A prospective, randomized study comparing morning to evening administration of gonadotropins in ART

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Abstract

Objective We sought to determine whether administering the daily gonadotropin dose in the morning (AM) or in the evening (PM) affects cycle outcome in patients undergoing IVF.

Design This is a prospective randomized study.

Setting The study is performed in a private assisted reproductive technology (ART) clinic.

Patient(s) The study included one hundred and twenty-seven women undergoing IVF.

Intervention(s) Morning (AM) and evening (PM) administration of gonadotropins (uFSH and hMG) was compared.

Main outcome measure(s) Live birth rate was the main outcome measured. Secondary outcomes including total IU use, days of stimulation, peak E₂, peak P₄, endometrial thickness, number of oocytes retrieved, MII oocytes, fertilization rates, #ET, IR, and clinical PR were all assessed.

Results A total of 127 cycles were included, 61 in the AM group and 67 in the PM group. Baseline and stimulation characteristics were similar in both groups. There was a trend for a higher implantation rate in the AM group vs. the PM group (60.3 vs. 47.2%, $P = 0.066$). The AM group had a higher chemical pregnancy rate compared to the PM group (81.7 vs. 65.6%, $P = 0.024$) and a higher clinical pregnancy rate (78.3 vs. 62.1%, $P = 0.048$), but the delivery rates were similar (68.3 vs. 56.1%, $P = 0.16$). The study was unfortunately prematurely terminated when uFSH (Bravelle©) was pulled out of the US market.

Conclusions AM administration of gonadotropins may be associated with a better ART outcome compared to PM administration. Larger studies are needed to confirm our findings.

Keywords Gonadotropins · Ovarian stimulation · AM administration · PM administration · Blastocyst · ART · Pregnancy rate · ET

Introduction

Historically most assisted reproductive technology (ART) programs administer the total daily gonadotropin dose in a single dose, usually in the evening, but many physicians still divide the daily dose into morning and evening doses if the total dose exceeds 300 IU despite the fact that a single FSH dose results in

sustained estradiol production for 24 h [1] and that we have previously shown in a prospective randomized trial using both FSH and Human Menopausal Gonadotropins hMG in a single injection that once-a-day administration of up to 450 IU does not compromise ART outcome [2]. Our study also showed that using once-a-day administration resulted in significantly less gonadotropin requirements (specifically FSH) compared to twice-a-day administration, resulting in cost savings [2]. Needless to say that a once-daily gonadotropin dose is also associated with increased patient satisfaction and comfort. However, surprisingly no prior studies to our knowledge have evaluated whether it is best to administer the daily gonadotropin dose in the morning or the evening, and whether there are any advantages or disadvantages to the specific timing of administration. In this study, we sought to determine whether there was a difference in cycle outcome if the once-daily gonadotropin dose was given in the AM or in the PM.

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Materials and methods

We conducted a prospective, randomized, controlled trial of 127 IVF/ICSI cycles with blastocyst transfers performed at our center between January 2013 and September 2015. The trial was registered at www.clinicaltrials.gov as NCT 01139593. Women were prospectively randomized on the day of gonadotropin start using a computer-generated randomization table in groups of 12. A power analysis was not performed a priori as our null hypothesis being no difference in the main outcome, live birth rate. Patients (age ≤ 41 , range 27–41) were randomized to receive either morning (AM) or evening (PM) injections of gonadotropins subcutaneous (SC). Specifically, morning administration was around 9 am (± 30 min), and evening administration was around 9 pm (± 30 min). All patients received a mixed injection of uFSH (Bravelle®, Ferring, Parsippany, NJ) and Highly-purified human menopausal gonadotropins (HP-hMG) (Menopur®, Ferring, Parsippany, NJ) at 1:1 ratio mixed into a single syringe using a Q-cap. The dose of gonadotropins was a minimal of 150 IU (1 vial (75 IU) Bravelle® and 1 vial (75 IU) Menopur®) to a maximum of 450 IU (3 vials of Bravelle® and 3 of Menopur®) [3]. Dose adjustments were made, as needed, by physician (FIS) preference 5 days after stimulation start as previously described [4]. All monitoring visits (ultrasound and hormonal measurements) were conducted in the morning (8–11:45 am). Patients in the AM arm seen after 9 am would have received the morning dosage prior to be their visits.

