Article title

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

Methods: This is a prospective, multicenter, randomized, double-blind, placebo-controlled, Phase IIIb clinical study, conducted in three University IVF Units. We studied whether the addition of 100 IU hCG/day to a short GnRH-agonist IVF protocol from the onset of the follicular phase (Group 1, n=40) or placebo (Group 2, n=41) had any impact on the number of high-quality transferred embryos at day three and clinical pregnancy rates. The comparison encompassed descriptive statistics, and univariate and multivariate analyses.

Ethical approval: All procedures performed were in accordance with the ethical standards of the Scientific Council and by the Bioethics Committee and the Scientific Council of the Hospital, prior to study initiation (EIRBM no 47/1/13-3-2017; 4/13-3-17) and the relevant committees, National Ethics Committee and National Organization for Medicines, respectively (EED 46-17/23-6-2017; EOF IS 44-17/40345/2017) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The protocol was registered in the relevant databases (NCT03423527; Study name: hCG-GR-001-2016; EudraCT Number 2016-005208-24).

Study population: Data was collected on 81 IVF/ICSI cycles, at the Assisted Reproduction Units of the Third Department of Obstetrics and Gynecology, "Attikon" University Hospital, the First Department of Obstetrics and Gynecology, "Alexandra" University Hospital, Athens, and the Department of Obstetrics and Gynecology of University of Thessaly, Greece, and from July 2017 to April 2019.

The study group (Group 1 [HCG [+] group]) indicated the application of the protocol by adding hCG with the initiation of standard short GnRH agonist protocol for IVF / ICSI with 200 IU rFSH (BEMFOLA, Fertilland Pharma/Gedeon Richter Plc, Hungary), while the control group (Group 2 [HCG [-] group]) indicated the application of the same protocol, adding placebo. The addition of hCG (Pregnyl®, MSD Greece) at a dose of 100 IU per day, was administered subcutaneously, and continued until the administration of hCG for follicular

maturation/triggering; flacons were prepared centrally and distributed to all involved Units, by one study midwife, using 5000 IU commercial ampoules. As for placebo, an identical flacon with 100 IU containing N/S 0.9% per day was injected.

Outcome measures

The primary outcomes were the number of top-quality transferred embryos and clinical pregnancy rate. Secondary endpoints included the number of follicles [> 11,> 14,> 18 mm] and endometrial thickness on the day of triggering, the total number of retrieved and MII oocytes, the total number of total, top-rated and frozen embryos, biochemical, ectopic, and multiple pregnancy, miscarriage and OHSS rates. Definitions of the outcomes were according to the International Glossary on Infertility and Fertility Care [37].

Statistical analysis and power calculation

As for the sample size calculation, using similar trials as references, we observed that the main differences of outcomes (not very profound in all studies), was achieved in the level of embryos, and especially their quality, leading to higher pregnancy rates [13, 17, 29, 30]. Thus, we calculated that samples of 50 participants from each arm would be required, to provide a significance of 0.05 and a power of 0.8 detecting a difference equal to 0.58*standard deviation in the top-quality embryos, including dropouts and missing data. So, the total number of participants required was 90.

Premature ending / reasons

Unfortunately, the study was prematurely ended (while relative authorities were notified officially), as one of the main investigators moved to another IVF Unit, and mainly because of the Covid-19 pandemic: IVF protocols in all Units worldwide changed from fresh to frozen, so that it was impossible to recruit more patients during the last year.

Table 1. Descriptive statistics in the two study arms.

