fortune Journals

Research Article

JOURNAL OF WOMENS HEALTH AND DEVELOPMENT

ISSN: 2644-2884

Intrafollicular Endocrine Milieu Hormonal Profile Using Four Different Ovarian Stimulation Protocols: HMG, HMG/hCG, rFSH, rFSH/hCG: A Single-Center Pilot Study

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Abstract

Objectives: The present single-center pilot study investigated for the first time the follicular fluid (FF) hormonal profile derived from four different ovulation induction protocols (HMG, HMG/hCG, rFSH, rFSH/hCG) in relation to the ovulation induction parameters, including number and quality of oocytes, and pregnancy rate.

Methods: FFs were pooled for analyses for each one of the 94 patients that underwent a GnRH antagonist protocol with HMG, HMG/hCG, rFSH, or rFSH/hCG treatment. Intrafollicular hormone concentrations in relation to treatment groups, number and quality of oocytes, and pregnancy rate were measured.

Results: Testosterone, FSH, androstenedione and estradiol levels were significantly higher in HMG/hCG-treated group (697±448 ng/ml, p=0.000, 8.75±2.82 IU/L, p=0.002, 6.90±2.77 ng/ml, p=0.000, and 38696391 ± 170049486 ng/ml, p=0.000, respectively). Progesterone and hCG levels were significantly lower in pregnant women treated with HMG compared to non-pregnant HMG-treated women (16184±3910 ng/ml vs 23338±8880 ng/ml, p=0.054 for progesterone, and 30.62±15.04 mIU/ml vs 63.00±25.61 mIU/ml, p=0.033 for hCG). Patients >34 years old that received HMG tended to have higher pregnancy rate (31.2%), while the rFSH-treated patients seemed to have a lower pregnancy rate (9.1%) (p=0.531).

Conclusions: Ultimately, the present study demonstrates that the different gonadotropin preparations have an impact on the intrafollicular endocrine milieu hormonal profile. HMG/hCG-treated group presents the strongest androgenic environment, revealing that the treatment with HMG creates a strong androgenic environment that is in favor of successful reproductive outcome.

Keywords: Intrafollicular endocrine milieu; Hormonal profiling; HMG; hCG; rFSH

Abbreviations: A: Androstenedione; ART: Assisted reproductive technology; cAMP: Cyclic adenosine 3'-5'-monophosphate; COS: Controlled ovarian stimulation; E2: estradiol; FSH: Follicle-stimulating hormone; hCG: Human chorionic gonadotropin; HMG: Human menopausal gonadotropin; LH: Luteinizing hormone; Prg: Progesterone; rFSH: Recombinant FSH; rhCG: Recombinant hCG; rLH: Recombinant LH; T: Testosterone; uFSH: Urinary FSH; uhCG: Urinary hCG

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Citation: Papamentzelopoulou Myrto-Sotiria, Liokari Emanouela, Mavrogianni Despoina, Dimitroulia Evagelia, Stavros Sofoklis, Potiris Anastasios, Loutradis Dimitris. Intrafollicular Endocrine Milieu Hormonal Profile Using Four Different Ovarian Stimulation Protocols: HMG, HMG/hCG, rFSH, rFSH/hCG: A Single-Center Pilot Study. Journal of Women's Health and Development. 7 (2024): 55-67.

Received: July 29, 2024
Accepted: August 07, 2024
Published: August 16, 2024

Myrto-Sotiria P, et al., J Women's Health Dev 2024
DOI:10.26502/fjwhd.2644-288400121

Introduction

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are responsible for the initial recruitment, growth, selection, and dominance of ovarian follicles as well as for maturation and ovulation, respectively. Both gonadotropins act in synergy and have been used in various forms for hormonal controlled ovarian stimulation (COS) in assisted reproductive technology (ART) to increase follicle count [1-4]. Furthermore, human chorionic gonadotropin (hCG) is a hormone that presents biochemical similarities with LH, binds to the same receptor as LH, and therefore, it has also been used in ART. Despite their similarities, LH and hCG are two distinct hormones and activate different affairs through mechanisms that can vary significantly between the two molecules [5, 6]. Thuesen et al., have published that hCG supplementation was associated with a significant stimulatory effect on the intrafollicular androgen and estradiol (E2) levels, with a shift toward a more androgenic milieu. Good quality embryos were developed upon higher intrafollicular concentrations of E2 and progesterone. As demonstrated, the estrogen to androgen ratio was higher in the follicles with oocytes giving rise to good quality embryos, supporting the importance of aromatization in order for the follicles to produce high quality oocytes [7]. Notably, advanced age reflects a change in the structure of the FSH and LH molecules [8]. 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 [9-11]. It is well recognized that an increase in the daily dose of gonadotropins 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 hypothesized that granulosa cells, which are resistant to gonadotropin stimulation, might benefit from LH or hCG stimulation. Further, LH or hCG may have beneficial effects through stimulation of theca cells. Interestingly, in situations where the ovaries are older or less sensitive to gonadotropins (biologically older), there is increasing evidence that the supplementation with LH or hCG activity is beneficial in the successful pregnancy outcome [12-15].

To address fertility issues, a variety of ovulation induction protocols have been introduced to reproductive medicine and assess the use of several forms of gonadotropins either of human purified urine or artificial recombinant origin. The urine analogues include Human Menopausal Gonadotropin (HMG) [16], urinary FSH (uFSH) [17] and urinary hCG (uhCG) [18]. On the other hand, the recombinant regimens that have been applied are recombinant FSH (rFSH) [19, 20], recombinant LH (rLH) [21], and recombinant hCG (rhCG) [22]. Selection of the most appropriate regimen among uFSH and rFSH still remains challenging with the currently available literature reporting no significant differences in the number of oocytes retrieved or pregnancy rates among the two types of FSH. The above gonadotropin preparations have been administered to induce COS in infertile women who undergo ART protocols. Hence, no concordance is available on the optimal regimens and preparations. Several studies support the addition of the LH-related regimens to FSH in COS, while LH, hCG, HMG and various combinations of them have been administered with or without FSH for COS [23-25]. Regarding combinations with hCG administration, Theofanakis et al., showed that the addition of hCG to rFSH was associated with better quality embryos and higher pregnancy rates, even in women over 40 years old, having higher basal FSH levels, and poor ovarian reserve [12]. The purpose of every ovulation induction protocol is to yield as many mature oocytes as possible; that is, to achieve the best possible synchronization of follicular maturation. For nuclear maturation, it has been suggested that the gap junction–mediated transmission of follicular-cell cyclic adenosine 3'-5'-monophosphate (cAMP) to the oocyte inhibits oocyte maturation [26-28], whereas gonadotropin stimulation ultimately terminates cumulus-oocyte communication and initiates resumption of meiosis by interruption of direct transfer of cAMP to the oocyte [27, 28]. Oocytes that mature in vitro appear to undergo nuclear and cytoplasmic maturation. For the assessment of their full in vitro maturation, it is necessary to examine not only their fertilizability, but also their potential for early embryonic development [26]. Moor et al., [29] and Ainsworth et al., [30] demonstrated that oocytes require a specific intrafollicular steroid environment for the inductive signals of meiotic resumption and the completion of the full maturational process. The aim of the present pilot study is the investigation of how hormonal follicular fluid (FF) profile derived from four different protocols of ovulation induction relates to the ovulation induction parameters, including number and quality of oocytes, and pregnancy rate.

Materials and Methods

Study population

The present pilot study was conducted from January 2022 until October 2022 at the Diagnostic and Therapeutic Fertility Institute S.A., Athens, Greece. The study group comprised 94 women that underwent controlled ovarian stimulation in a GnRH antagonist protocol with an age range of 24–45 years. Patient recruitment was accomplished using a computer-generated randomization table. Four different regimens were used: HMG (n=21) (Menopour Ferring), HMG/hCG (n=23), rFSH (n=29) (Gonal-F Merck) and rFSH/hCG (n=21). The addition of hCG (Pregnyl MSD) was a low dose of 100 IU/day. Protocol selection was based on age, Anti-Müllerian Hormone (AMH), FSH, LH, and antral follicle count (AFC). When the age of the patient was lower than 35 years rFSH was administered, while when the age was greater than 35 years HMG, rFSH+hCG, or HMG+hCG were administered. The rational for this decision was that women of advanced age are likely to achieve pregnancy using LH or hCG activity.

