9.3-21.9)	17.4(10.5)	15.9 (9.3-22.6)	0.795
AMH (ng/ml)	3.6(5.2)	2.2(1-4.1)	3.7(5.4)	2.1(0.9-4)	0.881
TSH (mIU/L)	2.8(3.9)	1.8(1.2-2.9)	2.7(4.1)	1.7(0.9-2.7)	0.328
Follicles	2.8(2.3)	2(1-4)	7(2.5)	7(5-9)	<0.001
Oocytes	2.2(2)	2(1-3)	6.2(2.5)	6(4-8)	<0.001
Embryos	1.4(1.5)	1(0-2)	0.9(0.2)	1(1-1)	0.039
Pregnancy rate %	121%			267%	0.004

number of embryos (Table 1). Comparison of hormone levels is shown in Table 1 and no significant differences were recorded. The pregnancy rates were 12.1% and 26.7% in group 1 and group 2, respectively (p = 0.004) (Table 1).

Regarding the subgroup of patients over 40 years of age, the statistical analysis results are shown clearly in Table 2. Thus, hormonal profile based on FSH and AMH levels was similar, the number of follicles and oocytes retrieved were significantly higher in group 1, while the number of embryos was not significant different. The pregnancy rate in group 2 was higher than group 1 (21.6% vs 5%, p = 0.017, Table 2).

Discussion

The role of hCG in follicular maturation and ovulation is essential, particularly in order women [3,7]. Studies have clearly demonstrated the favorable effect of the combination of hCG-rFSH or rLH [4], when administered during the early follicular phase, regardless whether a long/short GnRH agonist or GnRH antagonist protocol is in use [18].

The aim of this study was to determine if the use of hCG-HMG in a short GnRH agonist or GnRH antagonist protocol has beneficial effect in the pregnancy rates. Regarding our results between the two different clinics that participated in this study, multiple logistic regression analysis after adjusting for age, it showed that group 2 had 2.2 times greater odds for pregnancy (95% CI: 1.05–4.58, p = 0.036) than group 1 (26.7% and 12.1%, respectively, Table 1).

Advanced age reflects a change in the structure of the FSH and LH molecule secreted by the pituitary gland [14]. This may be related to the biological expression at the target cell level, where an imbalance in the FSH and LH ovarian receptors was found [11,19,20]. It is well recognised that simply by raising the daily dose of gonadotrtopins only partially compensates for the age-related decline in gonadotropin sensitivity.

Granulosa cells in antral follicles develop LH receptors, hence they become sensitive to LH/hCG stimulation. Thus, it could be hypothesised that granulosa cells, which are resistant to gonadotropin stimulation, might benefit from r-hLH or hCG stimulation. Further, r-hLH or hCG may have beneficial effects through stimulation of theca cells. Based on that notion, it is interesting to note that in situations where the ovaries are older or less sensitive to gonadotropins (biologically older), there is increasing evidence to suggest that the use of r-hLH or hCG may be beneficial. We clearly validated this hypothesis because the addition of hCG to rFSH was associated with better quality embryos and higher pregnancy rates, even in women of advanced reproductive age, over 40-years-old, with higher basal FSH levels, which are characterized by poor ovarian reserve [7].

Thus, if the age of the patient is >40 years of age, the number of follicles, oocytes retrieved, embryos, and PR were significant higher in group 2 (Table 2). This observation highlights the fundamental role of hCG in ovulation induction, even when rFSH or HMG gonadotropins are used.

An explanation for the beneficial action of hCG could be that the gonadotropin receptor allocation in follicular cells is in line with the two-cell two-gonadotrophin theory [21]. According to this theory, LH/hCG induces androgen production by the theca cells and FSH promotes aromatase enzyme activity and thus the utility of androgens as a substrate for estrogen biosynthesis in the ff, after the treatment of hCG where the follicular environment was androgenic [16]. In fact, FSH and LH act synergistically and complementally in the process of follicular growth given that FSH drives recruitment, selection and dominance, whereas LH contributes to dominance, maturation and ovulation [20,22].

A question about lower pregnancy rates observed in group 1 is pending. The only difference between clinics was the use of GnRH (antagonist vs short). An explanation could lie on the receptivity of the endometrium. Orvieto et al., examined whether the choice of the GnRH analogues used during controlled ovarian hyperstimulation (COH), may influence endometrial receptivity, in patients undergoing COH with GnRH agonist or antagonist and with the transfer of at least one top-quality embryo. The GnRH agonist group showed significantly higher endometrial thickness and higher pregnancy rate, suggestive of a higher endometrial receptivity, compared to the GnRH antagonist group [23].

