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ASSISTED REPRODUCTION TECHNOLOGIES

The value of delaying hCG administration to enable maturation of medium-sized follicles in patients undergoing superovulation for IVF/ICSI

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Abstract

Purpose The purpose of the study is to determine whether continued stimulation of mature follicles to allow "catch up" growth of medium-sized follicles in assisted reproductive technology compromises the clinical pregnancy (CPR) and live birth (LBR) rates in IVF/ICSI cycles.

Methods This retrospective cohort study reviewed 200 first IVF ± ICSI cycles out of a total of 340 cycles with complete data. Women underwent stimulation protocols with gonadotropins (Gn) and GnRH antagonist. Treatment cycles were divided into two groups (Gp): hCG administration delayed despite the presence of two mature follicles, defined as ≥ 18 mm [Gp1, $n = 79$] and hCG administration given when there were two mature follicles [Gp2, $n = 121$].

Results The patients in Gp1 were significantly younger than those in Gp2 [32.9 (4.5) vs. 34.3 (4.8), $p = 0.04$] and needed a median of one more day of superovulation before ovulation was triggered with hCG. The extra days was associated with the use of 450 [75–2025] more Gn, such that at the time the hCG was administered, patient's in group 1 had developed significantly greater number of follicles ≥ 18 mm [mean (SD), 4.9 (1.8) vs. 3.4 (1.7), $p < 0.0001$]. The clinical pregnancy (48.1 vs. 38.0%, [OR (95% CI)] [1.6 (1.0–2.5), $p = 0.09$]) and live birth (43.0 vs. 35.5%, [1.4 (0.9–2.3), $p = 0.21$]) rates per cycle started were not significantly different between the two groups. Forward stepwise logistic regression showed that only maternal age ($p = 0.04$) influenced clinical pregnancy rates (OR = 0.88, CI 0.78–0.99) and only the number of days for superovulation influenced live birth rates (OR = 0.65, CI 0.486–0.869).

Conclusion This study demonstrated that delaying hCG administration to allow further growth of the medium-sized follicles added further days of superovulation and cost without improvement in CPR and LBR.

Keywords IVF stimulation · Superovulation · hCG · Follicles · Oocyte retrieval

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Introduction

The tremendous improvement in the success rates of assisted reproductive technology (ART) cycles, despite the transfer of increasingly fewer embryos [1], can be attributed to better superovulation protocols, embryo culture media, and embryo transfer technique. Nevertheless, the pregnancy rates still vary between infertility centers, thus warranting measures to maximize success rates. The number of oocytes collected during ART is one of the most important determinants of overall success. However, there is considerable variability in the criteria used for inducing final oocyte maturation in IVF, which reflects on the fact that the basis for such practice in part is both arbitrary and empirical.

Following superovulation, ovulation triggering is often advised when more than two or three follicles ≥ 18 mm have developed on ultrasound [2, 3]. Continued stimulation to allow medium-sized follicles to grow further may increase the

number of oocytes retrieved; however, such practice may adversely affect endometrial receptivity [3] or predispose patients to developing ovarian hyperstimulation syndrome (OHSS). The point at which the advantages of multifollicular development outweigh potential complications such as OHSS is difficult to determine [4]. Although OHSS is more likely to occur with higher estradiol levels on the day of hCG administration, this is not invariable [5]. Two studies have suggested that superovulation, with the intent to collect more oocytes to improve pregnancy and live birth rates, is futile when greater than 15 oocytes are retrieved [4, 6].