Citation: Papamentzelopoulou Myrto-Sotiria, Liokari Emanouela, Mavrogianni Despoina, Dimitroulia Evagelia, Stavros Sofoklis, Potiris Anastasios, Loutradis Dimitris. Intrafollicular Endocrine Milieu Hormonal Profile Using Four Different Ovarian Stimulation Protocols: HMG, HMG/hCG, rFSH, rFSH/hCG: A Single-Center Pilot Study. Journal of Women's Health and Development. 7 (2024): 55-67.

Myrto-Sotiria P, et al., J Women's Health Dev 2024
DOI:10.26502/fjwhd.2644-288400121

Inclusion criteria were women with no uterine or ovarian anomalies, having normal hormonal profile (according to WHO guidelines), a regular menstrual cycle of 25–30 days and both ovaries intact. The indications for fertility treatment included male factor, tubal factor, 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 participants, anthropometric data, such as age and BMI, and early follicular phase FSH, LH, PRL, AMH, TSH, T3, T4, TPO, TG, A, and DHEA-S levels within the preceding 6 months were recorded. The study protocol was approved by the review board of the Diagnostic and Therapeutic Fertility Institute S.A., (11/2020, date:20/12/2020). All participants provided informed consent for their medical records to be used in the study.

Ovarian stimulation protocol

The study participants underwent controlled ovarian stimulation in a GnRH antagonist protocol according to the strict routine practice of our institute. On day 5 of the menstrual cycle, daily administration of GnRH-antagonist (Orgalutran, MDS Hellas) was initiated and maintained until triggering of final oocyte maturation with rhCG (Ovitrell, Merck Hellas). Gonadotropins were administered on day 2 at a dose of 200 IU which was adjusted according to ovarian response on a daily basis, 6 days after the onset of gonadotropins administration. hCG (Pregnyl, MSD Hellas) was administered intramuscularly at a dose of 100 IU per day along with gonadotropins, starting on day 2 of the menstrual 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 the menstrual cycle) until the day of triggering the final oocyte maturation with subcutaneous administration of 250 μg rhCG (Ovitrell, Merck Hellas). Follicular tracking started on day 6 of stimulation (day 8 of the menstrual cycle) and subsequent ultrasound scans were performed every day until oocyte retrieval.

Follicular fluid sample

Follicular aspiration and oocyte retrieval were scheduled 36 hours after rhCG triggering via transvaginal ultrasound-guided puncture. The procedure was performed using a needle single lumen (Cook Medical, USA) with manual aspiration of each single follicle using a 20-mL syringe. Only FFs from follicles of approximately 12 mm and greater were aspirated, centrifuged and supernatants were aliquoted and stored at -20°C for subsequent analysis. IVF/ICSI procedure followed. Embryo quality evaluation was blinded according to treatment and included assessment of blastomere number, degree of fragmentation, blastomere uniformity, and multinucleation [26]. Embryo transfer was performed on day 5 after oocyte retrieval. Luteal phase support was provided with 200 mg of micronized progesterone administered intravaginally three times daily starting from the day after egg collection onwards and serum beta-hCG was measured 14 days later. Clinical pregnancy was defined as the presence of a gestational sac on ultrasound at 6 gestational weeks. Ultrasound scans, oocyte retrievals and embryo-transfers were conducted by either of the two fertility specialists of the institute. Similarly, oocyte grading, fertilization, early embryo development and embryo grading were performed by either of the two senior embryologists of the institute.

Follicular fluid hormonal determination

All hormone measurements in FFs, including progesterone (Prg), testosterone (T), androstenedione (A), hCG, FSH and estradiol (E2), were performed at the Laboratory Genes Lab (Athens, Greece). Estradiol and progesterone required a 1:1000 dilution. RIA method was applied for analysis of androstenedione. For all the other hormones COBAS 6000 analyzer (COBAS 6000, Roche Diagnostics) was used. The sensitivity for each measurement was as follows: hCG 0.1 IU/L; FSH, 0.1 IU/L; LH 0.1 IU/L; E2 0.02 nmol/L; progesterone 0.1 nmol/L; T 0.087 nmol/L; and androstenedione, 0.1 nmol/L. The ratios of E2 to Prg, E2 to A, E2 to T, and A to T were calculated to evaluate the activity of the major steroidogenic hormones in the follicle. The main outcome measures were intrafollicular hormone concentrations in relation to treatment groups, number and quality of oocytes, and pregnancy rate.

Statistical Analysis

The qualitative data were presented as frequency and percentage. Associations were explored by the Fisher's exact test between protocol and categorical variables. The quantitative data were presented as mean, standard deviation (or 95% Confidence Interval for mean). Comparisons between protocols with regards to follicular fluid hormonal profiling were performed using Analysis of Variance (F-test or Kruskal-Wallis H test). In addition, the comparison between the protocols regarding the qualitative and quantitative variables was repeated for the age group >34 years. To investigate the relationship between oocyte quality and follicular fluid measurements, the Pearson's correlation coefficient was applied. Graphical display using scatter plots and applying a linear trend equation to the data was conducted. The coefficient of determination was used for the degree of equation fit to the data. The correlations of embryo quality with follicular fluid measurements through all four protocols were investigated using Mann-Whitney U test. Statistical analysis was performed on SPSS version 20 software. The criterion of statistical significance was set at 5%.

Results

Study group demographic and clinical characteristics

The study group consisted of 94 women with a mean age of 36.3 ± 4.5 years (min=24, max=45). Of them, 21 were

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DOI:10.26502/fjwhd.2644-288400121

treated with HMG, 23 with HMG/hCG, 29 with rFSH and 21 with rFSH/hCG. Demographic and clinical characteristics for the four distinct subgroups are presented in Table 1. Women treated with HMG/hCG were statistically significantly older (38.09 ± 4.25 years, p=0.000), while the group of women with rFSH treatment were statistically significantly younger (33.03 ± 3.57 years, p=0.000). Moreover, serum FSH level at day 3 [9.65 ± 4.60 IU/L, p=0.029] and the number of previous attempts (1.96 ± 1.46, p=0.000) were statistically significantly higher in the HMG/hCG treated group. On the contrary, all the other serum hormone levels did not present statistically significant differences among the four treatment groups.

Follicular fluid hormone profile in HMG, HMG/hCG, rFSH, rFSH/hCG-treated groups

Hormonal profiling in the four treated groups presented statistically significant differences in terms of testosterone, androstenedione, estradiol and FSH levels, as demonstrated in Table 2. In details, testosterone, FSH, androstenedione and estradiol levels were significantly higher in HMG/hCG-treated group compared to the other three groups (697±448 ng/ml, p=0.000, 8.75±2.82 IU/L, p=0.002, 6.90±2.77 ng/ml, p=0.000, and 38696391 ± 170049486 ng/ml, p=0.000, respectively). On the other hand, progesterone and hCG levels did not differ significantly in the four groups. As presented

Table 1: Demographic and clinical characteristics of the HMG, HMG/hCG, rFSH, rFSH/hCG-treated groups.