	Group 1 (n=40)	Group 2 (n=41)	p
Sociodemographic and lifestyle features			
Age, years, median [IQR]	38 [4]	38 [3]	0.980[T]
BMI, kg/m^2, median [IQR]	22.6 [4.6]	23.9 [6.1]	0.292 [M]
Previous parity, n (%)	3 (7.5)	4 (9.8)	0.718[C]
Smoking, >4 cigarettes/day, n (%)	12 (30.0)	16 (39.0)	0.393 [C]
Alcohol consumption, >4 cups/week, n (%)	0 (0.0)	1 (2.4)	>0.999[F]
Reproductive and clinical history			
Chronic disease, n (%)	14 (35.0)	12 (29.3)	0.581 [C]
Age at menarche, years, median [IQR]	13.0 [1.5]	13.0 [1.0]	0.313 [T]
Duration of menstruation, days, median [IQR]	5 [0]	5 [1.5]	0.906 [T]
Type of infertility, n (%) Tubal Male Anovulatory Male and tubal Unexplained	 4 (10.0) 16 (40.0) 0 (0.0) 4 (10.0) 16 (40.0)	 4 (9.8) 17 (41.5) 1 (2.4) 6 (14.6) 13 (31.7)	0.887[F]
First IVF/ICSI cycle, n (%)	26 (65.0)	33 (80.5)	0.117 [C]
Findings at baseline			
Heart rate, bpm, median [IQR]	75 [10]	77 [9]	0.217 [M]
Systolic blood pressure, mmHg, median [IQR]	116 [11]	115 [11]	0.892 [T]
Duration of infertility, years, median [IQR]	3.5 [2.0]	3.0 [4.0]	0.977 [M]
Baseline hormonal measurements			

FSH, (mIU/ml), median [IQR]	7.4 [5.1]	8.0 [3.4]	0.882 [T]
TSH, (mIU/lt), median [IQR]	1.7 [1.0]	1.7 [0.9]	0.627 [T]
LH, (mIU/ml), median [IQR]	5.7 [4.3]	6.1 [3.5]	0.685 [M]
E2, pg/ml, median [IQR]	39.5 [16.2]	43.3 [21.3]	0.806 [M]
AMH, ng/mL, median [IQR]	1.4 [1.5]	1.0 [1.3]	0.106 [M]
PRL, ng/mL, median [IQR]	14.9 [10.5]	13.0 [10.3]	0.887 [M]
AFC, median [IQR]	8.5 [4.0]	7.0 [4.0]	0.518 [M]
Features of the male partner			
BMI, kg/m^2, median [IQR]	27.5 [6.5]	27.6 [4.7]	0.702 [M]
Smoking, >4 cigarettes/day, n (%)	20 (50.0)	23 (56.1)	0.582 [C]
Alcohol consumption, >4 cups/week, n (%)	1 (2.5)	2 (4.9)	>0.999[F]
Chronic disease, n (%)	10 (25.0)	7 (17.1)	0.381 [C]
Proven fertility, n (%)	10 (25.0)	15 (36.6)	0.259 [C]

[C]: p-value derived from Pearson's chi-squared test; [F]: p-value derived from Fisher's exact test; [M]: p-value derived from Mann-Whitney-Wilcoxon test for independent samples; [T]: p-value derived from t-test; AFC: antral follicle count; AMH: anti-Müllerian hormone; BMI: body mass index; E2: estradiol; FSH: follicle-stimulating hormone; IQR: interquartile range; LH: luteinizing hormone; PRL: prolactin; TSH: thyroid-stimulating hormone

Table 2. Primary and secondary outcomes in the two study arms.

	Group 1 (n=40)	Group 2 (n=41)	p
Primary outcomes			
Clinical pregnancy, n (%)	10 (25.0)	10 (24.4)	0.949[C]
Excellent quality embryos on day 3 (≥2), n (%)	21 (52.5)	14 (34.2)	0.095[C]
Secondary outcomes			
Number of follicles >11mm at triggering, median [IQR]	6 [6]	6 [5]	0.996[M]
Number of follicles >14mm at triggering, median [IQR]	5 [5]	6 [4]	0.377[M]
Number of follicles >18mm at triggering, median [IQR]	3 [4]	3 [4]	0.568[M]
E2 at triggering, pg/ml, median [IQR]	1770 [1742]	1100 [1182]	0.144[M]
P4 at triggering, ng/ml, median [IQR]	1.1 [1.1]	0.9 [0.8]	0.116[M]
Endometrial thickness at triggering, mm, median [IQR]	9.0 [2.0]	9.0 [2.2]	0.917[M]
Number of oocytes (≥2), n (%)	32 (80.0)	34 (82.9)	0.735[C]
Number of MII oocytes, median [IQR]	3 [5]	3 [2]	0.735[M]
Moderate quality embryos on day 3 (≥2), n (%)	5 (12.5)	7 (17.1)	0.562[C]
Poor quality embryos on day 3 (≥2), n (%)	1 (2.5)	7 (17.1)	0.057[F]
ET embryos, median [IQR]	2 [1]	2 [1]	0.545[M]
Frozen embryos, median [IQR]	0 [3]	0 [1]	0.894[M]
Biochemical pregnancy, n (%)	11 (27.5)	12 (29.3)	0.860[C]
Cycle cancellation, n(%)	8 (20.0)	7 (17.1)	0.735[C]
Spontaneous abortion, n (%)*	1 (3.1)	0 (0.0)	0.485[F]
Ovarian hyperstimulation syndrome, n (%)	3 (7.5)	1 (2.4)	0.359[F]

