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Recombinant FSH Versus HP-HMG for Controled Ovarian Stimulation in Intracitoplasmic Sperm Injection Cycles

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The aim of this study was to make a conclusion about aplicability of two differnet gonadothropins in COS (rFSH versus HP-hMG). The primary conclusion for the success as a result of COS are the mean number of retrived oocytes, mature oocytes, fertilization rate, mean number of quality embrios, and criopreverzed embrios. The secondary conclusions were clinical pregnancy rate and delivery rates. **Methods:** The study was a retrospective case–control study,. A total of 1238 fresh, non donor, IVF cycles with COS were analyzed, but to minimize the bias, only the first cycle for each patient below 40 yaears old, in that period was analyzed. This selection composed the group of respondents that was analyzed which in total amounted to 760 patients.(rFSH = 422, HP-hMG = 338). The patients underwent COS by long luteal protocol using two differnt inducers of COS (rFSH and HP-hMG). **Results:** The average starting dose of rFSH used was significantely lower (152.7±41.1IU), whereas with HMG it was (228.8±68.7 IU, p=000000). The average number of IU gonadothropin used in therapy, statistically highly is significantly lower when r- FSH is used as an inducer. (1639.2 ± 476.9 IU, rFSH vs 2356.4 ± 955.1IU, HP–hMG, p <0.001). We received significantly higher average number of oocytes and mature oocytes in the group of r–FSH (oocytes; rFSH v HP-hMG–11.8 ± 7.1 v 10.7 ± 6.5, p = 0.028 ; mature oocytes: rFSH v HP-hMG 9.9 ± 6.2 v8.7 ± 5.5 p = 0.009). However, we did not find a significant difference in the use of the COS inductors regarding the clinical pregnancy rate (rFSH v HP-hMG 49.5% vs 48.9% p=0.92) and delivery rate (rFSH vs HP-hMG 42.9% vs 43.4% p=0.96). **Conclusions:** Our study showed that rFSH is more powerful and more applicable in individualized dosing then HP-hMG and brings better results from COS (more oocytes, more matured oocytes). **Key words:** Controled ovarian stimulation (KOS), rekominant folikulostimulative hormon (rFSH), high purity human menopausal gonadothropin (HP-hMG), intracitoplasmic sperm injection (ICSI), retrived oocytes.

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1. INTRODUCTION

The success of the in vitro fertilization (IVF) depends directly on the optimization of ovarian stimulation with a result of obtaining oocytes and embryos of excellent quality in the IVF process which would later be utilized for in vitro insemination. In regards to the COS inducers, there is still no clear distinction or advantage about the result of COS in terms of their applicability. The question of the dominance of recombinant FSH as most new medications over other forms of gonadothropins is not yet defined. There are studies that highlight the benefits of r-FSH, as well as many other studies which rebut it. Smaller studies give advantage to one over the other inducer, while large multicentre studies show little nuances of advantage that make all kinds of inducers applicable in the process of COS (1, 2, 3, 4, 5).

2. MATERIAL AND METHODS

The study was a retrospective case–control study, of all patients undergoing IVF from 2008 - 2010 in to the IVF Centre - Remedika. A total of 1238 fresh, non donor, IVF cycles with COS were analyzed, but to minimize the bias, only the first cycle for each patient in that period was analyzed.

In order to avoid the influence of certain factors that may influence the results, subsequent selection was made with the following inclusion criteria: Normal menstrual cycle 23 to 35 days, normal values of basal gonadothropins measured on the third day of the cycle, body mass index–greater than 18 and less than 30 kg/m2, age of female below 40 years. Criteria that excluded from the group: clinically relevant systemic and/or endocrine conditions, high basal levels of FSH measured on the third day of the cycle (above 25 uu/L).

This selection composed the group of respondents that was analyzed which in total amounted to 760 patients. The

parameters were obtained from the medical documentation of each patient. The study was approved by the Ethics committee of the institution.