Characteristics	HMG protocol (n=21)	HMG/hCG protocol (n=23)	rFSH protocol (n=29)	rFSH/hCG protocol (n=21)	p-value
Age	37.19 ± 4.03	38.09 ± 4.25	33.03 ± 3.57	37.86 ± 4.08	0.000
Years of infertility	4.48 ± 4.35	5.96 ± 4.75	3.17 ± 2.50	4.05 ± 2.82	0.191
No previous attempts	0.86 ± 1.42	1.96 ± 1.46	0.38 ± 0.86	0.62 ± 1.02	0.000
BMI	23.49 ± 4.02	22.44 ± 4.20	24.09 ± 5.54	24.68 ± 4.67	0.253
FSH (IU/L)	7.24 ± 3.04	9.65 ± 4.60	6.55 ± 1.90	7.64 ± 2.38	0.029
LH (IU/L)	5.90 ± 2.89	6.56 ± 3.82	7.14 ± 2.95	7.18 ± 3.00	0.498
PRL(ng/ml)	15.24 ± 12.10	13.75 ± 4.94	19.21 ± 8.48	18.90 ± 12.99	0.076
T (ng/dl)	22.25 ± 22.19	15.50 ± 6.89	18.02 ± 16.40	13.51 ± 12.66	0.931
AMH (ng/ml)	2.87 ± 1.78	2.37 ± 2.05	6.14 ± 11.02	2.69 ± 1.61	0.005
T3 (ng/ml)	19.07 ± 53.60	1.26 ± 0.38	18.30 ± 43.51	1.27 ± 0.71	0.718
T4 (μg/dl)	7.98 ± 3.29	26.81 ± 38.68	117.16 ± 359.80	14.34 ± 20.74	0.873
FT3 (ng/dl)	2.98 ± 1.51	3.55 ± 0.91	3.62 ± 0.94	3.19 ± 0.77	0.512
FT4 (pg/ml)	3.05 ± 4.83	3.84 ± 5.73	5.18 ± 6.46	3.77 ± 5.39	0.545
a-TPO (IU/ml)	193.60 ± 410.41	304.34 ± 603.93	28.50 ± 65.81	957.61 ± 2900.09	0.128
a-TG (IU/ml)	78.15 ± 174.24	73.34 ± 115.16	46.62 ± 79.35	108.33 ± 191.02	0.91
Tubal factor	0 (0%)	2 (9.1%)	0 (0%)	3 (14.3%)	
Male factor	16 (76.2%)	12 (54.5%)	18 (62.1%)	12 (57.1%)	
Unexplained	5 (23.8%)	8 (36.4%)	11 (37.9%)	6 (28.6%)	0.22
Values are mean ± SD or number (column percentage)					
One-way ANOVA or Kruskal-Wallis test and Fisher's exact test is used					

Table 2: Follicular fluid hormonal profile in HMG, HMG/hCG, rFSH, rFSH/hCG-treated groups.

Characteristics	HMG protocol	HMG/hCG protocol	rFSH protocol	rFSH/hCG protocol	p-value
Prg (ng/ml)	21350 ± 8374	15923 ± 8887	223222 ± 13703	21683 ± 12944	0.275
T (ng/ml)	480 ± 368	697 ± 448	139 ± 93	469 ± 338	0.000
A (ng/ml)	5.98 ± 2.79	6.90 ± 2.77	3.47 ± 1.80	5.88 ± 2.49	0.000
hCG (mIU/ml)	54.48 ± 27.17	46.45 ± 30.98	60.69 ± 44.30	51.34 ± 27.53	0.529
FSH (IU/L)	7.18 ± 1.98	8.75 ± 2.82	6.33 ± 3.82	5.43 ± 1.89	0.002
E2 (ng/ml)	1568768 ± 950108	38696391 ± 170049486	3891468 ± 15362008	6497325 ± 20472202	0.000
Values are mean ± SD					
One-way ANOVA or Kruskal-Wallis test is used					

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DOI:10.26502/fjwhd.2644-288400121

in Table 3, the statistically significantly higher ratios for the combinations of Prg/T, Prg/A, Prg/E2, A/hCG, hCG/FSH, hCG/E2, FSH/E2 were found in the rFSH-treated group. Moreover, for Prg/FSH, T/FSH and A/FSH combinations, the higher ratios were detected in the rFSH/hCG-treated group, while for T/A, T/hCG and T/E2 combinations, the higher ratios were found in the HMG/hCG-treated group, revealing a stronger androgenic environment in the FF of women treated with HMG/hCG.

Ovulation induction profiles and pregnancy rates in HMG, HMG/hCG, rFSH, rFSH/hCG-treated groups

As presented in Table 4, the numbers of follicles >12 mm (12.00 ± 2.59, p=0.000), mature oocytes (MII) (10.00 ± 2.87, p=0.000), and embryos (8.79 ± 3.06, p=0.000) obtained were statistically significantly higher in rFSH-treated group compared to the other three groups, as expected given the younger average age of rFSH-treated women. Moreover, those women that were treated with rFSH achieved a higher pregnancy rate (31.0%; 9/29) without, though, being statistically significant (p=0.669). Another observation is that in the HMG group with the older women of the study the pregnancy rate was higher (28.6%; 6/21) compared to the HMG/hCG (17.4%; 4/23) and rFSH/hCG (19.0%; 4/21) groups, and relatively similar with the pregnancy rate of the younger rFSH-treated women. As presented in Table 5, ovulation induction profiling and pregnancy rates in HMG, HMG/hCG, rFSH, rFSH/hCG-treated groups with age adjustment >34 years were recorded. In particular, the number of follicles >12 mm, the number of mature oocytes (MII), and the number of embryos were statistically significantly higher in rFSH-treated women, as also expected (11.82 ± 3.71, p=0.001; 11.27 ± 4.17, p=0.002;

Table 3: Follicular fluid hormonal profile ratios in HMG, HMG/hCG, rFSH, rFSH/hCG-treated groups.

Characteristics	HMG protocol	HMG/hCG protocol	rFSH protocol	rFSH/hCG protocol	p-value
Prg/T	71.3 ± 64.6	31.8 ± 27.7	207.3 ± 171.7	63.0 ± 43.7	0.000
Prg/A	4729.2 ± 3644.7	2707.7 ± 2219.9	8463.9 ± 6943.9	4440.5 ± 3078.7	0.002
Prg/hCG	510.4 ± 299.7	466.4 ± 368.7	520.1 ± 422.2	519.7 ± 317.6	0.96
Prg/FSH	3149.8 ± 1067.2	1853.8 ± 1210.1	4203.9 ± 2803.0	4345.3 ± 2527.1	0.001
Prg/E2	17453.3 ± 10285.3	9276.0 ± 8224.3	36479.6 ± 28894.5	14026.9 ± 9848.8	0.003
T/ A	78.8 ± 37.8	98.6 ± 39.6	44.1 ± 24.8	82.0 ± 36.8	0.000
T/hCG	11.6 ± 12.1	20.2 ± 16.3	17.0 ± 51.4	11.9 ± 10.8	0.000
T/FSH	75.0 ± 64.9	89.2 ± 69.2	28.8 ± 25.4	98.2 ± 85.5	0.000
T/E2	291.4 ± 92.6	324.5 ± 174.6	204.7 ± 119.2	285.8 ± 184.7	0.032
A /hCG	0.162 ± 0.183	0.210 ± 0.148	0.683 ± 2.383	0.154 ± 0.108	0.011
A /FSH	0.990 ± 0.780	0.911 ± 0.525	1.006 ± 2.140	1.322 ± 0.971	0.033
A /E2	4.723 ± 3.035	3.659 ± 2.058	6.786 ± 6.793	4.674 ± 5.571	0.317
hCG/FSH	7.512 ± 2.946	5.494 ± 2.903	10.785 ± 7.292	9.309 ± 2.922	0.002
hCG/E2	45.174 ± 31.283	25.971 ± 24.681	134.615 ± 151.118	33.007 ± 24.033	0.002
FSH/E2	6.369 ± 4.404	5.112 ± 3.409	12.161 ± 11.795	3.484 ± 1.971	0.001
Values are mean ± SD					
One-way ANOVA or Kruskal-Wallis test is used					

Table 4: Ovulation induction profiles and pregnancy rates in HMG, HMG/hCG, rFSH, rFSH/hCG-treated groups.