Tan and colleagues [7] evaluated the impact of delaying ovulation triggering for 1 or 2 days when criteria for hCG were met (mean diameter of the largest follicle had reached 18 mm, and at least two other follicles were greater than 14 mm) in a randomized study and found no significant differences in the clinical pregnancy rates. However, the follicular criteria used to decide when hCG was given in the study by Tan and colleagues [7] were conservative (mean diameter of the largest follicle of 18 mm and at least two other follicles > 14 mm), and hCG injections were delayed for a maximum of 2 days. Nevertheless, the ability to vary the timing of hCG administration may also allow greater flexibility in scheduling of oocyte collection to meet logistical needs of the patient and her partner as well as the ART center, without compromising the clinical pregnancy rates (CPRs) [8–12]. Reproductive endocrinologists sometimes sacrifice lead follicle(s) when the remaining cohort is further behind to allow additional follicles to reach maturity. It remains to be determined, however, whether continued stimulation of mature follicles to allow "catch up" growth of medium-sized follicles (14–16 mm) in ART compromises the CPR and live birth rates (LBRs) in IVF/ICSI cycles. The objective of this study was to determine whether continued stimulation of mature follicles to allow "catch up" growth of medium-sized follicles in ART compromises the CPR and LBR in IVF/ICSI cycles.

Materials and methods

Following approval from the Wayne State University Institutional Review Board, we undertook a retrospective analysis of 340 consecutive IVF ± ICSI cycles in 240 patients who achieved the development of at least two or more follicles (> 18 mm) between January 2003 and December 2009. Of these, 237 patients who underwent their first IVF/ICSI treatment at our institution were reviewed of which 37 cycles in 37 patients were excluded. The reasons for their exclusion are enumerated in Fig. 1. Women underwent standard stimulation protocols with gonadotropins (Gn) (Gonal-F®, Serono; and Menopur® or Repronex®, Ferring) and GnRH antagonist for pituitary

down-regulation (Ganirelix acetate, Organon). Down-regulation with GnRH antagonists is the standard protocol in our institution. Patients were started on oral contraceptives for 14–30 days prior to and were stopped 3 days before starting ovarian stimulation. GnRH antagonist (0.25 mg daily) was added when at least one follicle reached 14 mm in diameter and was continued until day of hCG administration. An additional 75 IU of LH and FSH (Menopur® or Repronex®) was added to the Gn. regimen when GnRH antagonist was started. At our center, the criteria to proceed to oocyte aspiration are the presence of at least four mature follicles between 16 and 20 mm in size with a minimum of two follicles 18 mm or greater. Treatment cycles were divided into two groups (Gp), based on the day the patients first met the criteria for hCG administration. hCG administration was delayed despite patients having met these criteria [Gp1, $n = 79$] and hCG administration given when these criteria were met [Gp2, $n = 121$]. The decision to administer or withhold hCG and for how long was based solely on the attending physician's discretion after reviewing the stimulation parameters. For the purpose of this study, the number of days that elapsed before hCG was eventually given was extracted and recorded. Oocyte collection was performed 36 h after hCG trigger. Embryo transfer was performed 2 to 5 days after oocyte collection. Implantation rate was defined as the number of gestation sacs per embryo transferred, while CPR was defined as the number of cycles with intrauterine gestational sac(s) with fetal heart pulsation at 4–5 weeks from the day of transfer. Live birth rate was defined as the number of pregnancies that resulted in live births per cycle started.

All statistical analyses were performed using Statistical Package for Social Sciences (SPSS, Version 18 for Windows; SPSS, Inc., Chicago, IL, USA). All tests were conducted using p value less than 0.05 for statistical significance. The data were expressed as mean + standard deviation (SD), and median [range] for continuous variables, and the number of cases (n) and percentage of occurrence (%) for categorical variables. Between group, differences were analyzed with chi-square test and Fisher's exact test for categorical data, and Student's t test and Mann-Whitney U test for continuous variables as appropriate. We used binary logistic regressions to model CPRs and LBRs with forward stepwise selection according to the likelihood ratio method based on inclusion criteria of $p \leq 0.05$–$p \leq 0.10$. The variables of maternal age, serum estradiol when decision was made to delay hCG administration, the total number of days of superovulation, the number of follicles ≥ 18 mm on day of hCG, the size of the lead follicle and the day of embryo transfer (Tables 1, 2, and 3), and whether the cycle was delayed or not delayed were included in the model. Statistical significance was defined as $p < 0.05$.

