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Addition of low-dose hCG to rFSH during ovarian stimulation for IVF/ICSI: is it beneficial?

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Summary

Purpose: The aim of the study was to assess the effect of the addition of low-dose human chorionic gonadotropin (hCG) to ovarian stimulation with recombinant follicle stimulating hormone (rFSH) on *in vitro* fertilization/intracytoplasmic sperm injection (IVF/ICSI) outcome. *Materials and Methods:* This retrospective clinical study was conducted on 141 women undergoing ICSI through a short GnRH-agonist protocol with rFSH and the addition of low-dose (100 IU/day) hCG. The control group consisted of 124 women undergoing ovarian stimulation with a similar protocol devoid of hCG. Statistical analysis in the study population along with a subgroup analysis for age ≥ 35 years and ≥ 36 years was performed. *Results:* Women in hCG group were statistically significant older and with higher basal FSH compared to control group. This can be attributed to the Centre's latent tendency to add hCG in the stimulation protocol in poor prognosis patients. Despite this fact and the fact that several ovarian stimulation parameters, such as peak estradiol levels, number of oocytes retrieved, number of mature oocytes, and fertilization rates were in favor of the control group, the quality of transferred embryos and pregnancy rates were in favor of hCG group. Similar results were obtained in the subgroup analyses apart from peak estradiol levels, which did not differ among the study groups. *Conclusions:* The addition of hCG to rFSH may be associated with better quality embryos and higher pregnancy rates, even in women of advanced reproductive age with higher basal FSH levels, which are often considered to have poorer ovarian reserve.

Key words: hCG; ICSI; IVF; LH activity.

Introduction

The diversity of ovarian response among women undergoing controlled ovarian stimulation (COS) for *in vitro* fertilization (IVF), especially in cases with a history of previous failed attempts, has led researchers to investigate the factors that determine and potentially improve this response [1].

In physiology, it is uniformly recognized that luteinizing hormone (LH) is drastically involved in follicle maturation from the antral stage onwards. Basically, primordial and primary preantral follicle development is considered gonadotropin independent, given that both cumulus cells and theca cells are devoid of follicle stimulating hormone (FSH) and LH receptors. However, in cumulus cells, the presence of FSH receptors and LH receptors has been confirmed from the secondary preantral and from the antral stage onwards, respectively. With regards to the theca cells, although FSH receptors are lacking, LH receptors are present from the secondary preantral stage onwards. Gonadotropin receptor allocation in follicular cells is in line with the two-cell two-gonadotropin theory [2], according to which, LH 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 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 [3-4].

On the basis of this theory, preantral stage can be reached in the absence of LH. However, this hormone is considered essential for antral formation and further follicle development and differentiation from the antral stage onwards. In this context, hypogonadotropic hypogonadal women respond to FSH alone with follicle development with blunted estradiol production and poor luteinization following human chorionic gonadotropin (hCG) administration for triggering final follicle maturation [5, 6]. However, in gonadotropin-releasing hormone (GnRH)-agonist downregulation cycles, despite gonadotropin suppression, residual endogenous LH secretion is considered adequate for effective ovarian stimulation [7]. Basically, in assisted reproductive technology (ART), follicle development can be accomplished in the absence of LH in the stimulation protocol, suggesting that the addition of this hormone in COS protocols for IVF may be optional, determined by clini-

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cian's preferences.

Currently, LH activity can be provided by human menopausal gonadotropin (hMG), recombinant LH (rLH), human derived chorionic gonadotropin (hCG) or recombinant human chorionic gonadotropin (rhCG). hMG is a urinary product, which has been estimated to provide around 75 IU of FSH and 75 IU of LH activity per ampoule. Studies have shown that the hCG content of the hMG preparation is around five IU per ampoule. Given that hCG is around six-fold more potent than LH [8], it is concluded that of the 75 IU of LH activity provided in the hMG preparation, actually, about 30 IU are provided by hCG. Thus, hCG content contributes considerably to hMG-mediated LH activity [9].