Characteristics	HMG protocol	HMG/hCG protocol	rFSH protocol	rFSH/hCG protocol	p-value
No follicles > 12mm	9.95 ± 2.40	7.39 ± 2.13	12.00 ± 2.59	9.48 ± 2.96	0.000
No oocytes	9.38 ± 2.54	6.78 ± 2.07	11.00 ± 2.67	8.95 ± 3.12	0.000
No mature oocytes (MII)	8.05 ± 1.99	6.00 ± 1.91	10.00 ± 2.87	7.57 ± 2.82	0.000
No embryos	7.14 ± 1.65	5.30 ± 1.72	8.79 ± 3.06	7.00 ± 3.11	0.000
Pregnancy					
Yes	6 (28.6%)	4 (17.4%)	9 (31.0%)	4 (19.0%)	
No	15 (71.4%)	19 (82.6%)	20 (69.0%)	17 (81.0%)	0.669
Values are mean ± SD or number (column percentage)					
One-way ANOVA and Fisher's exact test is used					

Citation: Papamentzelopoulou Myrto-Sotiria, Liokari Emanouela, Mavrogianni Despoina, Dimitroulia Evagelia, Stavros Sofoklis, Potiris Anastasios, Loutradis Dimitris. Intrafollicular Endocrine Milieu Hormonal Profile Using Four Different Ovarian Stimulation Protocols: HMG, HMG/hCG, rFSH, rFSH/hCG: A Single-Center Pilot Study. Journal of Women's Health and Development. 7 (2024): 55-67.

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DOI:10.26502/fjwhd.2644-288400121

11.45 ± 4.55, p=0.004, respectively). However, in the rFSH-group pregnancy rate was lower (1/11; 9.1%) compared to the other three groups, while the higher pregnancy rate was observed in the HMG-group (5/16; 31.2%); though, such finding did not reach statistical significance (p=0.531).

Follicular fluid hormonal profile in relation to pregnancy status in HMG, HMG/hCG, rFSH, rFSH/hCG-treated groups

The hormonal profile of follicular fluid in HMG, rFSH, HMG/hCG and rFSH/hCG-treated women with respect to pregnancy status is presented in Table 6. As shown, follicular fluid progesterone and hCG levels were statistically significantly lower in pregnant women treated with HMG compared to non-pregnant women who also received HMG (16184±3910 ng/ml vs 23338±8880 ng/ml, p=0.054 for progesterone, and 30.62±15.04 mIU/ml vs 63.00±25.61 mIU/ml, p=0.033 for hCG). For women treated with rFSH and proceeded to pregnancy, only follicular fluid progesterone levels were statistically significantly lower compared to the non-pregnant ones (11570±6049 ng/ml vs 25152±13829 ng/ml, p=0.051). On the contrary, women that received HMG in combination with hCG and achieved pregnancy had higher progesterone levels compared to non-pregnant women that were treated with the same regimen (24377±4944 ng/ml vs 14338±8634 ng/ml, p=0.034). As for rFSH/hCG-treated group, pregnant women had higher follicular fluid androstenedione levels compared to the non-pregnant women of the group (8.30±1.40 ng/ml vs 5.27±2.34 ng/ml, p=0.017).

Follicular fluid profiling in relation to oocyte quality in HMG, HMG/hCG, rFSH, rFSH/hCG-treated groups

Next, the possible associations between testosterone and estradiol levels in follicular fluid of women treated with HMG/hCG, rFSH/hCG, HMG and rFSH, and mature oocyte (MII) quality were investigated via Pearson's correlation coefficient. As shown in Figures 1 and 2, high testosterone levels tended to be positively correlated with an increased number of mature oocytes in both HMG/hCG (r=0.309, p=0.162) and rFSH/hCG (r=0.253, p=0.295) protocols, respectively; however, such observations did not reach statistical significance. On the contrary, high levels of testosterone seemed to be slightly inversely related to maturation rate in HMG and rFSH protocols, respectively, whereas, no statistical significance was observed (data not shown). In addition, estradiol levels were weakly positively related to MII oocytes in both HMG/hCG (r=0.155, p=0.502, Fig.3) and rFSH/hCG (r=0.056, p=0.821, Fig.4) protocols, respectively; however, such findings were not statistically significant. Similarly, a weak negative not statistically significant correlation was detected between high estradiol levels and maturation rate in HMG and rFSH protocols, respectively (data not shown).

Follicular fluid hormonal profile in relation to quality of embryos in HMG, HMG/hCG, rFSH, rFSH/hCG-treated groups

The possible effect of the different gonadotropin preparations and follicular fluid profiling on the quality of embryos was consecutively studied. As presented in Table 7a, good quality embryos outperformed poor quality ones in all ovulation induction regimens, with HMG-treated group having the highest number of good quality embryos with only one poor quality embryo (HMG: 95.0% vs 5.0%, HMG/hCG: 65.2% vs 34.8%, rFSH: 74.1% vs 25.9%, and rFSH/hCG: 66.7% vs 33.3%). Moreover, none of the four different ovulation induction protocols affected the quality of embryos. Similarly, there were no statistically significant differences in the FF profile between good quality and poor quality embryos. The conclusion about the absence of correlation between FF profile and embryo quality was observed in all four protocols (Table 7b).

Table 5: Ovulation induction profiles and pregnancy rates in HMG, HMG/hCG, rFSH, rFSH/hCG-treated groups with age adjustment >34 years

Characteristics	HMG protocol	HMG/hCG protocol	rFSH protocol	rFSH/hCG protocol	p-value
No follicles > 12mm	9.69 ± 2.57	7.65 ± 1.84	11.82 ± 3.71	9.39 ± 2.70	0.001
No oocytes	9.06 ± 2.72	6.95 ± 1.85	11.91 ± 3.88	8.83 ± 2.79	0.003
No mature oocytes (MII)	7.75 ± 2.18	6.25 ± 1.55	11.27 ± 4.17	7.39 ± 2.66	0.002
No embryos	7.00 ± 1.79	5.50 ± 1.47	11.45 ± 4.55	6.82 ± 3.05	0.004
Pregnancy					
Yes	5 (31.2%)	3 (15.0%)	1 (9.1%)	3 (16.7%)	
No	11 (68.8%)	17 (85.0%)	10 (90.9%)	15 (83.3%)	0.531
Values are mean ± SD or number (column percentage)					
One-way ANOVA and Fisher's exact test is used					

Citation: Papamentzelopoulou Myrto-Sotiria, Liokari Emanouela, Mavrogianni Despoina, Dimitroulia Evagelia, Stavros Sofoklis, Potiris Anastasios, Loutradis Dimitris. Intrafollicular Endocrine Milieu Hormonal Profile Using Four Different Ovarian Stimulation Protocols: HMG, HMG/hCG, rFSH, rFSH/hCG: A Single-Center Pilot Study. Journal of Women's Health and Development. 7 (2024): 55-67.

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DOI:10.26502/fjwhd.2644-288400121

Table 6: Follicular fluid hormonal profile in relation to pregnancy status in HMG, HMG/hCG, rFSH, rFSH/hCG-treated groups.