Fig. 1 Depicts the number of cycles included and excluded from analysis
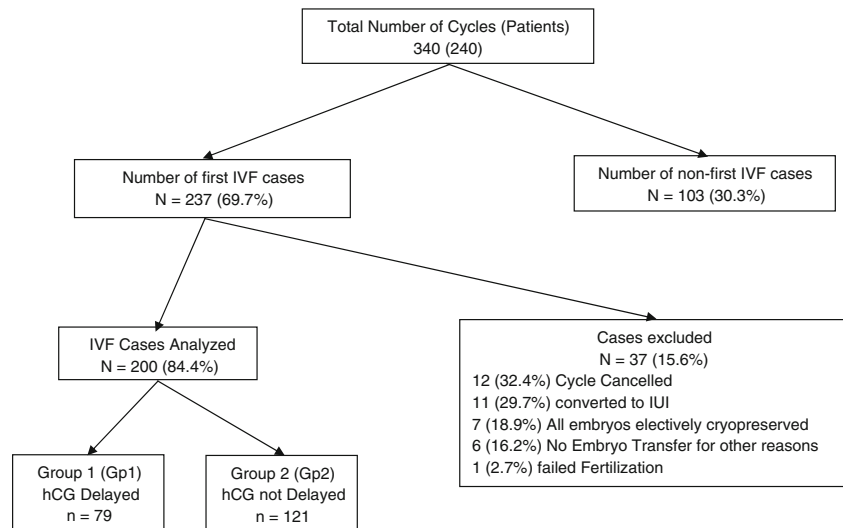
Results

During the study period, 237 women underwent their first IVF ± ICSI cycle. Of these, 37 cycles were excluded from further analyses and the reasons for exclusion are enumerated in Fig. 1. Of the remaining 200 cycles, 79 had hCG administration withheld despite the presence of 4 follicles ≥ 16 mm when 2 were ≥ 18 mm and in 121 cycles, hCG was given when these criteria were met. The overall clinical pregnancy (intrauterine sac and fetal heart activity) and livebirth rates per cycle started were 48.1 and 38.0%, respectively. Among patients who had their hCG administration delayed, a greater proportion, 68 (86.1%) needed 2 further days of superovulation, 4 (5.1%) needed 1 more day, 6 (7.6%), and 1 (1.3%) needed 3 and 4 more days, respectively. However, the clinical pregnancy ($p = 0.37$) and the livebirth ($p = 0.28$) rates did not differ in relation to the number of days the patient was delayed before hCG trigger was administered. The CPR (48.1 vs. 38.0%) and

Table 1 Baseline characteristics of patients in whom hCG administration was delayed (group 1) compared with those not delayed (group 2)

	Group 1 $n = 79$	Group 2 $n = 121$	p
Age (years)	32.9 (4.5) 33.0 [20–43]	34.3 (4.8) 34 [17–44]	0.04
BMI (kg/m^2)	27.0 (5.9)	27.9 (6.0)	0.27
Duration of infertility (months)	30.0 [4–132]	36 [5–204]	0.19
Gravidity [n (%)]			0.46
0	44 (55.7)	59 (48.8)	
1–2	29 (36.7)	47 (38.8)	
≥ 3	6 (7.6)	15 (12.4)	
Parity [n (%)]			0.25
0	62 (78.5)	87 (71.9)	
1–2	14 (17.7)	32 (26.4)	
≥ 3	3 (3.8)	2 (1.7)	
Etiology of infertility [n (%)]			0.90
Male factor	43 (54.4)	60 (49.6)	
Tubal	15 (19.0)	28 (23.1)	
Ovulatory dysfunction	14 (17.7)	19 (15.7)	
Unexplained	4 (5.1)	8 (6.6)	
Others	3 (4.5)	6 (5.0)	
Day 3 FSH (mIU/L)	7.5 (2.4)	7.1 (3.3)	0.53
Day 3 estradiol (pg/mL)	41.1 [11.0–169.0]	41.3 [20.0–249.0]	0.83
Antral follicle count	13 [2–40]	11 [0–43]	0.15