Theoretically, LH activity mediated by hCG sounds quite attractive in the clinical setting due to its unique characteristics. In particular, hCG shares structural similarities with LH and function through the same receptor, LH/CGR. However, hCG has a longer half-life of 36 hours [10] compared with recombinant LH whose elimination half-life is estimated to be around ten to 12 hours [11], has stronger LH/CGR receptor binding affinity probably due to differences in the carbohydrate moiety, which may make the molecule more sensitive to the binding receptor [12], and is much more potent than LH [8].

Taking into consideration the accumulating evidence of a potential beneficial effect of hCG-mediated LH activity in ART outcome, the authors retrospectively collected data of ICSI cycles, in which as low as 100 IU of hCG were added in COS with rFSH and compared them with cycles, in which COS was conducted with rFSH only.

Study endpoints

The aim of this study was to assess the effect of the addition of low-dose hCG to rFSH throughout the follicular phase in COS conducted with a short GnRH-agonist protocol on ART outcome. In particular, the primary endpoint was to assess the effect on pregnancy rates, whereas secondary endpoints were the effect on various COS parameters, such as total rFSH dose used, duration of stimulation, peak serum estradiol levels, number of oocytes retrieved, number of mature oocytes, fertilization rates, and embryo quality.

Materials and Methods

Patients

This retrospective clinical study was conducted in 141 women undergoing intracytoplasmic sperm injection (ICSI) through a short GnRH-agonist protocol with rFSH and the addition of low dose (100 IU/day) hCG (hCG group). The control group consisted of 124 women undergoing COS with a similar protocol devoid of hCG. In fact, from July 2012 to June 2014, the medical records of a total of 645 women, who underwent in vitro fertilization (IVF)/ICSI in Fertility Institute, Diagnostic and Therapeutic Centre S.A., a private Fertility Centre in Athens, were assessed for eligibility. Among them, 283 were found eligible and finally 265 agreed to participate in the study, 141 in the hCG group and 124 in the control group (Figure 1). The inclusion criteria were premenopausal women ≤ 48 years-old, who underwent a short GnRH-agonist protocol with either rFSH or rFSH along with hCG, having normal hormone profile (according to WHO guidelines), a regular menstrual cycle of 21-35 days, and with both ovaries intact. The indications for fertility treatment for the 265 patients 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.

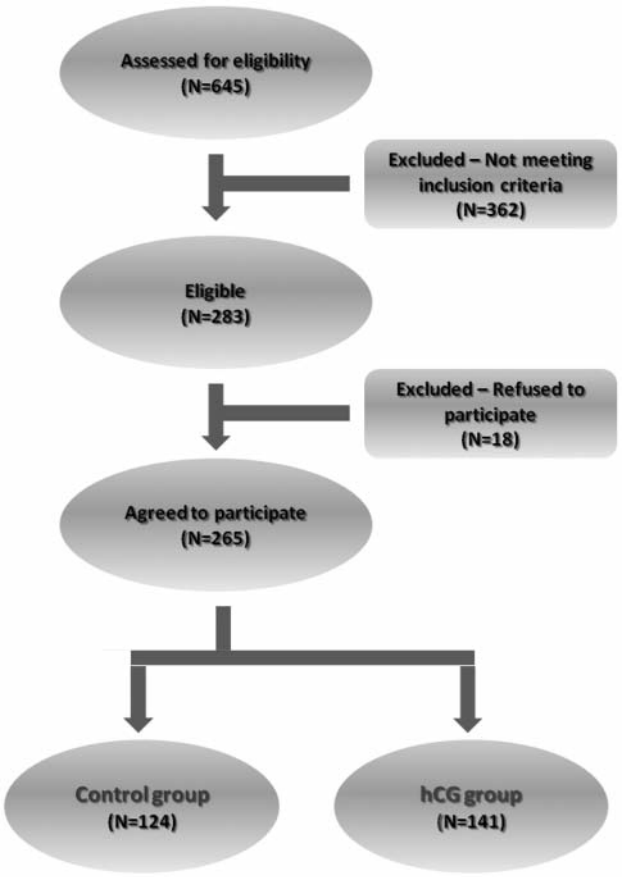

Figure 1. — Flow chart. Medical records of a total of 645 women, who underwent IVF/ICSI were assessed for eligibility. Among them, 283 were found eligible and finally 265 agreed to participate in the study, 141 in the hCG group and 124 in the control group.