*regarding spontaneous abortion, the denominator was the number of women on whom cycles were not cancelled (32 and 34 in groups 1 and 2, respectively); [C]: p-value derived from Pearson's chi-squared test; [F]: p-value derived from Fisher's exact test; [M]: p-value derived from Mann-Whitney-Wilcoxon test for independent samples; E2: estradiol; ET: embryo transfer; IQR: interquartile range; P4: progesterone

Table 3. Univariate and multivariate logistic regression analysis examining outcomes in the two study arms. Group 1 was set as reference category.

	Outcome Compared categories	Crude OR (95%CI)	Adjusted OR (95% CI)*
Primary outcomes			
Clinical pregnancy	Yes vs. no	0.97 (0.35-2.66)	0.89 (0.29-2.75)
Excellent quality embryos on day 3	≥2 vs. <2	0.47 (0.19-1.15)	0.54 (0.21-1.42)
Secondary outcomes			
Number of follicles >11mm at triggering§	≥6 vs. <6	1.28 (0.52-3.13)	1.50 (0.54-4.19)
Number of follicles >14mm at triggering§	≥6 vs. <6	1.73 (0.72-4.16)	2.38 (0.86-6.60)
Number of follicles >18mm at triggering§	≥3 vs. <3	1.16 (0.48-2.78)	1.30 (0.51-3.32)
E2 at triggering, pg/ml §	≥1300 vs <1300	0.47 (0.19-1.15)	0.60 (0.24-1.56)
P4 at triggering, ng/ml, §	≥0.95 vs. <0.95	0.60 (0.25-1.46)	0.63 (0.24-1.64)
Endometrial thickness at triggering, mm, §	≥9 vs. <9	0.94 (0.39-2.28)	0.94 (0.37-2.41)
Number of oocytes	≥2 vs. <2	1.21 (0.39-3.73)	1.59 (0.47-5.43)
Number of MII oocytes§	≥3 vs. <3	0.95 (0.40-2.27)	1.27 (0.48-3.34)
Moderate quality embryos on day 3	≥2 vs. <2	1.44 (0.42-4.98)	1.69 (0.44-6.49)
Poor quality embryos on day 3	≥2 vs.<2	8.03 (0.94-68.60)	11.69 (1.29-106.19)
ET embryos§	≥2 vs. <2	0.75 (0.30-1.87)	0.84 (0.32-2.20)
Frozen embryos§	≥1 vs. 0	1.20 (0.48-3.00)	1.51 (0.53-4.30)
Biochemical pregnancy	Yes vs. no	1.09 (0.41-2.87)	1.05 (0.36-3.04)
Cycle cancellation	Yes vs. no	0.82 (0.27-2.53)	0.81 (0.25-2.66)
Ovarian hyperstimulation syndrome	Yes vs. no	0.31 (0.03-3.10)	0.45 (0.04-4.79)

*adjusted for age (≥38 vs. <38 years), BMI (≥25 vs. <25 kg/m^2), baseline AMH (≥1 vs. <1 ng/mL), and AFC (>4 vs. ≤4); §the median was set as the cut-off values for these outcomes; No analysis on spontaneous abortion was presented, as only one event was noted.