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Research Article

Evaluate the Efficacy of Double Dose rhCG Comparing to uhCG Plus rhCG for Ovulation Induction in Term of Embryo Quality

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Abstract

Background: Early technology in recombinant hCG (r-hCG) production initially focused on manufacturing biological molecules in bacterial cells, typically *Escherichia coli*. Subsequently, these molecules were produced by introducing genetic material into non-human cell lines (Chinese hamster ovary cells) capable of replicating identical amino acid sequences to the human compound. Recombinant hCG (rhCG) has recently emerged as an alternative to urinary hCG for the final maturation of oocytes in women undergoing IVF and ICSI treatments. rhCG is derived from genetically engineered Chinese hamster ovary cells using recombinant DNA technology.

It is evident these days that recombinant hCG (r-hCG) is well tolerated and offers remarkable consistency from batch to batch, high purity, and high specific activity. However, there is no consensus regarding the dose and the form of HCG that should be utilized.

Design and Methods: This is a retrospective, single-center study conducted at the British-Syrian IVF and Fetal Medicine Centre, AL Rasheed Hospital, Syria.

The study initially included 356 women who underwent the ICSI procedure with the GnRH Antagonist protocol due to various subfertility factors. The women were divided into 2 groups: Group 1, who received 500 mcg (Ovitrelle) of rhCG as an oocyte maturation trigger, and group 2, who received a combination of 250 mcg rhCG (Ovitrelle) plus 5000 IU uhCG (Hucog).

No special considerations were taken for distributing the two groups regarding the administration of ovulation-triggering drugs. However, the availability of these drugs in the Syrian market during that period was the controlling factor, particularly considering the Syrian sanctions.

The exclusion criteria included couples with azoospermia and severe male factor, high BMI (> 30), previous poor responders, endometriosis, and women with a history of ovarian hyperstimulation syndrome.

Results: Results showed that the differences between the two groups favored group 1 in terms of the number of fertilized oocytes, the number of embryos, and the quality of the embryos, i.e. Top quality embryos (TQE).

Conclusion: Regardless of cost-effectiveness, we demonstrated that rhCG at a dose of 500 mcg is more effective than a combination of uhCG at 5000 IU with rhCG at 250 mcg when used for final oocyte maturation in ICSI cycles. The number of top-quality embryos was higher in Group 1, which used only rhCG at a dose of 500 mcg.

Keywords: *Ovulation Triggering; Recombinant hCG; Urinary hCG; Embryo Quality; Grading*

Introduction

The assisted reproductive medicine has progressed at an astounding pace over the past three decades as new techniques have been developed, medications, testing, and strategies to treat infertile couples. Now, many previously sterile couples can conceive, carry, and deliver healthy children of their own. Although the first baby born after *in vitro* fertilization, without stimulated factors and in natural cycle [1]. It was soon accepted that the role of IVF, could be better served through multifollicular development, achieved with gonadotropins. The GnRH is produced in a pulsatile manner by hypothalamic neurons in the area of the arcuate nucleus and controls pituitary-ovarian function. The main problem with gonadotropin stimulation is premature LH surge because of the positive feedback signal from estradiol to the pituitary gland, consequences are premature ovulation, a reduction in oocyte and embryo quality, and thus a reduced pregnancy rate, therefore, there was a need for further pharmacological research to develop a drug inhibiting a premature rise in LH [2].

The aforementioned premature LH surge side effects in addition to ovarian hyperstimulation syndrome justified the move away from the standard GNRH agonist long protocol to GNRH antagonist protocol [3].

Due to the considerable structural similarities between hCG and human (h) LH, and hence both hormones stimulate the same receptors, for years hCG had been used for final oocyte maturity and triggering of ovulation [4]. The hCG major function is to maintain the progesterone production of corpus luteum during early pregnancy [5] and Independently of FSH, low dose hCG can support development and maturation of larger ovarian follicles that have acquired granulosa cells LH/hCG receptors, potentially providing effective and safer ovulation induction regimens. hCG seems to be capable of improving uterine receptivity by enhancing endometrial quality and stromal fibroblast function. Furthermore, through its actions on insulin-like growth factor binding protein-1 and vascular endothelial growth factor, hCG might stimulate endometrial angiogenesis and growth and extend the implantation window, thus making pregnancy more likely [5].