For all women demographic data such as age, years of infertility, number of previous IVF/ICSI attempts, and BMI were recorded. Reference values for early follicular phase FSH, LH, estradiol (E2), and prolactin (PRL) levels performed within the preceding six months, were also recorded. In addition, total FSH dose, duration of stimulation, peak estradiol levels, number of oocytes retrieved, number of mature oocytes, fertilization rates, embryo quality, and pregnancy rates were recorded for each participant in the study.

Table 1. — *Analysis of clinical and laboratory results of all cases.*

	Control group (n=124)	hCG group (n=141)	t-test *p*-value	Mann-Whitney U *p*-value	Fisher's exact test *p*-value
Age (years)	33.99 ± 3.73	37.02 ± 4.63	0.000	0.000	N/A
Previous failed attempts	0.764 ± 0.950	1.596 ± 1.454	0.000	0.000	N/A
FSH (mIU/ml)	6.67 ± 2.13	8.52 ± 3.70	0.000	0.000	N/A
Total rFSH dose (IU)	2692.92 ± 1053.99	2702.51 ± 1034.62	0.941	0.982	N/A
Duration of stimulation (days)	10.02 ± 1.33	9.78 ± 1.80	0.208	0.272	N/A
Peak E_2 levels (pg/ml)	2152.76 ± 1104.26	1850.07 ± 1331.19	0.045	0.002	N/A
Number of oocytes retrieved	8.85 ± 2.71	6.31 ± 2.68	0.000	0.000	N/A
Number of mature oocytes	7.323 ± 2.69	5.738 ± 2.37	0.000	0.000	N/A
Fertilization rate (%)	89.4 ± 0.113	81.7 ± 0.141	0.000	0.000	N/A
Quality of transferred embryos	2.871 ± 0.382	3.355 ± 0.575	0.000	0.000	N/A
Pregnancy rate (%)	26.6	39.7	N/A	N/A	0.027

N/A: not applicable; Statistical significance: p-value < 0.05.

The study protocol was approved by the Institutional review board of Fertility Institute and all participants were approached and provided consent for their medical records to be used in the study.

Ovarian stimulation, IVF/ICSI and embryo transfer

Short GnRH-agonist protocol was conducted according to the strict routine practice of Fertility Institute. On day 2 of cycle, a baseline ultrasound scan was performed. Serum E2 and progesterone levels were determined and provided that they were reassuring, daily subcutaneous injections of buserelin acetate were started on cycle day 2 at a dose of 0.5 mg and continued until triggering of final oocyte maturation with hCG. Recombinant FSH administration started on day 3 at a dose of 200 IU and the dose was adjusted according to ovarian response on a daily basis, six days after the onset of rFSH administration.

In hCG group, hCG was administered intramuscularly at a dose of 100 IU per day along with rFSH, 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 from day 5 of ovarian stimulation with gonadotropins (day 7 of cycle) until the day of triggering final oocyte maturation. Follicular tracking began on day 6 of stimulation (day 8 of cycle) and subsequent ultrasound scans were performed daily until oocyte retrieval. Follicular aspiration and oocyte retrieval took place 35-36 h ours after the intramuscular administration of 10,000 IU hCG, by transvaginal ultrasound-guided puncture. Oocytes were assessed for their maturation under the microscope following stripping and among them, mature metaphase II oocytes were used for ICSI.