Protocol	FF profile	No Pregnancy	Pregnancy	p-value
HMG	Prg (ng/ml)	23338 ± 8880	16184 ± 3910	0.054
	T (ng/dl)	505 ± 426	409 ± 105	0.643
	A (ng/ml)	5.83 ± 2,84	6.40 ± 2,90	0.672
	hCG (mIU/ml)	63.00 ± 25.61	30.62 ± 15.04	0.033
	FSH (IU/L)	7.72 ± 1.71	5.66 ± 2.06	0.078
	E2 (ng/ml)	1723471 ± 1070853	1135600 ± 130410	0.578
HMG/hCG	Prg (ng/ml)	14338 ± 8634	24377 ± 4944	0.034
	T (ng/dl)	700 ± 467	683 ± 407	0.932
	A (ng/ml)	6.76 ± 2,80	7.55 ± 2.90	0.503
	hCG (mIU/ml)	44.65 ± 30.82	54.55 ± 35.03	0.443
	FSH (IU/L)	8.93 ± 2.86	7.95 ± 2.91	0.798
	E2 (ng/ml)	46610033 ± 188030311	3085000 ± 1972112	0.444
rFSH	Prg (ng/ml)	25152 ± 13829	11570 ± 6049	0.051
	T (ng/dl)	137 ± 85	146 ± 117	0.898
	A (ng/ml)	3.13 ± 1.44	4.33 ± 2.38	0.231
	hCG (mIU/ml)	61.36 ± 44.50	59.04 ± 46.80	0.899
	FSH (IU/L)	5.91 ± 2.69	7.40 ± 5.89	0.508
	E2 (ng/ml)	1172595 ± 1484101	10688650 ± 28815812	0.373
rFSH/hCG	Prg (ng/ml)	22787 ± 13478	17268 ± 10977	0.45
	T (ng/dl)	427 ± 340	640 ± 312	0.156
	A (ng/ml)	5.27 ± 2.34	8.30 ± 1.40	0.017
	hCG (mIU/ml)	53.96 ± 28.48	40.83 ± 23.71	0.321
	FSH (IU/L)	5.75 ± 1.86	4.15 ± 1.61	0.17
	E2 (ng/ml)	7617281 ± 22892495	2017500 ± 768327	0.637
Values are mean ± SD				
Mann - Whitney U test is used				

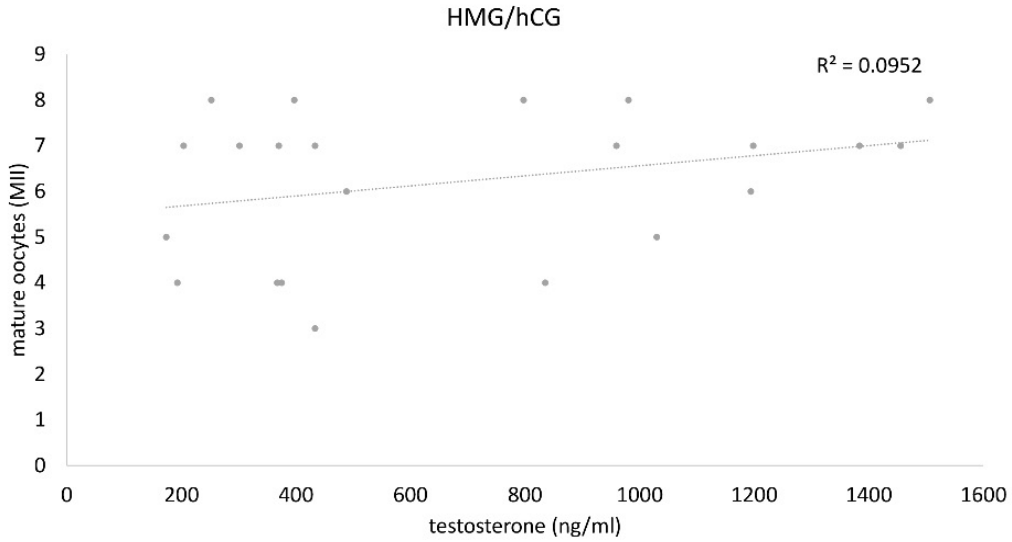

Figure 1: Testosterone vs mature oocytes (MII) in HMG/hCG protocol. Scatter plot showing the association between testosterone levels in follicular fluid of HMG/hCG-treated women and mature oocyte (MII) quality using Pearson's correlation coefficient.

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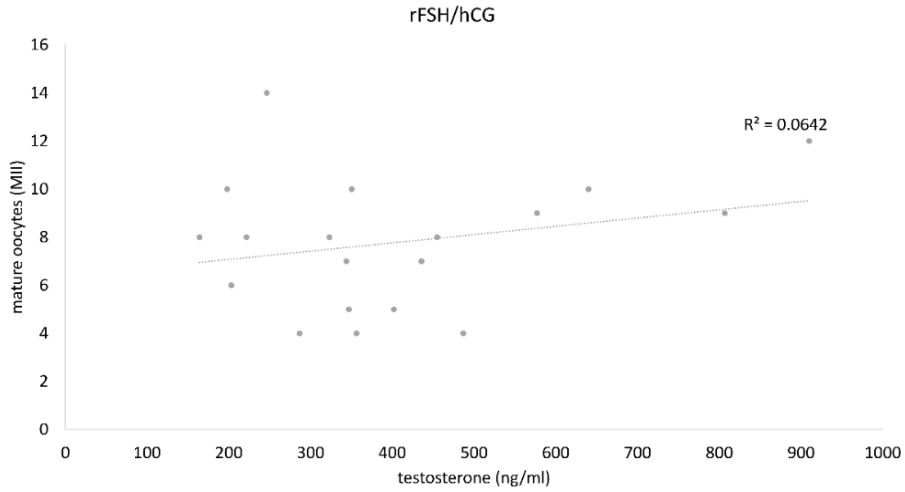

Figure 2: Testosterone vs mature oocytes (MII) in rFSH/hCG protocol. Scatter plot showing the association between testosterone levels in follicular fluid of rFSH/hCG-treated women and mature oocyte (MII) quality using Pearson's correlation coefficient.

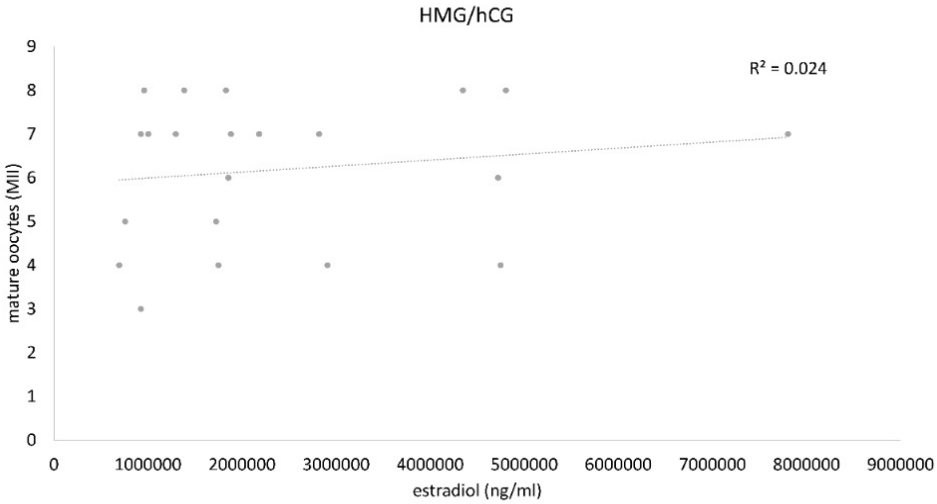

Figure 3: Estradiol vs mature oocytes (MII) in HMG/hCG protocol. Scatter plot showing the association between estradiol levels in follicular fluid of HMG/hCG-treated women and mature oocyte (MII) quality using Pearson's correlation coefficient.

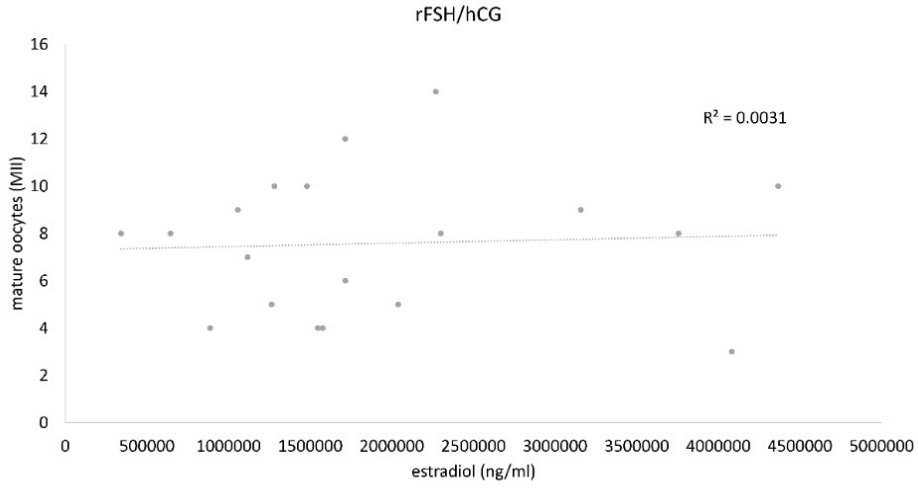

Figure 3: Estradiol vs mature oocytes (MII) in HMG/hCG protocol. Scatter plot showing the association between estradiol levels in follicular fluid of HMG/hCG-treated women and mature oocyte (MII) quality using Pearson's correlation coefficient.