For more than 25 years, hCG extracted from urine from pregnant women has provided a therapeutic analog for LH. Urinary preparations, however, are associated with several disadvantages, including an uncontrolled source, lack of purity, and batch-to-batch variation in activity leading to variable clinical results [6].

Recombinant hCG (rhCG) was recently introduced as an alternative to urinary hCG for the final maturation of oocytes in women treated with IVF and ICSI. rhCG is derived from genetically engineered Chinese hamster ovary cells through recombinant DNA technology. This product has a high purity that facilitates characterization and quantitation by phsycochemical means, reducing the need for animal bioassays [7,8].

Aim of the Study

This retrospective study aimed to compare the efficacy of using urinary hCG with recombinant hCG versus recombinant hCG for ovulation triggering and final oocyte particularly focusing on embryo quality in a group of women who underwent the ICSI process.

Methods and Statics

We used a fixed antagonist protocol, started the stimulation in early follicular phase, human gonadotropins was given in (225 - 300 IU) daily, with (Menogon®, Ferring Pharma) ovarian response was monitored with vaginal ultrasound, for LH antagonist (Cetrotide®, Merck, Germany) 0.25 SC daily from the 6th day of stimulation was given.

The duration of stimulation varies from 9 to 15 day as maximum. Monitoring was performed with vaginal ultrasound, when 2 follicles or more reach 18 mm trigger was given, with at least 10 days of stimulation guided by estradiol level (E2).

Transvaginal ultrasound-guided oocyte retrieval was scheduled within 34 - 36h after the hCG injection. The needle used in oocyte retrieval was done with 16-gauge needle. MII oocytes were identified by the presence of a polar body. ICSI was performed on MII oocytes only. The oocytes checked after 13 hr to check the fertilization forming, when two pronuclei were visible fertilization confirmed.

As each center have its own scoring system, we perform a scoring based on morphological evaluation on day 3 after oocytes retrieval, we evaluate the grade of fragmentation, cytoplasmic appearance, and number of blastomeres per embryo, variation in the zona pellucida thickness, presence of multinucleated blastomeres, location of fragments and size of blastomeres in relation to each other [9].

Embryo quality has been reported to correlate with oocyte and zygote morphology, e.g. appearance of the cytoplasm, pronuclei and polar bodies [10]. Recent reports have suggested that an examination of the morphology of human zygotes can predict the outcome of IVF [11-14].

The study sample

The study sample consisted of 356 patients, divided into two groups, The first group was treated with medication (OVITRELLE®, Serono Labs, Germany) in dose of 500 mcg (Group 1), it represented 33.1% of the study sample. The second group was treated using combination of (OVITRELLE®, Serono Labs, Germany) in single dose 250 mcg plus urinary (HUCOG®, Bharat Serums and Vaccines Ltd, India) with 5000 iu, (Group 2) their percentage reached 66.9% of the study sample. Both groups aged between 17 and 45 years.

Statistical treatment used

Our researchers used the following statistical methods, based on the statistical package for social sciences (SPSS) package for the social sciences statistical version 25 for data analysis:

- **Descriptive statistics:** To know the arithmetic mean and standard deviation of the quantitative variables included in the tests, in addition to the percentages and frequencies of the qualitative variables.
- **Normal distribution test:** Using Kolmogorov-Smirnov test when the sample size is greater than 50 in order to find out which tests are best suited for study.
- **Difference test:** Using Mann-Whitney nonparametric variant of the test independent t-test to study the difference between the two study groups. (The difference was tested using a test independent t-test Its results matched the results of the test Mann-Whitney so far).
- **Correlation study:** Using Spearman test to study the strength and direction of the relationship between the study variables.