Unless otherwise stated, results are mean (SD) or median [range]

Table 2 Superovulation parameters in patients in whom hCG administration was delayed (group 1) compared with those not delayed (group 2)

	Group 1 $n = 79$	Group 2 $n = 121$	p
Parameters when hCG withheld	hCG withheld	hCG not withheld	
E2 at decision to delay (pg/mL)	1692.3 (728.1)	2431.3 (1012.3)	< 0.0001
Follicles 14–17 mm	6.1 (2.8), 6 [0–14]	12.0 (7.5), 6 [0–34]	< 0.0001
Follicles 17 mm	1.5 (1.0), 1 [0–5]	1.6 (1.3), 2 [0–8]	0.43
Follicles > 18	2.0 (1.0), 2 [0–4]	3.5 (1.6), 3 [0–13]	< 0.0001
Extra days on Antagonist	2.1 (0.4), 2 [1–4]		
Extra days on Gn	1.4 (0.5), 2 [1–4]		
Extra dose of Gn used (IU)	450 [75–2025]		
Parameters on the day hCG was administered			
Gn (IU) used for superovulation	3563 [1238–14,625]	3525 [1162–17,025]	0.30
Number of days of superovulation	9.9 (1.8)	10.3 (1.8)	0.09
Serum estradiol (pg/mL)	2443.9 (830.1)	2436.8 (1013.0)	0.93
Total number of follicles	20.2 (10.0)	21.9 (10.8)	0.29
Number of follicles > 18 mm	5.0 (1.9)	3.4 (1.6)	< 0.0001
Number of follicles 14–17 mm	6.1 (2.9)	13.0 (6.6)	< 0.0001
Size lead follicle (mm)	22.3 (2.3)	20.2 (1.6)	< 0.0001
Endometrium thickness (mm)	10.6 (2.4)	10.0 (2.1)	0.12
Day hCG trigger was given	10.7 (1.7)	10.7 (1.9)	0.78

Unless otherwise stated, data are in mean (standard deviation) and median [range]

n = number

LBR (43.0 vs, 35.5%) were higher in the delayed compared with the non-delayed group; however, the odds ratio [OR (95% CI)] of achieving a clinical pregnancy [1.56 (1.00–2.52)] and live birth [1.39 (0.85–2.27)] was not significantly different. Apart from the age of patients, there were no significant differences in demographic variables (body mass index, duration or etiology of infertility, gravidity, and parity) and baseline measurements (day 3 serum FSH, E_2, and antral follicle count) between the two groups (Table 1).

At the time hCG was administered to patients in group 2 and withheld in patients in group 1, the mean serum estradiol,

and number of follicles that measured 14–17 mm and those that were > 18 mm were significantly lower in group 1 compared with group 2 ($p < 0.0001$) (Table 2). The patients in group 1 needed a median of two [1–4] extra days of antagonist, and one [1–4] more day of superovulation, which was associated with the use of 450 IU [75–2025] more of Gn, to catch up and overtake their group 2 counterparts in the number of follicles ≥ 18 mm at the time of hCG. However, on the day hCG trigger was administered, the total dose of Gn used for superovulation in group 1 compared to that in group 2 was not statistically significant. Also, not significantly different on the

Table 3 Treatment outcome in patients in whom hCG administration was delayed (group 1) compared with those not delayed (group 2)

	Group 1 $n = 79$	Group 2 $n = 121$	p
Number of oocytes collected	15 [4–34]	14 [2–40]	0.89
Percentage of oocyte collected per follicle aspirated	72.0 (21.1)	69.2 (21.0)	0.38
Percentage of mature oocytes per oocyte collected	80.5 (14.2)	81.1 (15.4)	0.76
Fertilization rate	61.5 (18.6)	62.2 (20.0)	0.79
Number of embryo(s) transferred	2.9 (0.9)	2.8 (1.1)	0.82
Number of embryos cryopreserved	5 [0–32]	4 [0–27]	0.46
Day of embryo transfer	3.1 (0.6)	3.0 (0.4)	0.10
Moderate/severe OHSS [n, (%)]	3 (3.8)	0 (0.0)	0.06
CPR per cycle [n, (%)]	38 (48.1)	46 (38.0)	0.19
Live birth per cycle [n, (%)]	34 (43.0)	43 (35.5)	0.30