Embryos were scored based on normal cleavage rate, absence of fragmentation, and even-sized blastomeres on a scale from 4 (the best) to 1 (the worse) under a light microscope on the day of embryo transfer [13]. Two to three embryos were transferred according to embryo quality assessment and patient's preferences. Luteal phase support was provided with 200 mg of micronized progesterone administered intravaginally three times daily from the day after egg collection onwards and serum hCG was measured 14 days after that. 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.

Statistical analysis

Statistics Package for Social Sciences was employed to analyze the data of the study. Two independent samples *t*-test was used for quantitative data and chi-square test (Fisher exact test) for qualitative data. Due to the deviation from normality, non-parametric Mann-Whitney U test was applied in order to evaluate the univariate association of demographic and biochemical factors. This test was used as a complementary statistical test due to the relatively limited number of women comprising each group and subgroup to double check the results obtained by the parametric test.

Bombarded by progressively accumulating evidence of a tentative beneficial effect of hCG when added to rFSH, fertility specialists of Fertility Institute tended to mobilize hCG in women in which they expected a rather suboptimal ovarian response to ovarian stimulation. Among them, women of more advanced reproductive age were anticipated. Thereby, given the retrospective methodology of the study, in addition to statistical analysis in the study population [control group n=124 and hCG group n=141], a subgroup analysis was performed for age ≥ 35 years [control group n= 62 and hCG group n= 98] as well as for age ≥ 36 years [control group n=44 and hCG group n=87]. Statistical significance was set at the level of 5% (p < 0.05).

Results

Indications for fertility treatment with IVF/ICSI (male factor, tubal factor, unovulatory cycles due to polycystic ovaries, other causes of infertility, complex etiology, and unexplained infertility) did not differ among the study groups (data not shown).

Taking into consideration the Centre's latent tendency to add hCG in expected poor responders, it is not surprising that women in hCG group were statistically significant older (37.02 *vs.* 33.99 years) and with higher basal FSH levels (8.52 *vs.* 6.67) compared to control group. However, despite this and the fact that several ovarian stimulation parameters, such as peak estradiol levels, number of oocytes retrieved, number of mature oocytes, and fertilization rates

Table 2. — *Previous IVF/ICSI attempts and pregnancy rates.*

	Pregnancy	n	Mean	Std. Deviation	*t*-test *p*-value
Previous failed attempts	No	175	1.14	1.31	0.216
	Yes	89	1.35	1.31	

Statistical significance: *p*-value < 0.05.

were in favor of the control group, the quality of transferred embryos were in favor of hCG group as were pregnancy rates (Table 1). Other parameters such as height, weight, BMI, and basal hormonal profile (excluding FSH) did not differ among the study groups (data not shown). Furthermore, no difference was seen in total rFSH dose, and duration of ovarian stimulation (Table 1). However, it should be mentioned that in the hCG group, average number of previous failed IVF/ICSI attempts was statistically significant higher compared to control group (1.596 *vs.* 0.764 respectively). Although, it could be assumed that the higher the number of previous cycles, the more experience is gained on each individual case's response to ovarian stimulation, which may lead to a more tailored to each patients needs approach in a future IVF/ICSI cycle, this assumption is not supported in the present study, when examining the correlation between the number of previous IVF/ICSI attempts and pregnancy rates (Table 2). Similar results were obtained in the subgroup analyses aside from peak estradiol levels measured on the day of triggering final oocyte maturation, which did not differ among the subgroups (Tables 3 and 4).