Descriptive statistics

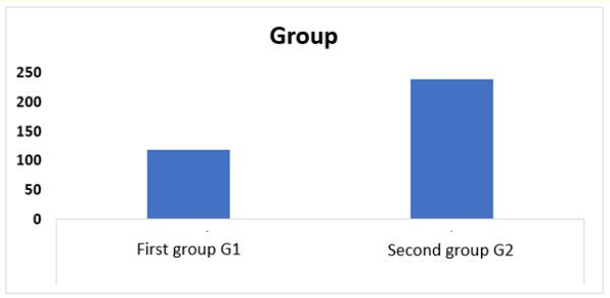
The following table shows basic information about the sample and the data collected to identify the elements of the study sample as follows.

Variable	Category	Number of cases	The ratio %
Groups	First group G1	118	33.1
	second group G2	238	66.9
Cause of infertility	Unexplained	56	16.0
	Pgd	22	6.3
	Male factor	148	42.3
	Low ovarian reserve	66	18.9
	Tubal factor	12	3.4
	Anovulation	10	2.9
	Age over 35 years	36	10.3
Ovulation trigger	Ovitrelle + hucog	238	66.9
	Ovitrelle	118	33.1

Table 1: *Description of the study sample for categorical variables.*

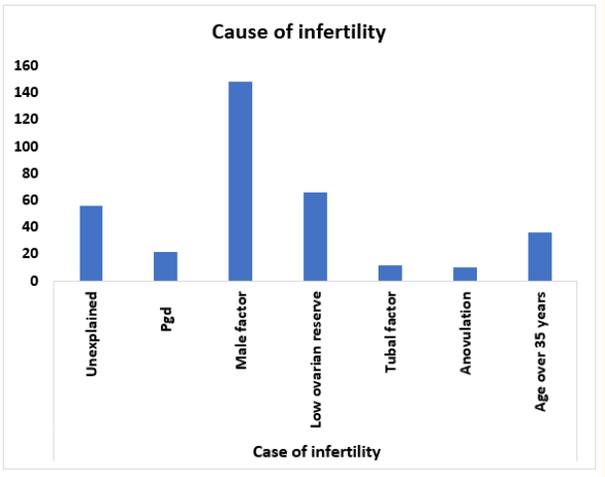
According to the results, 66.9% of the study participants were female patients from the second group who received treatment with Ovitrelle + Hucog. The causes of infertility varied, but the highest percentage (42.3%) was attributed to male factor.

The following graphs show the distribution of the study sample by group, cause of infertility, and type of medication used to stimulate ovulation.

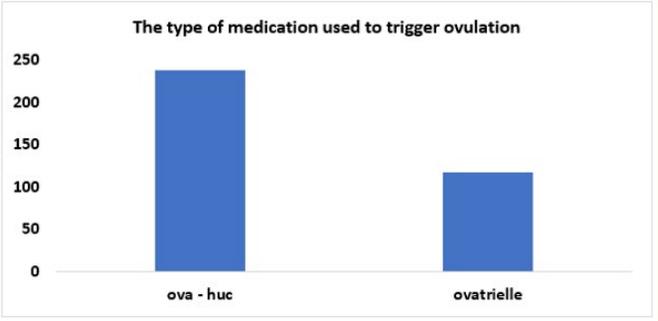


Graph 1: *Sample distribution according to the group.*

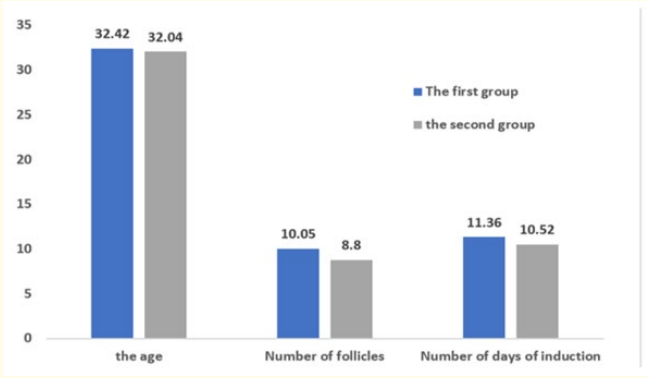
The following graphs are scatterplots to study the relationship between embryo quality G4, age, number of follicles, number of days of induction, number of retrieved oocytes, number of mature oocytes, number of fertilized oocytes, number of embryos, and embryo quality G.