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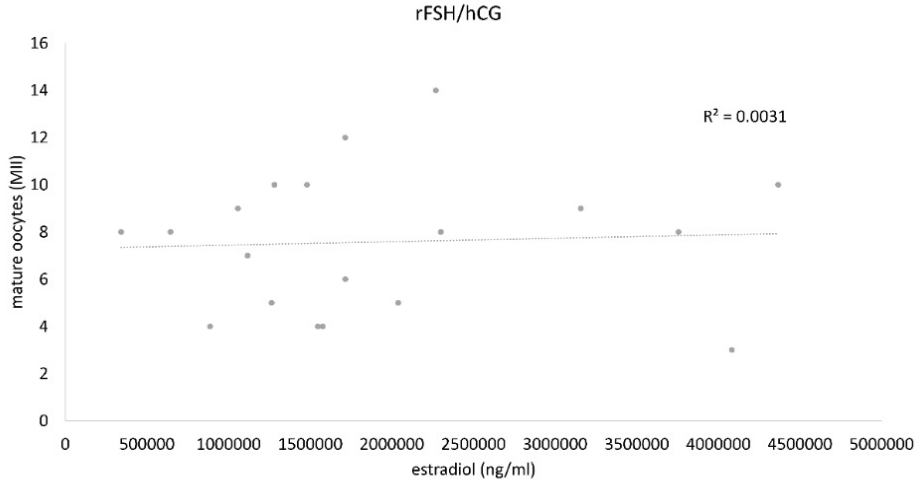

Figure 4: Estradiol vs mature oocytes (MII) in rFSH/hCG protocol. Scatter plot showing the association between estradiol levels in follicular fluid of rFSH/hCG-treated women and mature oocyte (MII) quality using Pearson's correlation coefficient.

Table 7a: Good and poor quality embryo distribution in HMG, HMG/hCG, rFSH, rFSH/hCG-treated groups.

Protocol	Good quality	Poor quality	Total	p-value
HMG	19 (95.0%)	1 (5.0%)	20 (100.0%)	
HMG/HCG	15 (65.2%)	8 (34.8%)	23 (100.0%)	
r-FSH	20 (74.1%)	7 (25.9%)	27 (100.0%)	
r-FSH/HCG	14 (66.7%)	7 (33.3%)	21 (100.0%)	
	68 (74.7%)	23 (25.3%)	91 (100.0%)	0.103
Chi-square test is used				

Table 7b: Follicular fluid hormonal profile in relation to quality of embryos in HMG, HMG/hCG, rFSH, rFSH/hCG-treated groups.

Protocol	FF profile	Good quality	Poor quality	p-value
HMG	Prg (ng/ml)	21494 ± 8608	18910	0.63
	T (ng/dl)	465 ± 373	740	0.361
	A (ng/ml)	5.81 ± 2.76	9	0.228
	hCG (mIU/ml)	55.71 ± 27.40	32.2	0.201
	FSH (IU/L)	7.18 ± 2.04	7.1	0.855
	E2 (ng/ml)	1490088 ± 911739	2985000	0.201
HMG/hCG	Prg (ng/ml)	15476 ± 6479	16690 ± 12607	0.933
	T (ng/dl)	697 ± 459	696 ± 458	0.916
	A (ng/ml)	6.55 ± 3.12	7.66 ± 1.76	0.553
	hCG (mIU/ml)	53.44 ± 34.32	31.47 ± 15.07	0.052
	FSH (IU/L)	9.31 ± 2.66	7.55 ± 2.99	0.13
	E2 (ng/ml)	55882707 ± 205863554	1868571 ± 1389151	0.217
rFSH	Prg (ng/ml)	21827 ± 14797	27304 ± 11265	0.269
	T (ng/dl)	136 ± 92	150 ± 112	0.908
	A (ng/ml)	3.68 ± 2.06	3.30 ± 0.96	0.839
	hCG (mIU/ml)	66.06 ± 49.97	48.60 ± 28.40	0.452
	FSH (IU/L)	6.52 ± 4.43	6.00 ± 2.49	0.908
	E2 (ng/ml)	5363847 ± 18617392	579371 ± 404423	0.326
rFSH/hCG	Prg (ng/ml)	20124 ± 10808	25320 ± 17617	0.564
	T (ng/dl)	428 ± 224	566 ± 536	0.741
	A (ng/ml)	5.54 ± 2.51	6.67 ± 2.45	0.406
	hCG (mIU/ml)	53.46 ± 30.30	46.37 ± 21.23	0.967
	FSH (IU/L)	5.59 ± 2.04	5.07 ± 1.59	0.71
	E2 (ng/ml)	8231886 ± 24504883	2450017 ± 1807919	0.934
Values are mean ± SD				
Mann - Whitney U test is used				

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DOI:10.26502/fjwhd.2644-288400121

Discussion

To our knowledge, this is the first study that compares the endocrine profile of follicular fluid generated after gonadotrophin stimulation using four different regimens (HMG, HMG/hCG, rFSH and rFSH/hCG) in a GnRH antagonist protocol. As disclosed herein, testosterone, androstenedione, estradiol and FSH levels are higher in HMG/hCG-treated group. On the contrary, progesterone and hCG present similar levels in the four groups. Regarding the follicular fluid hormonal ratios, Prg/T, Prg/A, Prg/E2, A/hCG, hCG/FSH, hCG/E2, FSH/E2 ratios are higher in the rFSH-treated group, indicating a different balance of steroid/androgenic milieu of FF with rFSH regimen with lower FF levels of androgens and estradiol. Our results confirm the lower levels of follicular fluid androgens and estradiol in rFSH-treated women compared to women that received HMG, as disclosed in previous studies [13, 31]. Therefore, the strongest androgenic environment is observed in the HMG/hCG-treated group, which includes the oldest recruited women with the most previous failed attempts and the highest FSH level on day 3, suggesting that the LH/hCG activity enhances the androgenic environment of follicular fluid. This finding is in line with literature where the androgen production increased with the effect of LH or hCG supplementation and the FF environment became more androgenic. Moreover, a previous study demonstrated high androgen concentrations upon hCG supplementation, which resulted in a more androgenic milieu with good quality oocytes [7]. A recent study showed that the addition of hCG to rFSH in a short GnRH-agonist protocol, throughout the follicular phase, was associated with better quality embryos and higher pregnancy rates. The significance of these findings was enhanced by the fact that women who received hCG were significantly older and with higher basal FSH levels, thereby with expectant poorer ovarian reserve [14]. In our study such beneficial effect of hCG supplementation even in rFSH or HMG treatment was not observed, since the pregnancy rates were 19.0% and 17.4%, respectively, as compared with the pregnancy rate of 28.6% in the HMG-treated group. A potential explanation of this observation could be the type of ovulation induction; herein GnRH antagonist protocol was used, while Partsinevelos et al., used GnRH analogs short protocol [14]. On the other hand, it was recently demonstrated that the addition of low-dose hCG (100 IU of hCG/day) from the start of stimulation with rFSH in a GnRH agonist short protocol did not increase significantly the rate of top-quality embryos and clinical outcomes [15].