2.1. Treatment

The patients underwent COS by one standard protocol: long luteal protocol with a gonadotroping releasing hormone agonist (GnRh - agonist - buserelin acetate, Suprefact ®, Aventis Pharma). The third day of the spontaneous or deprivationed bleeding patients started with injectible FSH recombinant gonadotropin, beta folitropin (Puregon ® NVOrganon), or HP-hMG, menotropin at a differnt dose depending on the patient's age and number of preantral follicles. Criteria for application of human chorion gonadotropin (hCG, Pregnyl ®) as a trigger of maturation of oocites were at least two follicles larger than 18 mm, mean diameter. Transvaginal ultrasound guided oocyte retrievalwas performed 32 - 36 h after hCG injection in a short intravenous anaesthesia. In all oocytes obtained, or in 100% of the cases, the process of fertilization was realized with the method of intracytoplasmic sperm insemination (ICSI), without considering the quality of seed. The transfer of embryos was performed on the second, third or fifth day of development of embryos. With all patients progesterone supplementation was given with (Utrogestan ®). Pregnancy tests were done on the 14th day after ET. Two weeks after the positive test was detected, there was a vaginal ultrasound examination for detection of clinical pregnancy.

2.2. Analysis

Statistical analysis made in the program SPSS 13,0 for Windows. Descriptive measures (mean and SD) were used to display continuous variables. The absolute and relative numbers are shown as frequencies of categorical variables. In order to test the significance of the differences between continuous variables with symmetric distribution the t-test was used for independent samples, and for asymmetric distribution the Mann-Whitney U test was used. The significance of differences in the frequency distribution of categorical variables was performed with Chi-square test, Yates Chi-square and Kolmogorov-Smirnov test. p <0.05 was considered as significant for all values. The primary conclusion for the success as a result of COS are the mean number of oocytes, mature oocytes, fertilization rate, mean number of embrios, quality embrios, and mean number of criopreverzed embrios. The secondary conclusions were clinical pregnancy rate and delivery rates. Definitions of terms: pregnancy, biochemical pregnancy, clinical pregnancy, are defined by the revised terminology dictionary for terms of assisted reproduction prepared by the International Committee for Monitoring assisted reproduction technologies (ICMART) and the World Health Organization (WHO) (6).

variable	Gonadothropins		p-level
	rFSH	HP-hMG	
Number of patients	422(55.5%)	338(44.5)	
BMI ± SD,(kg/m2)	24.3±3.9	24.8±4.4	0.12*
age ± SD,(yaears)	31.8±4.3	32.0±4.2	0.58*
Tobaco consumption			
Non – smokers	261(61.8%)	200(59.2%)	0.013**
smokers	150(35.5%	114(33.7%)	
without data	11(2.7%)	24(7.1%)	
Primary cause of infertility			
tubal	74(17.5%)	63(18.6%)	0.14***
endometriosis	11(2.6%)	6(1.8%)	
male	140(33.2%)	134(39.6%)	
female disfunctional	22(5.2%)	10(2.9%)	
premature ovarian failure–relative	8(1.9%)	10(2.9%)	
Unexplained	105(24.9%)	82(24.4%)	
Multifactorial	62(14.7%)	33(9.8%)	
Infertility type			
primary	361(85.5%)	288(85.2%)	0.98
secondary	61(14.6%)	50(14.8%)	
Hormonal basal profile before COS			
2	40.3±30.7	40.9±28.5	0.8*
FSH	7.8±3.2	7.4±2.7	0.1*
LH	5.8±3.0	5.9±4.9	0.62*
Basal Ultrasound before start of COS – number of preantral follicles			
until 5 follicles	70(16.6%)	61(18.1%)	0.81**
5 – 10 follicles	325(77.0%)	258(76.3%)	
> 10 follicles	27(6.4%)	19(5.6%)	
Duration of infertility (years)	6.3±3.6	6.1±3.9	0.48*
Number of previouse IVF attempt	Rang 1-9	Rang 1-5	0.84****

Table 1. Demographic and baseline characteristic of patients in the study p*(t-test for independent samples) p**(Pearson Chi-square) p***(Yates Chi-square) p****Mann-Whitney U

3. RESULTS

There were 422 (55.5%) patients that participated in the survey, with whom as an inductor for controlled ovarian stimulation recombinant gonadotropins (rFSH) was used and 338 (44.5%) patients with inducers of high purity gonadothropins (uHMG). These two groups of patients insignificantly (p> 0.05) differ in age and BMI, type of primary or secondary infertility, the average basal values of E2, FSH and LH, the number of preantral follicles, years of infertility and number of previous IVF (Table 1) .

The average number of I.U. gonadotropin spent in therapy, statistically highly significant (1639.2±476.9 rFSH versus 2356.4±955.1 HP-hMG, p <0.001) is greater when HP-hMG was used (Table 2). Controlled ovarian stimulation (COS) expressed in days, lasted significantly longer at high induction with r-FSH. Average starting dose of rFSH significantly is lower (152.7±41.1rFSH verus 228.8±68.7HP-hMG p <0.001), than that of HP-hMG (Table 2). In terms of average values of oestradiol E2, during the first control they are significantly higher in the group of patients where as inducer HP-hMG was used, (279.9±247.1rFSH versus 321.9±334.4 HP-hMG) but are slightly different on the day of HCG. At the last day of stimulation, the number of follicles of 18

mm and more, and thickness of the endometrium in mm are insignificantly different in both groups (Table 2).