Graph 2: *Distribution of the sample according to the cause of infertility.*



Graph 3: *Distribution of the sample according to group and type of medication used in triggering of ovulation.*



Graph 4: *The average of women age, number of follicles, days of stimulation.*

The following graphic table shows a description of the quantitative variables for the study sample.

Variable	Number of cases	Minimum	The highest rate	SMA	Standard deviation
The age	356	17	45	32.17	6.462
Number of follicles	354	1	20	9.21	4.356
Number of days of induction	354	9	15	10.8	1.12
Number of oocytes retrieved	356	1	40	9.57	6.865
Number of mature oocytes	356	1	35	7.03	5.226
Number of fertilized oocytes	356	0	20	4.79	3.956
Number of embryos	356	0	15	3.74	3.105
Embryo quality g1	356	0	0	0.00	0.00
Embryo quality g2	356	0	3	0.16	0.453
Embryo quality g3	356	0	5	1.04	1.06
Embryo quality g4	356	0	5	1.48	1.281

Table 2: *Description of the study sample for quantitative variable.*

The age of the study sample ranged between 17 and 45 years, with an average of 32 years. The number of follicles ranged between 1 and 20 follicles, with an average of 9 follicles. The number of days of induction ranged between 9 and 15 days, with an average of 10 days, and the number of oocytes retrieved ranged between 1 and 40 oocyte, whoever worth to mentioned that every case retrieved over 18 oocytes where ended up in frozen embryos. An average of 9 oocytes, and the number of mature oocytes ranged between 1 and 35 mature oocytes, with an average of 7 mature oocytes. The number of fertilized oocytes ranged between 0 and 20 fertilized oocytes, with an average of 4 fertilized oocytes. The number of embryos ranged between 0 and 15 embryos, with an average of 3 embryos. It also ranged Embryo quality g2 between 0 and 3, with an average of 0.16, and embryo quality grade3 It ranged between 0 and 5, with an average of 1.04, and the quality of the embryo also varied g4 between 0 and 5, with an average of 1.48.

Data normality

The quantitative variables of the study were tested using Kolmogorov-Smirnov to investigate their distribution, and all variables are not normally distributed as their P-value is smaller than 0.05, but since the sample size is large (356), mediation tests can be used by the Central Limit Theorem (CLT). The CLT states that as the sample size increases, the distribution of sample means becomes more normal, regardless of the Patients distribution. Typically, a sample size of 30 or more is sufficient to meet the requirements of the CLT.

Studying the relationships between study variables

In this section, the relationship between quantitative variables will be studied, and Spearman test will be used When studying the relationship between a quantitative variable and a qualitative variable consisting of only two categories, Mann-Whitney test will be used to study the difference between the level of the qualitative variable in the quantitative variables studied.

Study the relationship between the two groups and study variable

In this section, the difference between the two groups in the study variables will be studied using Mann-Whitney Nonparametric test knowing that an independent t-test can be used because the sample size (356) is large according to the central limit theorem, the results of which are largely consistent with the results of the non-parametric test. The Central Limit Theorem (CLT) suggests that larger sample sizes lead to more normally distributed sample means, regardless of the Patients distribution. A sample size of 30 or more is usually sufficient for the CLT.