Our center's Institutional Review Board approved the study. The protocol included both long luteal downregulated cycles ($n = 100$) and antagonist cycles ($n = 27$). Only cycles with blastocyst transfers were included as our center performs blastocyst transfers exclusively. Exclusion criteria included women with one ovary, donor oocyte cycles, surrogacy, PGD/PGS, and FET cycles, but otherwise, all infertility diagnoses were allowed into the study. When at least 3 follicles reached 16–18 mm in diameter, 5000–10,000 IU of urinary hCG (Novarel®, Ferring, Parsippany, NJ) was administered SC that evening, regardless of gonadotropin administration in both the morning or evening groups. Oocyte retrieval was performed 35 h later in the early morning (7:30–9:30 am). For luteal support, all patients received IM P in oil (50 mg) along with vaginal estradiol (2 mg bid) starting the day after oocyte retrieval. Oocytes were rinsed and denuded using a hyaluronidase solution combined with mechanical stripping, and ICSI was performed 4–6 h later. All embryos were cultured under 37 °C in a 5% O₂, 5% CO₂ environment in groups under mineral oil in droplets of culture media (Continues Single Culture (CSC) Media, Irvine Scientific, Santa Ana, CA) with 10% serum substitute supplement. All ET were performed 5 days later under ultrasound guidance using a Wallace Sure-view catheter (Marlow, Irvine Scientific, Santa Ana, CA) by physician (FIS). A pregnancy test was performed 9–12 days later. Biochemical pregnancies were recorded as

negative. An ultrasound was performed in pregnant women at 6–7 weeks, and the number of gestational sacs was recorded (to define implantation rate).

Live birth rate was the main outcome measured. Secondary outcomes including total IU use, days of stimulation, peak E₂, peak P₄, endometrial thickness, number of oocytes retrieved, MII oocytes, fertilization rates, #ET, IR, and clinical PR were all assessed.

Statistical analysis was performed using a two-way ANOVA, chi-square, and *t* test to test for significance as needed. $P < 0.05$ was considered significant. Unfortunately the study was prematurely terminated when Ferring Pharmaceuticals decided to withdraw Bravelle® from the US market in October 2015. The company claims that several batches of Bravelle® did not meet potency specifications through established expiry period. As a precautionary measure, Ferring initiated a recall of all distributed lots of Bravelle®.

Results

A total of 127 stimulation cycles were analyzed by the time the study was stopped, of which 61 cycles were randomized to the daily gonadotropin dose in the AM, while 66 cycles received their dose in the PM (Fig. 1). Baseline characteristics including age, BMI, basal FSH, basal E₂, basal LH, TSH, prolactin, AMH, vitamin D, and antral follicle counts (AFC) were similar between the both AM and PM groups (Table 1). In terms of stimulation regimen, 100 used the long agonist protocol (47 in AM and 53 in the PM, $P = \text{NS}$), and 27 used an antagonist-based protocol (14 in AM and 13 in PM, $P = \text{NS}$). With respect to the stimulation parameters, the total IU of gonadotropins, stimulation days, peak estradiol and progesterone levels (day of hCG administration), and endometrial thickness were similar between the AM and PM groups (Table 2). The total number of oocytes retrieved, mature, and fertilized oocytes were also similar between the two groups (Table 2). There were no differences between the numbers of embryos transferred or cycles frozen between the AM and PM groups. There was a higher implantation rate in the AM group vs. the PM group that nearly reached statistical significance (60.3 vs. 47.2%, $P = 0.066$). However, the chemical and clinical pregnancy rates were significantly higher in the AM group compared to the PM group (81.7 vs. 65.6%, $P = 0.024$, and 78.3 vs. 62.1%, $P = 0.048$, respectively). There was a trend for a higher delivery rates in the AM group compared to the PM which did not reach statistical significance (68.3 vs. 56.1%, $P = 0.16$). As expected, there were no differences in outcomes between agonist- or antagonist-based protocols within each and between both groups: the live birth rate in the AM group compared to the PM group was 63.8% (30/47) and 52.8% (25/53), respectively, in the agonist group and

Fig. 1 CONSORT flow diagram

71.4% (10/14) and 69.2% (9/13), respectively, in the antagonist group ($P=NS$) (Table 3).