Variable	Type of gonadotrophin		p-level
	rFSH	HP-hMG	
Mean total days of tretman per cycle (days)	10.4±1.9	10.1±2.1	0.009*
Mean total dose of gonadothropin per cycle (IU)	1639.2±476.9	2356.4±955.1	0.0000*
Mean starting total dose per cycle (IU)	152.7±41.1	228.8±68.7	0.00000*
Mean E2 level 6 –th day of COS	279.9±247.1	321.9±334.4	0.047*
Mean E2 level – day of HCG	1445.4±848.5	1542.3±911.5	0.13*
Mean number of follicles 18 E and moore on HCG day	6.6±4.5	6.5±5.7	0.68*
Mean endomterium in mm on HCG day	9.9±1.7	10.0±2.3	0.33*

Table 2. Clinical parameters during COS p*(t-test fot independent samples).

The group of HP-hMG had a significantly greater number of canceled cycles and cycles without embrio transfer (Table 3). The reason was the poor response of COS as well as the small number of oocytes obtained et HP-hMG group (Table 3). Statistical analysis showed that in the group of recombinant gonadotrophins there was a significantly higher percentages of patients with embryo transfer realized. In this group of respondents there was a highly significantly greater average number of oocytes retrived, quality oocytes, fertilizirated quality oocytes and embryos, while the difference in fertilization rate and implantation rate are insignificantly different.

variable	rFSH	HP-hMG	p
Total cycles started	422	338	
Cycles with embrio trasnfer	410(97.2%)	311(92%)	0.001**
Cancelled cycles	8(1.9%)	9(2.7%)	
Cycles without embrio trasnfer	4(0.9%)	18(5.3%)	
Mean number of retrived oocyte per cycle	11.8±7.1	10.7±6.5	0.028*
Mean number of mature oocytes per cycle	9.9±6.2	8.7±5.5	0.009*
Mean number of fertilizated oocytes per cycle	7.3±4.6	6.4±4.1	0.006*
Fertilization rate	0.76±0.2	0.7±0.2	0.73*
Mean number of frozen embrios	1.01±2.6	0.56±2.01	0,008***
Mean number of transfered embrios	2.6±0.6	2.5±0.7	0.029*
Number of quality embrios transfered	2.2±0.9	2.0±1.0	0.011*
Implantation rate	0.23±0.3	0.25±0.3	0.41*

Table 3. Clinical parameters – oocyte and embryo parameters from retrival to transfer p*(t-test fot independent samples) p**(Pearson Chi-square) p***(Yates Chi-square)

The type of inducer of controlled ovarian stimulation does not significantly affect the outcome of the pregnancy test rate, clinical pregnancy rate, biochemical pregnancy rate, and delivery rate (Table 4).

4. 4. DISCUSION

Controlled ovarian stimulation (COS) is an important part in the process of in vitro fertilization. Its purpose is to obtain a greater number of oocites that would be later selected in the IVF process. Adequate response of the ovary to the COS is important for the ultimate success of the IVF process. Poor ovarian response, as seen in the small number of oocytes and quality oocytes delivered results in suboptimal success of IVF. The debate was started by multiple studies comparing the success of in vitro fertilization of both inducers urinary hMG and r-FSH. A problem in making the final conclusion of applicability regarding the different types of gonadotrophins, represents the small number of patients analyzed in smaller studies and clinical heterogeneity of large multicentre studies. Large studies have various types of protocols of COS analyzed, a different types of fertilization (IVF or ICSI), different doses and types of agonists, and certainly different types of inducers as u–hMG, HP-hMG, u–FSH, r–FSH. (1, 2, 7, 8). In our study, there was only one type of protocol analyzed, with one type of fertilization (ICSI), in all oocytes with two types of gonadotropins (HP- hMG vs r FSH). Different from some prospective studies where starting doses of gonadotropins used in COS are identical with rFSH and with HMG, (1, 7). Our study uses different starting dosages of gonadotropins. The protocol for our patinents has an individual approach for the starting dose of gonadothropins during the COS, respecting two important markers in the COS - the patient's age and number of preantral follicles measured before the start of COS. The analysis showed that the number of average starting dose of rFSH used was significantely lower (152.7±41.1IU), whereas with HMG it was (228.8±68.7 IU) (Table 2). This starting dosage of rFSH is common in clinical practice (9). As to the process of COS, in our study the average number of IU gonadothropin used in therapy, statistically highly is significantly lower when r- FSH is used as an inducer (Table 2). This data is in contrast with the results of certain systematic reviews and meta analyses which did not find differences in the mean compara-