Variables	The first group		The second group		P-value
	Number of cases	Mean ± Sd	Number of cases	Mean ± Sd	
the age	118	32.42 ± 6.374	238	32.04 ± 6.528	0.935
Number of follicles	116	10.05 ± 4.244	238	8.80 ± 4.368	0.058
Number of days of induction	116	11.36 ± 0.986	238	10.52 ± 1.080	< 0.001*
Number of eggs retrieved	118	10.83 ± 6.934	238	8.94 ± 6.773	0.050
Number of mature eggs	118	7.64 ± 4.802	238	6.73 ± 5.418	0.123
Number of fertilized eggs	118	5.59 ± 3.620	238	4.39 ± 4.067	0.008*
Number of embryos	118	5.56 ± 3.631	238	2.83 ± 2.345	< 0.001*
Embryo quality g1	118	0 ± 0	238	0 ± 0	1.00
Embryo quality g2	118	0 ± 0	238	0.24 ± 0.536	< 0.001*
Embryo quality g3	118	1 ± 1.017	238	1.06 ± 1.084	0.787
Embryo quality g4	118	1.88 ± 1.205	238	1.29 ± 1.277	0.002*
*Significant at the 0.05 level.					

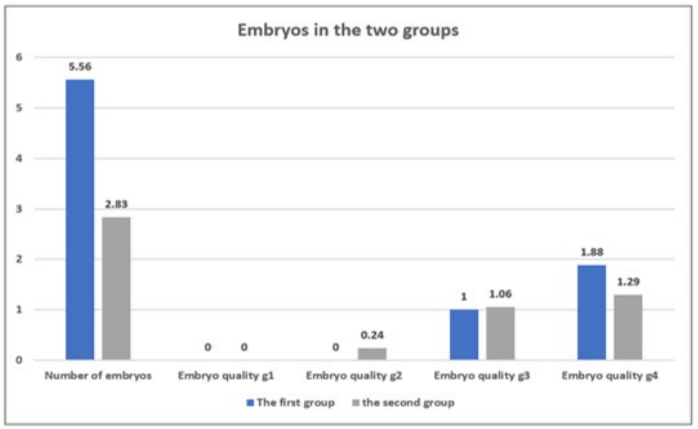
Table 3: *Results of studying the difference between the two groups in the study variables.*

The analysis of the previous table, in comparison two groups we recognized that the test's significance exceeds that 0.05 threshold. This implies a lack of statistically significant differences in variables such as age, number of follicles, oocytes retrieved, mature oocytes, embryo qualities g1and g3, with a 95% confidence level.

However, when examining the differences between the groups, the test's significant falls below 0.05. This suggests genuine statistically significant variation in days of induction and embryo quality G2 and G4, with a 95% confidence level.

Upon examining average value, the first group demonstrates advantages in days of induction, number of fertilized oocytes, total embryo, and the quality of embryo G4, significantly surpassing the second group. Conversely, the second group exhibits a notably higher quality of embryo G2 compared to the first group.

This is illustrated in the following graphs.



Graph 5

Study the relationship between quantitative variables

In this section, we examine the relationship between embryo quality (grade 4) and quantitative variables using the Spearman test. The table below presents the test results.

The results of the previous table showed:

- The test significance of the relationship between embryo quality g4 and age is smaller than the significance level of 0.05, with 95% confidence, there is a real, statistically significant relationship between embryo quality g4 and age.
- The test significance of the relationship between embryo quality g4 and the number of follicles is smaller than the significance level of 0.05, with 95% confidence, there is a real, statistically significant relationship between embryo quality g4 and the number of follicles.
- The test significance of the relationship between embryo quality g4 and the number of days of induction is smaller than the significance level of 0.05, with 95% confidence, there is a real, statistically significant relationship between embryo quality g4 and the number of days of induction.
- The test significance of the relationship between embryo quality g4 and the number of eggs retrieved is smaller than the significance level of 0.05, with 95% confidence, there is a real, statistically significant relationship between embryo quality g4 and the number of eggs retrieved.
- The test significance of the relationship between embryo quality g4 and the number of mature eggs is smaller than the significance level of 0.05, with 95% confidence, there is a real, statistically significant relationship between embryo quality g4 and the number of mature eggs.
- The test significance of the relationship between embryo quality g4 and the number of fertilized eggs is smaller than the significance level of 0.05, with 95% confidence, there is a real, statistically significant relationship between embryo quality g4 and the number of fertilized eggs.

- The test significance of the relationship between embryo quality g4 and the number of embryos is smaller than the significance level of 0.05, with 95% confidence, there is a real, statistically significant relationship between Embryo quality g4 and the number of embryos.