Discussion

Since the introduction of COS in IVF, the goal has been gradually shifted from "quantity" to "quality". It is well known that the first successful IVF was conducted through a natural cycle. Thenceforth, the objective of developing as many follicles as possible in a single ovarian cycle became quite attractive. The rationale was that the more the follicles developed, the more the eggs collected and the more the embryos produced, among which the best would be selected for transfer. However, soon after increasing clinical application of this aspect, the advent of ovarian hyperstim-

Table 3. — *Subgroup analysis of clinical and laboratory results for age ≥ 35 years.*

	Control group (n=62)	hCG group (n=98)	*t*-test *p*-value	Mann-Whitney U *p*-value	Fisher's exact test *p*-value
Age (years)	36.96	39.47	0.000	0.000	N/A
Previous failed attempts	0.758 ± 1.066	1.755 ± 1.534	0.000	0.000	N/A
FSH (mIU/ml)	6.66 ± 1.88	8.69 ± 3.70	0.000	0.000	N/A
Total rFSH dose (IU)	2711.82 ± 1110.37	2636.73 ± 962.06	0.651	0.702	N/A
Duration of stimulation (days)	9.72 ± 1.50	9.53 ± 1.76	0.472	0.629	N/A
Peak E_2 levels (pg/ml)	1909.64 ± 1037.56	1754.67 ± 1365.59	0.446	0.088	N/A
Number of oocytes retrieved	8.33 ± 2.69	5.83 ± 2.64	0.000	0.000	N/A
Number of mature oocytes	7.27 ± 2.69	5.43 ± 2.29	0.000	0.000	N/A
Fertilization rate (%)	89.3 ± 0.100	83.0 ± 0.134	0.002	0.002	N/A
Quality of transferred embryos	2.952 ± 0.335	3.418 ± 0.555	0.000	0.000	N/A
Pregnancy rate (%)	19.4	36.7	N/A	N/A	0.022

N/A: Not Applicable. Statistical significance: *p*-value < 0.05.

Table 4. — *Subgroup analysis of clinical and laboratory results for age ≥ 36 years.*

	Control group (n=44)	hCG group (n=87)	*t*-test *p*-value	Mann Whitney U *p*-value	Fisher's exact test *p*-value
Age (years)	37.72	40.04	0.000	0.000	N/A
Previous failed attempts	0.909 ± 0.137	1.759 ± 1.525	0.001	0.001	N/A
FSH (mIU/ml)	6.83 ± 2.03	8.76 ± 3.22	0.000	0.001	N/A
Total rFSH dose (IU)	2730.68 ± 1081.35	2593.39 ± 878.37	0.437	0.652	N/A
Duration of stimulation (days)	9.59 ± 1.54	9.51 ± 1.75	0.814	0.909	N/A
Peak E_2 levels (pg/ml)	1847.77 ± 901.14	1721.12 ± 1342.65	0.574	0.128	N/A
Number of oocytes retrieved	8.15 ± 2.71	5.83 ± 2.61	0.000	0.000	N/A
Number of mature oocytes	7.15 ± 2.65	5.83 ± 2.25	0.000	0.000	N/A
Fertilization rate (%)	88.9 ± 0.101	83.1 ± 0.127	0.006	0.008	N/A
Quality of transferred embryos	2.955 ± 0.371	3.425 ± 0.563	0.000	0.000	N/A
Pregnancy rate (%)	11.4	37.9	N/A	N/A	0.002

N/A: not applicable. Statistical significance: *p*-value < 0.05.

ulation syndrome as a result of massive ovarian stimulation, as well as premature luteinization shifting endometrium out of phase for embryo implantation, challenged popularity of "quantity" over "quality". Instead, mild stimulation and even modified natural cycle protocols emerged as alternative approaches. The addition of regimens in COS that could improve follicular milieu and/or endometrial receptivity was proposed. Among them, hCG has shown a beneficial effect, although large-scale well designed prospective randomized studies are still lacking.

hCG has been recently considered "the wonder of today's science" [10]. Actually, it is the most acid protein in humans, the most sialylated and the most glycosylated glycoprotein and has the longest circulating half-life of 36 hours [10]. hCG provides LH activity as it acts through the same receptor with LH, LH/CG receptor. However, substantial differences in the action of these two hormones seen contribute to hCG's well-favored profile as opposed to LH [12].