Discussion

To our knowledge, this is the first prospective randomized study that evaluated IVF cycle outcome based on daily

AM compared to daily PM gonadotropin administration in ART. We found an unexpected trend towards higher implantation rate, and a significantly higher chemical and clinical pregnancy rates in the AM dosing compared with the PM dosing group, but the live birth rates were similar in the two groups. The study was unfortunately prematurely terminated but it is not inconceivable that with few more cases, a statistical significance could have been achieved between the two groups.

Table 1 Baseline characteristics of the 127 cycles. Values are mean $\pm$ SD or mean $\pm$ SEM

	AM ($N=61$)	PM ($N=66$)	P
Age (years)	34.4 $\pm$ 4.9	35.0 $\pm$ 3.8	0.39
BMI (kg/m ²)	25.0 $\pm$ 4.7	24.2 $\pm$ 3.3	0.31
Basal FSH (ng/ml)	7.1 $\pm$ 2.3	7.5 $\pm$ 2.4	0.33
Basal E ₂ (pg/ml)	40.8 $\pm$ 23.2	41.1 $\pm$ 26.3	0.88
Basal LH (ng/ml)	5.7 $\pm$ 3.2	5.1 $\pm$ 2.1	0.23
TSH (ng/ml)	1.6 $\pm$ 0.7	1.9 $\pm$ 1.4	0.14
PRL (ng/ml)	9.9 $\pm$ 8.8	11.1 $\pm$ 7.0	0.39
AMH (ng/ml)	4.2 $\pm$ 3.8	3.1 $\pm$ 3.6	0.11
Vit D (ng/ml)	29.2 $\pm$ 11.5	33.5 $\pm$ 15.1	0.21
AFC right	8.3 $\pm$ 4.2	8.5 $\pm$ 4.0	0.87
AFC left	8.4 $\pm$ 3.9	8.2 $\pm$ 4.3	0.84

Table 2 Stimulation parameters for the 127 cycles. One patient in the AM group had an ovarian cyst at stimulation start and was canceled

	AM ($N=61$)	PM ($N=66$)	P
Cycles canceled before cycle start	1	0	
Gonadotropins (IU)	3257 $\pm$ 1099	3190 $\pm$ 1048	0.73
Stimulation days	9.6 $\pm$ 0.8	9.3 $\pm$ 0.8	0.057
Peak E ₂ (pg/ml)	2210 $\pm$ 1406	2013 $\pm$ 1175	0.41
Peak P ₄ (ng/ml)	0.88 $\pm$ 0.53	0.92 $\pm$ 0.56	0.68
Endometrial thickness (mm)	11.5 $\pm$ 2.4	11.4 $\pm$ 2.7	0.75
# oocytes	12.1 $\pm$ 4.5	12.3 $\pm$ 5.4	0.86
# MII	9.4 $\pm$ 4.0	8.9 $\pm$ 4.0	0.49
# fertilized	8.3 $\pm$ 4.0	7.6 $\pm$ 3.8	0.30

Table 3 ART outcome for the 126 cycles according to AM or PM administration

	AM (<i>N</i> = 60)	PM (<i>N</i> = 66)	<i>P</i>
#ET	1.95 ± 0.6	1.99 ± 0.7	0.77
# cryopreserved	1.45 ± 2.3	1.43 ± 2.5	0.96
IR (%)	60.3	47.2%	0.066
Chemical PR (%)	49/60 (81.7%)	42/66 (65.6%)	0.024
Clinical PR (%)	47/60 (78.3%)	41/66 (62.1%)	0.048
Twin rate (%)	16/60 (26.7%)	12/66 (18.2%)	0.27
Delivery rate (%)	41/60 (68.3%)	37/66 (56.1%)	0.16