Discussion

One of the most important predictive factors for the success of ICSI is the number and quality of oocytes and embryos available for fertilization and transfer. For this reason, researchers are investigating the best way of obtaining the highest number of mature oocytes in *in vivo* stimulation. For final oocyte maturation and development of other periovulatory events such as softening of the connective tissue of follicle for easy detachment of oocyte cumulus complex from the follicle wall, the physiological LH surge is essential, and this is brought about in ICSI cycles by hCG [15].

Although the use of uhCG significantly reduces the cost of hCG injections by ¾ (considering that the cost of rhCG is four times that of uhCG), these results should be taken into consideration when deciding which hCG option to choose [16]. Our study aimed to evaluate the efficacy of rhCG in a double dose of 500 mcg (Ovitrelle) compared to a mixture of 5000 IU of uhCG (HUCOG) given with 250 mcg of rhCG (Ovitrelle) for ovulation triggering, particularly concerning embryo quality. In the context of this study, we conducted analyses on several variables to attain our results.Top of Form

We found that the fertilization rate was higher in group 1, there was a significant difference in embryo quality which also was higher in group 1.

Many other research have studied different relationships between the type of triggers, there doses and the efficacies. Efhtkhar., *et al*. have studied the efficacy of recombinant versus urinary hcg in ART outcome they demonstrate that the injection of 250 µg of rhCG (Ovitrelle) is equal to an intramuscular injection of 10000 IU of uhCG in inducing final follicular maturation in ICSI cycles [17].

In other prospective, randomized, controlled, double-blind, double-dummy comparison of recombinant and urinary hcg for inducing oocyte maturation and follicular luteinization in ovarian stimulation, the study primary endpoint was comparison of the number of oocytes retrieved per patient receiving either compound. Secondary comparisons included the number of oocytes retrieved per follicles aspirated, the number of mature oocytes, normally fertilized oocytes, and cleaved embryo. There was no significant difference in embryo cleavage between the two groups of study [18].

In contrast to the previous study, a clinical trial comparing two doses of rhCG (Ovidrel^{R}) to urinary hCG (Profasi^{R}) for induction of final follicular maturation in *in vitro* fertilization-embryo transfer, they found that the number of 2PN fertilized oocytes on day 1 after oocyte retrieval, and the number of 2PN or cleaved embryos on the day of embryo transfer, were significantly higher in patients receiving 500 µg of rhCG than in those receiving the lower dose. However, the number of 2PN fertilized oocytes on day 1, 2PN, or cleaved embryos on the day of transfer, as well as implantation rate and mean serum P levels were not different between patients receiving 250 µg of rhCG and 10,000 U USP of urinary hCG [7]. Unfortunately, no existed researches compare the combination of uhCG in stander dose (5000) with lower dose of rhCG 250 mcg to the double dose of rhCG.

In another prospective randomized study comparing the standard dose of rhCG (250 mcg) with double dose (500 mcg) for final follicular maturation, researchers found that there is no difference in oocytes retrieved, mature oocytes, fertilization rate, and the number of produced embryos between the two groups. However, on the other hand, the double dose of rhCG for final follicular maturation in IVF cycles resulted in an improvement in the mean number of oocytes [19].

Our data show that women who receive rhCG in double dose (500 mcg) for ovulation triggering have a higher number of fertilized oocytes, a higher number of embryos, and a higher number of top-quality embryos compared to the women who receive the combination of rhCG and uhCG, taking into consideration that we didn't mention the pregnancy rate in our study.

Conclusion

Regardless of the cost effect, we found that there is no additional benefit of the combination uhCG and rhCG, as the data showed that the group of women who take rhCG in dose of 500 mcg have higher number of fertilized oocytes, embryo and top quality embryo. Further and more studies may be needed to clarify clinical correlation.

Limitations of the Study

This research was carried out in a single centre, and the participants were not randomized, which is a potential limitation of this research. In addition, this research only emphasized the advantages of rhCG versus rhCG and uhCG alone and did not compare the side effects, which hinders the generalizability of research outcomes.

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