The present study also discloses that follicular fluid progesterone levels are lower in HMG and rFSH-treated women that achieved pregnancy compared to women receiving the same regimens that did not proceed to pregnancy. On the contrary, HMG/hCG-treated women that achieved pregnancy have higher progesterone levels in the follicular fluid compared to non-pregnant ones, which could be attributed to the fact that hCG readily stimulates progesterone production in normally functioning corpus luteum [32]. Therefore, our data suggest that the low progesterone concentrations in the follicular fluid endocrine milieu itself of both HMG and rFSH-treated women are responsible for the higher pregnancy rates, whereas, upon hCG co-administration progesterone levels in the follicular fluid of pregnant women are increased. However, the potential effect of follicular progesterone level on fertilization is still under debate. In a recent study, the progesterone levels were significantly higher in follicular fluid in women that received only hCG compared to women that received either GnRH-agonist or both GnRH-agonist and hCG trigger; however, when patients were divided into pregnant and non-pregnant, progesterone concentrations in the follicular fluid were comparable [33]. In a recent meta-analysis, fertilized oocytes demonstrated to be derived from follicles with higher levels of follicular fluid progesterone; however, Nagy et al., suggest that due to the various progesterone measurement methods and different treatment protocols the results must be interpreted with caution [34]. HMG-treated women that achieved pregnancy had lower follicular fluid hCG levels compared to the non-pregnant women of the group. Such observation could be attributed to the continuously administrations of LH as composition of HMG during the follicular phase that could change the sensitivity of follicular cells [35]. Our data indicate that desensitization of granulosa cells by LH may play a positive role and that the low hCG concentration in the follicular fluid has beneficial effect in pregnancy rate. It should be mentioned that the HMG group, which presents herein a better clinical demographic profile, has the highest pregnancy rate. Such observation is confirmed by Smitz et al., who showed that HMG stimulation significantly increased intrafollicular testosterone and estradiol levels due to LH activity, resulting in high pregnancy rates [13]. For patients >34 years old, the higher numbers of collected oocytes, mature oocytes, and embryos are found in rFSH-treated women, who have the higher Prg/E2 ratio, confirming that the progesterone/estradiol ratio may be the best indicator of maturity [36] and oocyte fertilization potential [37]. However, in the rFSH-group, pregnancy rate is lower (9.1%) compared to the other three groups, while the higher pregnancy rate is observed in the HMG-group (31.2%). Such finding is confirmed by previous studies that highlight the importance of LH in older women during ovulation induction [38-41].

Concerning hormonal profiling and MII oocyte quality, high testosterone and estradiol levels in FF tend to be associated with increased number of mature oocytes in HMG/hCG and rFSH/hCG-treated women, while a slightly inverse relation was observed between high levels of FF testosterone and estradiol and maturation rate in HMG and rFSH-treated women. A possible explanation of the increased maturation

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rate in HMG/hCG and rFSH/hCG protocols is the beneficial effect of hCG administration on triggering final oocyte maturation [42]. However, there are contradictory disclosures upon follicular fluid hormonal profiling and oocyte maturity, with the majority supporting the beneficial effect of high testosterone and estradiol levels on the maturation rate of oocytes [7, 13, 43]. A recent study also displayed an increase in the estradiol levels in the FF with mature oocytes [44]. On the contrary, as disclosed in a previous study, E2 and testosterone levels were significantly higher in FF containing immature oocytes than in FF containing mature oocytes [45]. Upon further analysis on embryo quality, no association between follicular fluid hormonal profiling and embryo quality is demonstrated in all four ovulation induction protocols. Our observation is in line with an earlier study wherein embryo quality was not related to levels of follicular estradiol and progesterone [46]. Another study indicated that no robust association between follicular steroid hormones and embryo quality could be established [47]. Certain limitations are identified in our pilot study, which include the small number of the recruited patients and the significantly younger age of women that consisted the rFSH group. Another factor that along with the hormone levels in follicular fluid may influence main outcome measures, such as oocyte quality and the subsequent achievement of pregnancy, is the type of ovulation induction; herein, we used a GnRH antagonist protocol. Nevertheless, we tried by applying age adjustment and strict patient inclusion criteria to minimize potential biases.

To conclude, the strongest androgenic environment is observed in the HMG/hCG-treated group, which includes the older women of the study with the most previous failed attempts and the highest FSH level on day 3. Moreover, low progesterone concentrations in the follicular fluid of both HMG and rFSH-treated women are correlated with higher pregnancy rates, while hCG co-administration with either HMG or rFSH enhances oocyte maturation. For age >34 years, HMG-treated women have the higher pregnancy rate compared to rFSH-treated ones. However, more prospective studies with larger sample sizes are needed to provide firm evidence on the actual impact of the different gonadotropin preparations in ART protocols. Subsequently, the assisted reproduction experts will be provided with new, individualized therapeutic approaches based on the follicular fluid hormonal profiling of each patient; thus, increasing the probability for successful pregnancy.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the review board of the Diagnostic and Therapeutic Fertility Institute S.A., (11/2020, date:20/12/2020).

Acknowledgements

We would like to acknowledge the contribution of all patients in the present pilot study.

Author contributions

The conception and design of the study was performed by LD. The acquisition of data was carried out by MD, DE, VN, SS and PA, while MD, LE, PMS provided expertise on the analysis and interpretation of the data. PMS, MD, LD were responsible for drafting the article and revising it. The final approval of the submitted version was performed by LD. All authors approved the final version of the paper.

References

1. Couzinet B, Lestrat N, Brailly S, et al. Stimulation of ovarian follicular maturation with pure follicle-stimulating hormone in women with gonadotropin deficiency. J Clin Endocrinol Metab 66 (1988): 552-556.
2. Rabinovici J, Blankstein J, Goldman B, et al. In vitro fertilization and primary embryonic cleavage are possible in 17 alpha-hydroxylase deficiency despite extremely low intrafollicular 17 beta-estradiol. J Clin Endocrinol Metab 68 (1989): 693-697.
3. Lunenfeld B, Insler V. Future trends in infertility treatment: challenges ahead. Fertil Steril 68 (1997): 977-980.
4. Berger MJ, Taymor ML, Karam K, et al. The relative roles of exogenous and endogenous follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in human follicular maturation and ovulation induction. Fertil Steril 23 (1972): 783-790
5. Theofanakis C, Drakakis P, Besharat A, et al. Human Chorionic Gonadotropin: The Pregnancy Hormone and More. Int J Mol Sci 18 (2017).
6. Choi J, Smitz J. Luteinizing hormone and human chorionic gonadotropin: origins of difference. Mol Cell Endocrinol 383 (2014): 203-213.
7. Thuesen LL, Andersen AN, Loft A, et al. Intrafollicular endocrine milieu after addition of hCG to recombinant FSH during controlled ovarian stimulation for in vitro fertilization. J Clin Endocrinol Metab 99 (2014): 517-526.
8. Cole LA, Khanlian SA, Kohorn EI. Evolution of the human brain, chorionic gonadotropin and hemochorial implantation of the placenta: insights into origins of pregnancy failures, preeclampsia and choriocarcinoma. J Reprod Med 53 (2008): 549-557
9. Dufau ML. The luteinizing hormone receptor. Annu Rev Physiol 60 (1998): 461-496.
10. Loutradis D, Elsheikh A, Kallianidis K, et al. Results

Myrto-Sotiria P, et al., J Women's Health Dev 2024
DOI:10.26502/fjwhd.2644-288400121

of controlled ovarian stimulation for ART in poor responders according to the short protocol using different gonadotrophins combinations. Arch Gynecol Obstet 270 (2004): 223-226.

11. Ferraretti AP, Gianaroli L, Magli MC, et al. Exogenous luteinizing hormone in controlled ovarian hyperstimulation for assisted reproduction techniques. Fertil Steril 82 (2004):1521-1526.

12. Theofanakis C, Athanasiou V, Liokari E, et al. The impact of HCG in IVF Treatment: Does it depend on age or on protocol? J Gynecol Obstet Hum Reprod 48 (2019): 341-345.

13. Smitz J, Andersen AN, Devroey P, et al. Endocrine profile in serum and follicular fluid differs after ovarian stimulation with HP-hMG or recombinant FSH in IVF patients. Hum Reprod 22 (2007): 676-687.