variable	Type of gonadothropin		p-level
	rFSH	HP-hMG	
Number of patients with embrio transfer	N – 410	N – 311	
Pregnancy rate per cycle with embrio transfer	223 (54.4%)	175(56.3%)	0.67***
Biochemical pregnancy rate per cycle with embrio transfer	20(4.9%)	23(7.4%)	0.21***
Clinical pregnancy rate per cycle with embrio transfer	203(49.5%)	152(48.9%)	0.92***
Dellivery rate per cycle with embrio transfer			
Number of intiated cycles N – 422 N – 338			
Clinical pregnancy rate per initated cycle	203/422(48,1%)	152/338(44,9)	0.2*** 0,2 0,41
Number of cycles with retrived oocites	414	329	
Clinical pregnancy rate per retrival cycle	203/414 (49%)	152/329(46,2%)	

Table. 4. Pregnancy outcome p*(t-test fot independent samples) p***(Yates Chi-square) p****Mann-Whitney U

tive dose between rFSH and HP-hMG per IVF cycle (2, 3). It is important to stress that some of the studies the starting and daily dosage of rFSH was higher than the one commonly used in the routine practice (1, 7). That in turn brought greater number of follicles, number of oocytes in the group which used r-FSH as inducer but a smaller number of mature oocytes, fewer top quality embryos without significant impact on ongoing pregnancy rate when the two manners of fertilization are analysed (2, 3, 7). The main differences between the recombinant r-FSH and u-FSH urinary lie in the presence of LH in the latter. The main change is in terms of the level of LH which is necessary in follicular maturation. Raising the level of LH in the follicular phase of HP–hMG leads to atresia of a number of follicles and therefore results in fewer intermediate follicles, and significantly fewer retrieved oocytes (11). On the other hand significantly higher values of E2 a day of giving HCG which are detected in HP–hMG produce a better oocite maturity and receive a significantly higher number of mature oocites and higher average number of higher quality embryos (7, 11, 12). In HP-hMG which is maximally purified by LH peak main activity derived from hCG which is present with about 10 IU/amp (15). The same authors (7, 11, 12) focus on the quantity of HCG in preparations of HP-hMG which is higher than traditional u-hHMG. The point is the longer life of HCG from LH, and on the other hand, HCG has LH activity. This produces better maturity of the oocites (7, 11, 12). On the other hand, we received significantly higher average number of oocytes and mature oocytes in the group of r–FSH (oocytes; rFSH v HP-hMG–11.8 ± 7.1 v 10.7 ± 6.5, p level 0.028 ; mature oocytes: rFSH v HP-hMG 9.9 ± 6.2 v8.7 ± 5.5 p level 0.009). These findings are correlated with data from literature to significantly lower number of oocytes obtained in the group of urinary gonadotropins (7, 8). This finding is confirmed in previous studies (9) and confirm the notion that r -FSH as inducer of COS is a greater advantage of the individualized treatment itself (10). In our study the cancelled cycles and cycles without embryo transfer were significantly more common in the group of HP-hMG (Table 3). We have found a higher average number of fertilized oocytes in the r–FSH group (Table 4). We have had a greater average number of transferred embryos and significantly higher average number of embryos that were frozen (Table 4). This has been confirmed by other studies (2, 3). Certain levels of LH are necessary in cases where endogeniuous levels of LH are too low when rFSH is used. However, adding LH with rFSH in process of COS does still not have relevant evidence from the common practice (14). Studies where rLH is added do not show any significance in favor of achieving pregnancy, but reduced the total dose of gonadotropin in the process of COS. On the other hand, it showed that despite so high values of HCG there was no significance in the appearance of premature luteinisation. (14). Our study is the result of an analysis of everyday practice.This shows that rFSH is more powerful and more applicable in individualized dosing then HMG and brings better results from COS (more oocytes, more matured oocytes) (5). However, we did not find a significant difference in the use of the COS inductors regarding the achievement of pregnancy, clinical pregnancy and childbirth (Table 4). The lack of significance in implantation rate of both inducers raises the question of better understanding the impact of diversity of both inducers. In addition, this raises the need to understand the morphological and functional aspects of the quality of the embryo and its potential for implantation and the receptiveness of the endometrium, as well as the need for certain levels of LH and HCG during the COS, especially in the group of the r-FSH inducers.