The LH/CG receptor has been found to be ubiquitously distributed in reproductive organs. It is mainly located in gonads, ovary, and testis. Hence, it can be found in extragonadal reproductive organs, such as the uterus and the fallopian tubes [10, 14].

The favorable profile of hCG along with the widespread distribution of LH/CG receptor, especially in the female genital tract, suggest that the addition of hCG in the stimulation protocol may confer more extensive actions than previously thought. Among them, potential improvement of endometrial receptivity should be highlighted.

In the present study, women in the hCG group had statistically significant better quality embryos compared to the control group. These findings seemingly contrast other ovarian stimulation parameters, such as peak E2 levels, number of oocytes retrieved, number of mature oocytes, and fertilization rates, which were in favor of the control group. This contrast is reinforced taking into consideration that women in hCG group were statistically significant older, and with higher basal FSH levels compared to control group, due to the Centre's trend to enrich COS with low-dose hCG throughout the follicular phase in women thought to be candidates for poor ovarian response.

Herein, "quantity-to-quality" shift explanation may underlie. Contrary to the long-lasting unecdotal belief that good IVF results are associated with the number of oocytes retrieved, better quality embryos produced in hCG group in the present study are probably indirectly in line with a recent observation, that low response to ovarian stimulation is not apparently related to impaired oocyte quality, and thus embryo quality [15]. However, increased pregnancy rates found in hCG group in the present study contradicts findings by Nichi *et al.*, who reported that embryos derived from poor responder oocytes showed impaired implantation potential [15]. Nevertheless, hCG-mediated LH activity, which may enhance implantation capacity, was absent in that study. Furthermore, as the authors commented, the lower number of available embryos in the poor responder group may have compromised selection for transfer, thus influencing pregnancy rates.

In the present study, women in the hCG group had also statistically significant higher pregnancy rates compared to the control group. The difference in pregnancy rates between control and the hCG group remained significant, when analysis included subgroups based on woman's age. This may be ascribed to better embryo quality, as already discussed. Nevertheless, a potential favorable effect of hCG in the implantation process cannot be ignored.

Basically, apart from its well known favorable effect on corpus luteum steroidogenesis, promoting progesterone production, hCG has several distinct properties, which interfere with implantation and early pregnancy development [10, 16-36].

From an immunologic point of view, hCG has been found to promote immunotolerance in the maternal-fetal surface facilitating implantation [37-43] and also to lessen myometrial contractions, ensuring the establishment of pregnancy [44-47]. Besides increasing production of hCG by the trophoblastic tissue soon after implantation, this hormone is also produced by the blastocyst and may contribute in a paracrine manner to the implantation process [48]. Actually, it represents the first known human embryo–derived signal in maternal-fetal communication, through which the embryo influences the immunologic tolerance and angiogenesis at the maternal-fetal interface [49-50].

It has been speculated that hCG may induce endometrial receptivity, by improving the quality and adequacy of the fibroblast layer. It is probably the interaction of hCG with insulin-like growth factor binding protein-1 (IGFBP-1) and vascular endothelial growth factor (VEGF) that induces angiogenesis and endometrial growth, widening at the same time the implantation window [51].

The potential role of hCG in the implantation process has been pointed out through studies investigating the effect of hCG injected into the endometrial cavity before embryo transfer. Mansour *et al.* reported statistically significant higher pregnancy and implantation rates when injecting 500 IU of hCG as compared with the control group [52]. This was the case in a relevant study of Santibañez *et al.*, who used 500 IU of hCG as well as the study of Zarei *et al.*, who injected 250 mcg of rhCG instead [53, 54]. All these results point towards a beneficial effect of locally applied hCG in the implantation process. Extrapolating these data to systemic application of hCG throughout the follicular phase of COS, a potential direct or indirect beneficial effect in improving endometrial milieu, especially in maternal-fetal interface, cannot be ruled out.