14. Partsinevelos GA, Antonakopoulos N, Kallianidis K, et al. Addition of low-dose hCG to rFSH during ovarian stimulation for IVF/ICSI: is it beneficial? Clin Exp Obstet Gynecol 43 (2016): 818-825

15. Siristatidis C, Stavros S, Dafopoulos K, et al. A Randomized Controlled Trial on the Efficacy and Safety of Low-Dose hCG in a Short Protocol with GnRH Agonist and Ovarian Stimulation with Recombinant FSH (rFSH) During the Follicular Phase in Infertile Women Undergoing ART. Reprod Sci 29 (2022): 497-505.

16. Lunenfeld B, Bilger W, Longobardi S, et al. The Development of Gonadotropins for Clinical Use in the Treatment of Infertility. Front Endocrinol (Lausanne) 10 (2019): 429.

17. Palagiano A, Nesti E, Pace L. FSH: urinary and recombinant. Eur J Obstet Gynecol Reprod Biol 115 Suppl 1 (2004): S30-3.

18. Driscoll GL, Tyler JP, Hangan JT, et al. A prospective, randomized, controlled, double-blind, double-dummy comparison of recombinant and urinary HCG for inducing oocyte maturation and follicular luteinization in ovarian stimulation. Hum Reprod 15 (2000): 1305-1310.

19. Van Wezenbeek P, Draaijer J, Van Meel F, et al. Recombinant Follicle Stimulating Hormone: I. Construction, Selection and Characterization of a Cell Line. From clone to clinic (1990): 245-251

20. Loumaye E, Campbell R, Salat-Baroux J. Human follicle-stimulating hormone produced by recombinant DNA technology: a review for clinicians. Hum Reprod Update 1 (1995): 188-199.

21. Caglar GS, Asimakopoulos B, Nikolettos N, et al. Recombinant LH in ovarian stimulation. Reprod Biomed Online 10 (2005): 774-785.

22. Ludwig M, Doody KJ, Doody KM. Use of recombinant human chorionic gonadotropin in ovulation induction. Fertil Steril 79 (2003): 1051-1059.

23. Casarini L, Brigante G, Simoni M, et al. Clinical Applications of Gonadotropins in the Female: Assisted Reproduction and Beyond. Prog Mol Biol Transl Sci 143 (2016): 85-119.

24. van Wely M, Kwan I, Burt AL, et al. Recombinant versus urinary gonadotrophin for ovarian stimulation in assisted reproductive technology cycles. A Cochrane review. Hum Reprod Update 18 (2012): 111.

25. Shu L, Xu Q, Meng Q, et al. Clinical outcomes following long GnRHa ovarian stimulation with highly purified human menopausal gonadotropin plus rFSH or rFSH in patients undergoing in vitro fertilization-embryo transfer: a multi-center randomized controlled trial. Ann Transl Med 7 (2019):146.

26. Loutradis D, Drakakis P, Kallianidis K, et al. Oocyte morphology correlates with embryo quality and pregnancy rate after intracytoplasmic sperm injection. Fertil Steril 72 (1999): 240-244.

27. Dekel N, Beers WH. Development of the rat oocyte in vitro: inhibition and induction of maturation in the presence or absence of the cumulus oophorus. Dev Biol 75 (1980): 247-254.

28. Loutradis D, Drakakis P, Michalas S, et al. The effect of compounds altering the cAMP level on reversing the 2-cell block induced by hypoxanthine in mouse embryos in vitro. Eur J Obstet Gynecol Reprod Biol 57 (1994): 195-199.

29. Moor RM, Polge C, Willadsen SM. Effect of follicular steroids on the maturation and fertilization of mammalian oocytes. J Embryol Exp Morphol 56 (1980): 319-335

30. Ainsworth L, Tsang BK, Downey BR, et al. Interrelationships between follicular fluid steroid levels, gonadotropic stimuli, and oocyte maturation during preovulatory development of porcine follicles. Biol Reprod 23 (1980): 621-627.

31. Duijkers IJ, Willemsen WN, Hollanders HM, et al. Follicular fluid hormone concentrations after ovarian stimulation using gonadotropin preparations with different FSH/LH ratios. II. Comparison of hMG and recombinant FSH. Int J Fertil Womens Med 42 (1997): 431-435

32. Momoeda M, Tsutsumi O, Morita Y, et al. Differential effect of exogenous human chorionic gonadotrophin on progesterone production from normal or malfunctioning corpus luteum. Hum Reprod 13 (1998): 1907-1911.

33. Martazanova B, Mishieva N, Vedikhina I, et al. Hormonal profile in early luteal phase after triggering ovulation

Myrto-Sotiria P, et al., J Women's Health Dev 2024
DOI:10.26502/fjwhd.2644-288400121

with gonadotropin-releasing hormone agonist in high-responder patients. Front Endocrinol (Lausanne) 13 (2022): 834627.

34. Nagy B, Poto L, Farkas N, et al. Follicular fluid progesterone concentration is associated with fertilization outcome after IVF: a systematic review and meta-analysis. Reprod Biomed Online 38 (2019): 871-882.

35. Magoffin DA, Erickson GF. Direct inhibitory effect of estrogen on LH-stimulated androgen synthesis by ovarian cells cultured in defined medium. Mol Cell Endocrinol 28 (1982): 81-89.

36. Frederick JL, Francis MM, Macaso TM, et al. Preovulatory follicular fluid steroid levels in stimulated and unstimulated cycles triggered with human chorionic gonadotropin. Fertil Steril 55 (1991): 44-47.

37. Basuray R, Rawlins RG, Radwanska E, et al. High progesterone/estradiol ratio in follicular fluid at oocyte aspiration for in vitro fertilization as a predictor of possible pregnancy. Fertil Steril 49 (1988): 1007-1011.

38. Mochtar MH, Van der V, Ziech M, et al. Recombinant Luteinizing Hormone (rLH) for controlled ovarian hyperstimulation in assisted reproductive cycles. Cochrane Database Syst Rev (2007): CD005070.

39. Humaidan P, Bungum M, Bungum L, et al. Effects of recombinant LH supplementation in women undergoing assisted reproduction with GnRH agonist down-regulation and stimulation with recombinant FSH: an opening study. Reprod Biomed Online 8 (2004): 635-643.

40. Marrs R, Meldrum D, Muasher S, et al. Randomized trial to compare the effect of recombinant human FSH (follitropin alfa) with or without recombinant human LH in women undergoing assisted reproduction treatment. Reprod Biomed Online 8 (2004): 175-182.

41. Lisi F, Rinaldi L, Fishel S, et al. Evaluation of two doses of recombinant luteinizing hormone supplementation in an unselected group of women undergoing follicular stimulation for in vitro fertilization. Fertil Steril 83 (2005): 309-315.

42. Kolibianakis EM, Schultze-Mosgau A, Schroer A, et al. A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocyte maturation instead of HCG in patients undergoing IVF with GnRH antagonists. Hum Reprod 20 (2005): 2887-2892.

43. Hillier SG, De Zwart FA. Evidence that granulosa cell aromatase induction/activation by follicle-stimulating hormone is an androgen receptor-regulated process in-vitro. Endocrinology 109 (1981): 1303-1305.

44. Janati S, Behmanesh MA, Najafzadehvarzi H, et al. Comparison of Follicular Fluid Paraoxonase 3 Level, Ovarian Hormones and Oocyte Quality between Fertile and Infertile Women. J Reprod Infertil 23 (2022): 192-198.

45. Costa LO, Mendes MC, Ferriani RA, et al. Estradiol and testosterone concentrations in follicular fluid as criteria to discriminate between mature and immature oocytes. Braz J Med Biol Res 37 (2004): 1747-1755.

46. Asimakopoulos B, Abu-Hassan D, Metzen E, et al. The levels of steroid hormones and cytokines in individual follicles are not associated with the fertilization outcome after intracytoplasmic sperm injection. Fertil Steril 90 (2008): 60-64.

47. Carpintero NL, Suarez OA, Mangas CC, et al. Follicular steroid hormones as markers of oocyte quality and oocyte development potential. J Hum Reprod Sci 7 (2014): 187-193.