While there are to our knowledge no prior studies on morning compared to evening administration of gonadotropins in ART, data exist regarding once-a-day administration compared to twice-a-day administration of gonadotropins. We previously demonstrated in a prospective randomized trial that once-a-day administration of gonadotropins not only has equivalent ART outcomes compared to twice-a-day administration, but also results in significant cost savings [2], not to mention patient comfort. A later study in women with polycystic ovary syndrome (PCOS) undergoing IVF confirmed our previous findings [5]. In that retrospective study evaluating 254 embryos from an unreported number of PCOS and control women, 98 embryos from twice-a-day dosing were compared to 156 embryos from once-a-day dosing; no differences were noted in terms of embryo morphology, clinical and miscarriage rates, and the incidence of twins [5], suggesting that even in women with PCOS, there are no advantages for twice-a-day administration [5].

Since once-a-day administration is adequate, is it better to administer gonadotropins in the morning or evening? It is surprising that such an important question has not been previously raised. While we failed to show a statistical difference in implantation or live birth rates, an advantage was noted with AM administration in biochemical and clinical pregnancy rates. Despite the fact that there were no differences in baseline parameters, including vitamin D, or in the amount of gonadotropin used, peak estradiol or progesterone levels, endometrial thickness, number of mature oocytes, fertilization, or blastocysts obtained, transferred, and cryopreserved, the increased biochemical and clinical pregnancy rates in the morning group are quite intriguing. It has been previously shown that maximum estradiol production occurs 6 h after a single dose of FSH administration, but falls off 12 h later [1]. Is it only because the stimulation start happened 12 h before the evening group, leading to a possible early advantage in terms of follicular dynamics, and therefore better oocyte/embryo outcome, or is it due to some other factor(s) remains to be elucidated. A previous study evaluating the

addition of an “FSH boost” (450 IU uFSH (Bravelle®) administered along with the hCG trigger) showed an improvement in fertilization rate but not in pregnancy rates [7]. That study was unfortunately not powered to detect differences in pregnancy rates but the addition of the FSH boost did result in elevated serum and follicular fluid FSH levels [6, 7]. We did not evaluate serum or follicular fluid FSH concentrations but it is possible that these concentrations would have been higher in the morning group. Studies evaluating embryo competence, such as follicular fluid analysis for proteomics and aneuploidy testing, may be quite beneficial.

Another possible explanation is whether circadian rhythm in gonadotropin secretion could affect outcomes. Klingman et al. in a provocative study, conducted under a strict 24-h constant routine protocol investigating circadian rhythms of the reproductive axis with fully controlled exogenous cues, convincingly showed that, unlike temperature and TSH, there is no circadian rhythm of LH and FSH in the early follicular phase in reproductive age women [8], effectively ruling this out as a potential cause for the observations we noted.

One of the strengths of our study is the attempt to reduce as much as possible the variability in gonadotropin administration. All patients received their gonadotropins at exact times in the AM and PM, potentially resulting in a uniform effect throughout the duration of the IVF cycle. We believe this is an important variable that needs further assessment.

Criticism of our study includes few points. First, a power analysis was not performed as the null hypothesis was used. Second, we chose to use a combination protocol of FSH and hMG since this is the practice of more than 90% of IVF programs in the USA. Our study therefore needs to be replicated in other parts of the world where monotherapy is the norm; in addition, we used uFSH along with HP-hMG, and the available FSH preparations in the USA (after the withdrawal of Bravelle®) currently only include recombinant FSH, making generalizations using a combination of rFSH and HP-hMG inappropriate. Third, the study was only in blastocyst transfer cycles, while cleavage-stage transfers are more common in other parts of the world. Fourth, the number of cycles in each arm is relatively small that was unfortunately due to the premature termination of the trial because the uFSH used was withdrawn from the market. Fifth, the mean number of embryos transferred was close to 2, resulting in a high incidence of twins. While the practice of eSET is increasing in the USA [9], it is imperative that future trials evaluate only single embryo transfers. Nevertheless, while we are encouraged by the results of our study, a larger trial in the setting of single embryo transfer is clearly needed.

Compliance with ethical standards Our center's Institutional Review Board approved the study.

Conflict of interest The authors declare that they have no conflict of interest.

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