It appears that the individual approach to every patient, in terms of selecting the optimization of COS respecting to specific markers (the patient's age, number of preantral follicles, basal values of FSH and AM hormone, situation as endometriosis and PCO) can result in progress in improving the results in everyday practice.

REFERENCES

1. The European and Israeli Study Group on Highly Purified Menotropin versus Recombinant Follicle-Stimulating Hormone. Efficacy and safety of highly purified menotropin versus recombinant follicle-stimulating hormone in in vitro fertilization/intracytoplasmic sperm injection cycles: a randomized, comparative trial. Fertil Steril 2002; 78: 520-528.
2. Coomarasamy A, Afnan M, Cheema D, van der Veen F, Bossuyt PM, van Wely M. Urinary hMG versus recombinant FSH for controlled ovarian hyperstimulation following an agonist long down-regulation protocol in IVF or ICSI treatment: a systematic review and meta-analysis. Hum Reprod, 2008; 23: 310-315.
3. Al-Inany HG, Abou-Setta AM, Aboulghar MA, Mansour RT, Serour GI. Efficacy and safety of human menopausal gonadotrophins versus recombinant FSH: a meta-analysis. Reprod Biomed Online, 2008; 16: 81–88.
4. Platteau P, Smitz J, Albano C, Sørensen P, Arce JC, Devroey P. Exogenous luteinizing hormone activity may influence the treatment outcome in in vitro fertilization but not in intracytoplasmic sperm injection cycles. Fertil Steril, 2004; 81: 1401–1404.
5. Trew GH: Comparing highly purified hMG and rFSH in patientsundergoing IVF. Hum Reprod 2007, 22(6):1797-1798, author reply 1798-1800.
6. Zegers-Hochschild F, Adamson GD, de Mouzon J, et al. on behalf of ICMART and WHO The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, HumanReproduction. (2007) 24(11) :2683–2687.
7. Andersen AN, et al. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial. Human Reproduction 2006 Dec; 21(12):3217-27.
8. Hompes PG, Broekmans FJ, Hoozemans DA, Schats R for the FIRM group. Effectiveness of highly purified human menopausal gonadotropin vs. recombinant follicle-stimulating hormone in first-cycle in vitro fertilization intracytoplasmic sperm injection patients. Fertil Steril 2007. 6: 1206-12.
9. Trew HG, Brown PA, Gillard S, et al. In vitro fertilisation with recombinant follicle stimulating hormone requires less IU usagecompared with highly purified humanmenopausal gonadotrophin: results from a European retrospective observational chart review . Reproductive Biology and Endocrinology, 2010, 8: 137.
10. Popovic-Todorovic B, Loft A, Bredkjæer HE, et al. A prospective randomized clinical trial comparing an individual dose of recombinant FSH based on predictive factors versus a 'standard' dose of 150 IU/day in 'standard' patients undergoing IVF/ICSI treatment, Hum Reprod, 2003; 18: 2275-82.
11. Platteau P, Nyboe Andersen A, Balen A, et al. Similar ovulation rates, but different follicular developmentwith highly purified menotrophin compared with recombinant FSH inWHO group II anovulatory infertility: a randomized controlled study. HumReprod, 2006; 21:1798–1804.
12. Smitz J, Andersen AN, Devroey P, Arce JC. Endocrine profile in serum andfollicular fluid differs after ovarian stimulation with HP-hMG orrecombinant FSH in IVF patients. Hum Reprod 2007; 22: 676–687..
13. Ravhon A, Lavery S, Aurell R, Trew G, Margara R, Winston R. Cilinical experience with recombinant follicle-stimulating hormone (FSH) and urinary FSH: a retrospective case-controlled analysis. Fertil Steril. 2001; 75:92–95. doi: 10.1016/S0015-0282(01)01684-3.
14. Mochtar MMH, van Wely M, van der Veen F. Recombinant luteinizing hormone (rLH) for controlled ovarian hyperstimulation in assisted reproductive cycles. Cochrane Database Syst Rev, 2007; 2007: 1.
15. Wolfenson C, Groisman J, Couto AS, et al. Batch-to-batch consistency of human-derived gonadotrophin preparations compared with recombinant preparations, Reprod Biomed Online, 2005;10: 442–54.