Unless otherwise stated, data are in mean (standard deviation) and median [range]

n = number

day of hCG trigger were the numbers of days required for superovulation, the total number of follicles that developed, and the E_2 levels. However, the lead follicle was significantly larger ($p < 0.0001$) and the number of follicles ≥ 18 mm significantly higher in group 1 compared with that in group 2 (Table 2). Consistent with the development of a comparable total number of follicles, the number of oocytes collected and the percentage that were mature, fertilization rates, and the number of embryos transferred or cryopreserved were not significantly different between the two groups. Similarly, the CPR and LBR were not significantly different between the two groups (Table 3). However, when we performed forward stepwise logistic regression in order to determine whether any of the significant differences seen between the two groups influenced the CPRs and LBRs, only maternal age ($p = 0.04$) influenced CPRs (OR = 0.88, CI 0.78–0.99) and only the number of days for superovulation influenced LBRs (OR = 0.65, CI 0.486–0.869).

Discussion

In the present study, delaying hCG to allow further growth of medium-sized follicles did not result in any statistically significant difference in either clinical pregnancy rate or live birth rate. Logistic regression analysis controlling for confounding variables suggest that the only factor contributing to differences in CPR was maternal age, while only the length of superovulation influenced the LBR. Our data suggest that for each one-unit increase in maternal age, there is a 12% decrease in the odds of clinical pregnancy, and for each additional day of superovulation, there was a 35% decrease in the odds of a live birth. A major challenge in reproductive endocrinology has been to develop stimulation protocols that will "ideally" synchronize the development of a mature cohort of follicles. However, the advent of GnRH agonists [13], and antagonists [14], to prevent spontaneous LH surges and spontaneous ovulation has allowed flexibility in the timing of hCG administration. These have resulted in greater flexibility in allowing further growth of medium-sized follicles before hCG administration. Furthermore, the recovery of multiple fertilizable oocytes allows for cryopreservation of extra or surplus embryos, with the consequent reduction in the risk of multiple pregnancies and the improvement in the cumulative pregnancy rate following IVF cycles. In the present study, however, the number of embryos cryopreserved did not defer between those that had compared with dose that did not have their hCG trigger delayed.

In the present study, all patients underwent stimulation protocol using Gn and GnRH antagonist. There was no statistical significant difference in demographics apart from maternal age, the ovarian reserve parameters, and the etiology of infertility. Similar to previous studies on delaying hCG

administration, the total number of follicles that developed, the number of oocytes collected [7, 15], fertilization rates, and the number of embryos transferred were not significantly different between groups [7, 16]. Whereas, others [16] have found that a significantly higher dose of Gn was required for superovulation with a corresponding higher E_2 levels on the day of hCG in the delayed group; we found no such association in this study. The findings in our study are at variance with those of Tan et al. [7] who found similar pregnancy rates in patients whose stimulation continued for 1 and 2 days. Similarly, unlike the assertion by Clark et al. [15] that prolongation of follicular stimulation by 1 day and Kolibianakis et al. [16], by 2 days, significantly reduced the CPR, we found that only maternal age decreased CPR. The present study represents an advancement of the work by Tan and colleagues [7] as their study contained a relatively small number of patients (79, 84 and 84 in their 3 groups); the criteria for giving hCG was conservative (3 follicles measuring > 14 mm when the largest is 18 mm) and hCG injections were delayed for a maximum of 2 days. Our study also advances on the publications by Clark et al. [15] and Kolibianakis et al. [16], as we reported on live birth rate and we showed that only duration of superovulation influenced the LBRs.