To date, hCG has been used to supplement FSH in COS or even substitute FSH in the late follicular phase. Accumulating evidence suggests that simple increments in the daily dose of rFSH only partially compensate for the

ovaries, which are less sensitive to gonadotropins [55]. Previous studies have shown that regardless of FSH, low-dose hCG can support development and maturation of larger ovarian follicles, whose granulosa cells have acquired LH/CG receptors, rendering hCG a potentially safer and more effective regimen [51]. Besides, androgen production by theca cells as a result of LH activity, hCG has been suggested to increase follicular responsiveness to FSH, implying that granulosa cells resistant to rFSH stimulation might benefit from the addition of low-dose hCG, in terms of increased peak estradiol levels, number of oocytes retrieved, and number of mature oocytes. Unfortunately, this was not the case in the present study, where, on the contrary, these parameters were in favor of the control group. A plausible explanation can be found in the retrospective pattern of the study, according to which hCG group was not consisted exclusively of poor responders, although probably more such patients might have been included in this group due to common trend of the fertility specialists of the Centre to add hCG in patients of poor prognosis to ovarian stimulation.

Reviewing the literature, the addition of hCG at daily doses of 50-200 IU have been applied so far in COS during the early or late follicular phase or throughout the follicular phase [56-59], whereas a single dose of 1,250 IU of hCG have been used in a GnRH-antagonist protocol in combination with aromatase inhibitor in early-follicular phase [60].

A study conducted by the present group showed that the addition of 200 IU of hCG in a short GnRH-agonist protocol with rFSH for the first five days of ovarian stimulation yielded statistically significant higher number of follicles and oocytes and, most importantly, higher implantation and pregnancy rates compared to the addition of 200 IU of LH [12].

A recent randomized controlled dose-response pilot study came across similar results with the present study regarding embryo quality. In that study, a fixed dose of 150 IU/day of rFSH was selected and patients were randomized to receive daily hCG doses of 0, 50, 100 or 150 IU throughout stimulation in a short GnRH-agonist protocol [61]. Peak E2 levels were twice as high after 100-150 IU/day of hCG compared with no hCG administration, although the number of follicles and oocytes retrieved did not differ substantially. However, as in the present study, embryo quality was higher in the hCG group. With regards to pregnancy rates, daily doses of hCG up to 150 IU were compatible with good pregnancy rates, although the design of the study with the small sample size did not allow the detection of differences in pregnancy and live birth rates. Finally, a positive dose-response was seen for pre-ovulatory progesterone, but concentrations remained below values for which an impairment of endometrial receptivity has been previously reported.

Another study which is in line with the present study, assessed the effect of the addition of hCG to rFSH at a dose of 50 or 100 IU/day in a GnRH-antagonist protocol. Interestingly, lower total rFSH dose, fewer oocytes, and fewer embryos but higher implantation and pregnancy rates, were associated with hCG administration [62].

An obvious difference between these studies and the present study is the mean age of women, which did not differ among the study groups, whereas in the present study women in hCG group were significantly older. However, results imply that hCG-mediated LH activity may improve embryo quality.

In conclusion, the addition of hCG to rFSH in a short GnRH-agonist protocol, throughout the follicular phase, had a beneficial effect in terms of pregnancy rates. 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. Among the underlying explanations, hCG interaction with LH/CG receptors developed in granulosa cells of larger antral follicles, which can enhance follicle growth and maturation, as well as hCG properties in improving endometrial environment and subsequently implantation potential should be stressed. In fact, hCG-mediated LH activity sounds quite attractive due to its long acting profile, which can provide more prolonged and stable stimulation of LH/CG receptors compared to other means of LH activity.

Limitations of the present study include its retrospective design and the difference in the age among women, who constituted the hCG (older) and the control group (younger), although the latter may even reinforce findings of the study. Nevertheless, larger-scale prospective randomized studies in stratified age groups are welcome in order to clarify the role of hCG in contemporary ovarian stimulation protocols, given the wide availability and the low cost of this regimen.

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