In our results, especially in older patients, all parameters are in accordance with the study conducted by Malmusi et al., where they presented that the GnRH short protocol appears to be more effective than the GnRH-antagonist protocol in terms of mature oocytes retrieved, fertilization rate, and top-quality embryos transferred in patients with poor ovarian responce [24]. Finally a meta-analysis published by Franco et al., confirmed the superiority of GnRH short protocols. Analysis applied to the four trials that had used GnRH-antagonist versus GnRH short protocols showed a significantly higher number of retrieved oocytes was observed in the GnRHa protocols in poor responders patients [25].

On the other had, based on our results, we have to consider that the action of hCG doesn't affect positively only the ovulation induction regime and the microenvironment level of the follicle, but has effects on several levels; on the immune system, on the endometrium receptivity, and in the processes of oocyte nuclear maturation. These parameters are of high importance in human reproduction. However, hCG has effects on the follicular environment during ovulation hormonal changes. hCG supplementation markedly stimulated the intrafollicular concentration of both estradiol and androgens, with a shift toward a more androgenic milieu. In large follicles with oocytes giving rise to good-quality

Table 2
Subgroup analyses for women over 40 years of age.

	Group 1				Group 2				
	Mean	Standard Deviation	Median	Percentile 25	Percentile 75	Mean	Standard Deviation	Median	Percentile 25	Percentile 75	
Age (years)	42,9	2,1	43,0	41,0	44,0	42,6	2,3	42,0	40,0	45,0	
	Mean(SD)		Median(IQR)			Mean(SD)		Median(IQR)			P
FSH (mIU/ml)	8.3(3.3)		7.7(6.6-9.7)			8.7(3.9)		8.3(6.2-10.6)			0.717
LH (mIU/ml)	5.6(2.1)		5.4(4.2-6.7)			5.2(2)		5.3(3.5-6.4)			0.400
PRL (ng/ml)	17.3(10.8)		15.7(9.2-23.5)			18.5(10.8)		16(9.8-25)			0.562
AMH (ng/ml)	3.2(3.7)		2.1(0.9-3.9)			4.5(6.3)		2.5(1-5)			0.394
TSH (mIU/l)	2.9(4.2)		1.7(1.3-3.1)			2.2(2.2)		1.5(0.9-2.5)			0.196
Follicles	2.6(2.1)		2(1-4)			6.7(2.5)		6(5-9)			<0.001
Oocytes	1.9(1.8)		2(0-3)			5.9(2.6)		6(4-8)			<0.001
Embryos	1.3(1.2)		1(0-2)			1(0)		1(1-1)			0.720
Pregnancy rate %	5%					216%					0,017

embryos, the ff was significantly more estrogenic than in small follicles with oocytes developing into poor quality embryos [6].

Regarding the immune system, hCG promotes an anti-macrophage inhibitory factor or a macrophage migration inhibitory factor. This is a cytokine that modulates the immune response during pregnancy. It has also been shown that hCG may directly suppress any immune action against the invading foreign tissue by the mother, suggesting that hCG has an inhibitory or suppressive function on macrophage activity [26–30].

Regarding endometrium receptivity, intrauterine hCG infusion was associated with endometrial synchrony and reprograming of stromal development following ovarian stimulation. ESRI and PGR were significantly elevated in the endometrium of hCG-treated patients. hCG was also found to be increased, including C3 and NOTCHI, whose role in endometrial receptivity is already established. An additional possible role for hCG driven epithelial proliferation in preventing stromal advancement to maintain synchrony. Finally, in the process of follicular nuclear maturation, Dinopoulou et al., studied the effect of recombinant-LH and hCG in the absence of FSH on IVM, fertilization and early embryonic development of mouse germinal vesicle (GV)-stage oocytes. Nuclear maturation of GV-stage oocytes was evaluated after culture in the presence of r-LH or hCG. The LHCGR was expressed in all stages of in vitro matured mouse oocytes and in every stage of early embryonic development. Moreover, the addition of hCG in IVM cultures of mouse GV oocytes increased the nuclear maturation rates significantly [8].

If we compile the data from the aforementioned literature and take under consideration the systematic review from Chaca et al., the authors demonstrated that the relation to the final outcome - the clinical pregnancy rate - in the hCG group was significantly higher than that in the standard FSH group (RR, 1.32; 95% CI, 1.06–1.64). Although a lower number of metaphase II oocytes was obtained (MD, 0.3; 95% CI, 0.44 to 0.16), a better oocyte quality may be the reason for a higher number of clinical pregnancies [17].

Furthermore, in the current study, we clearly demonstrated the beneficial effect of hCG even when used with HMG, which contains a low amount of hCG. Our study confirmed the results of the current literature, where all studies agree that the fundamental role of hCG is expressed in the optimal dose of 100–200 IU [17].

`In conclusion, in the current study, we provided evidence that women of advanced age had good chances for achieving a pregnancy using the GnRH Short than GnRH antagonist, when HMG / hCG was used. Also HMG –hCG gonadotropins have the same potential as rFSH –hCG used in GnRH short protocol, especially when the target group of patients are more than 40 years old.

Disclosure of interest

The authors declare that they have no competing interest.

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