The differences between our study and those of others could be attributed to the use of different stimulation protocols [15]. Even with the use of Gn and GnRH antagonists for IVF, the criteria used for inducing final oocyte maturation during ovarian stimulation vary markedly among investigators, making comparisons of results difficult. Whereas some have used a combination of follicular size and E_2 levels [17, 18], most studies administered hCG when greater than or equal to three follicles ≥ 17 mm are present on ultrasound [2, 3]. Other studies trigger final oocyte maturation in the presence of greater than or equal to three follicles with a maximum diameter of 18 mm [19] or in the presence of greater than or equal to one follicle of ≥ 18 mm and three follicles of ≥ 15 mm [20]. In contrast, we used the presence of two mature follicles with a mean diameter > 18 mm with two additional follicles ≥ 16 mm as the criterion for hCG administration. Also, previous studies on delaying hCG administration delayed ovulation triggering for a specific number of days (1 or 2 days) [7, 15, 16]. In our study, hCG trigger was delayed between 1 and 4 days, per attending physician discretion. The specific reason for delaying hCG administration could not be determined on chart review; however, the proportion of cycles that were delayed were not significantly different among the two physicians that performed most of the oocyte retrievals during the study period (EP 52/126 (41.3%) and FY 26/64 (40.6%)).

The development of moderate to severe ovarian hyperstimulation syndrome (OHSS) is a concern when further stimulation continues in patients that have met the criteria for hCG as stipulated by individual units' protocol. In our study, 3.8% of our patients developed moderate to severe OHSS in delayed

group, compared to none in the non-delayed group, although the difference was not statistically significant. At the time hCG was withheld in group 1, the E_2 was significantly lower compared to group 2. This situation was reversed by the time hCG was eventually administered to patients in group 1. It is conjectural to speculate whether the OHSS that developed in this group could have been prevented given that no advantage was gained by delaying administration of hCG in this group in terms of CPR and LBRs after accounting for all the confounding variables. The fear that continuing ovarian superovulation and further rise in serum E_2 may put patients at high risk of OHSS should be another reason to avoid further ovarian stimulation once the criteria for administrating hCG trigger are reached.

Our study results are at variance with the finding of an earlier randomized study that suggested that a window of at least 3 days exists during which hCG can be given [7] without compromising the LBR. We found that an increase in the number of days of superovulation decreased the odds of livebirth by 35% after adjusting for confounding variables. Therefore, the practice of delaying hCG to allow further growth of medium size follicles when criteria for administering hCG has been met should be discouraged. Two studies [4, 6] have suggested that only approximately 31–38% of oocytes become useable embryos for transfer or cryopreservation, and that LBR is not necessarily increased when more than 15 oocytes are retrieved. The median number of oocytes collected in our study is in keeping with this suggestion. Therefore, the aim should be to stimulate patients with intent to collect not more than this number of oocytes. However, others have gone further and suggested that with good IVF laboratory conditions if a patient reaches embryo transfer the number of eggs retrieved should not affect the likelihood of pregnancy outcome [21].

A limitation of this study is its retrospective design with potential for missing data. In addition, no systematic criteria were applied to determine when to and who to delay before hCG was administered as this was done at the discretion of the attending physician taking care of the patient. However, the proportions of cycles that were delayed were not different among physicians who performed the patients' oocyte collections. Another limitation is the inability to evaluate the criteria used to determine the day of transfer. However, the default day of transfer at the time of this study was day 3 (180, 90.0%) with only a few patients having their transfer on day 2 (13, 6.5%) and day 5 (7, 3.5%). This variable did not influence CPR and LBR when included in the logistic regression model.

In conclusion, slightly asynchronous follicular development does not compromise CPR and LBR in IVF/ICSI cycles. Delaying hCG administration to allow further growth of medium-sized follicles should be discouraged as it added further days of superovulation and increases cost of treatment without improvement in CPR and LBR. Only maternal age

and the number of days for superovulation influenced CPR and LBR, respectively, in this study.

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