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Original Research

Oocyte yield of GnRH antagonist cycles scheduled with a short course of estradiol in the early follicular phase

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Scheduling in vitro fertilization cycles enables planning oocyte retrieval and embryology procedures in order to suit both patients' and medical staff's needs. Current methods to schedule ovarian stimulation cycles are either cumbersome, costly or provide minor flexibility. The aim of this study was to investigate if scheduling gonadotropin releasing hormone (GnRH) antagonist cycles with a short course of estradiol in the early follicular phase affects oocyte yield. Fifty-nine oocyte donors undergoing two GnRH antagonist stimulation cycles within 6 months, one with and one without follicular phase estradiol scheduling (FES), serving as their own control were included in this retrospective cohort study. FES was achieved by giving 6 mg/day estradiol valerate orally from the 2^{nd}--3^{rd} day of menstrual cycle until the desired day of gonadotropin start. Main outcome measures were number of cumulus oocyte complexes and metaphase two oocytes. A total of 118 cycles, 59 FES and 59 unscheduled GnRH antagonist, were included. Median duration of estradiol administration was 3 days in FES cycles. In the FES group, stimulation lasted significantly longer by one day (11 vs 10 days, P = 0.03) and total gonadotropin consumption (2497 vs 2404 IU, P = 0.03) was statistically significantly higher, albeit minimal absolute difference, which is probably short of clinical significance. Numbers of COC (21 vs 20) and metaphase-two oocytes (17 vs 17) were similar between the two groups. In conclusion, FES does not require planning in advance and involves shorter use of estradiol/oral contraceptive tablets and can be advantageous to scheduling with luteal estradiol/oral contraceptive administration.

Keywords

Assisted reproduction; Cycle scheduling; Estradiol; GnRH antagonist; In vitro fertilization

1. Introduction

Scheduling IVF cycles enables planning oocyte retrieval (OR) and embryology procedures in order to suit both patients' and medical staff's needs. While the commonly used Gonadotropin releasing hormone (GnRH) antagonist protocols require less injections than the long luteal gonadotropin releasing hormone agonist (GnRHa) protocol, the former is less flexible with regard to timing of OR. Two facts limit the flexibility of GnRH antagonist protocols, (i) gonadotropin injections must be started within the first 3 days of the menstrual cycle as opposed to any day during the constant down regulated state in the long GnRHa protocol, (ii) the ovulation trigger cannot be delayed further than one day after the follicles reach triggering criteria, without impairing implantation rates [1].

Several strategies have been employed to increase the flexibility of GnRH antagonist cycles. These include: (i) starting gonadotropin injections on the 2nd or 3rd day of the menstrual cycle [2]. (ii) programming the stimulation cycle with administration of combined oral contraceptives (COC) [3]. (iii) delaying gonadotropin start by the use of estradiol and/or GnRH antagonists from the luteal phase of the preceding cycle, and (iv) advancing or delaying ovulation trigger by one day [4]. Despite successful scheduling of the stimulation cycle and OR, these methods have several downsides, e.g., providing only limited flexibility of 1–2 days (methods i and iv), impairing implantation rates (method ii), increasing the gonadotropin consumption and/or number of GnRH antagonist injections (methods ii and iii), or requirement for advance planning or monitoring endogenous LH surge (method iii).

Given the drawbacks and limitations of the current scheduling methods in GnRH antagonist cycles, we pursued other options. Oral estradiol rapidly and reversibly suppresses endogenous follicle stimulating hormone (FSH) secretion and halts follicular growth [5]. In a retrospective cohort study involving 35 women who underwent cycle scheduling with oral estradiol administration during the early follicular phase and 35 controls without scheduling, we reported successful delay of gonadotropin start for a median duration of five days (quartiles 4–7 days) without a significant change in gonadotropin consumption, oocyte yield, implantation and pregnancy rates. Women who had their cycles scheduled by this method consumed only one additional GnRH antagonist injection on average [6]. We call this method follicular phase estradiol scheduling (FES). As such, FES seemed an easy, inexpensive and efficient method of

scheduling GnRH antagonist cycles. In this study, we aimed to investigate if scheduling GnRH antagonist cycles with FES affected oocyte yield by comparing oocyte donors who underwent two GnRH antagonist cycles, one with FES and one without.

2. Materials and methods

The protocol of this retrospective cohort study was approved by the Near East University School of Medicine Research Ethics Committee (Reference number: YDU-2019/66-751).

Electronic records of women who donated oocytes at Dunya IVF Clinic (Kyrenia, Cyprus) between January 2015 and September 2018 were screened to identify those who underwent two ovarian stimulation cycles with and without FES within six months. Thus, each donor served as her own control. Only women aged between 20 and 30 years who had no medical contraindication to oocyte donation were recruited as oocyte donors. In general, an antral follicle count of ≥ 20 was required to become an oocyte donor. Prior to recruitment, all women underwent routine screening for infectious diseases in accordance with the international guidelines [7]. In addition, all women underwent karyotype analysis and screening for thalassemia carrier status.

All women underwent a baseline scan on the second or third day of menstrual bleeding to exclude follicles larger than 12 mm or ovarian pathology that would preclude ovarian stimulation. Gonadotropin injections were started immediately in unscheduled cycles. In programmed cycles, 17 beta-estradiol valerate tablets (Estrofem, Novo Nordisk, Denmark) were administered orally at a dose of 6 mg/day in three divided doses until the scheduled date of starting gonadotropin injections. Estradiol was stopped and a second scan was done to ensure there were no growing follicles $>$ 12 mm at the time of gonadotropin start in FES cycles.

Gonadotropin starting dosage ranged between 225 and 300 IU/day (Gonal F, Merck Serono, Switzerland). Dose adjustments were made according to follicle measurements and serum estradiol levels. GnRH antagonist (Cetrotide, Merck-Serono, Switzerland) injections were commenced at a dose of 0.25 mg/day when the leading follicle reached a diameter of 14 mm. When two follicles reached a diameter of $>$ 17 mm and the majority of growing follicles were $>$ 14 mm, 1 mg leuprolide acetate (Lucrin Daily, Abbott, United States) was given subcutaneously to induce final oocyte maturation.

Transvaginal OR under general anesthesia was done 36 hours after leuprolide acetate injection. Oocyte maturation was assessed following denudation and all metaphase II oocytes were either fertilized by intracytoplasmic sperm injection or cryopreserved for oocyte banking.

All treatment procedures including ovarian stimulation and OR were done by the same attending physician (B Angun). B. Ata, B.U., S.Y., E.T. did not participate in the recruitment or clinical care of the oocyte donors or their recipients.

Table 1. Baseline characteristics of oocyte donors (n = 59).

Age (years)	23.17 (2.75)
Body Mass Index (kg/m^2)	21.55 (3.56)
Antral Follicle Count	33.25 (10.62)

Values are mean (standard deviation).

Outcome measures were determined as total gonadotropin and GnRH antagonist consumption, the duration of stimulation, number of cumulus oocyte complexes and metaphase two oocytes were compared between the cycles. Since some of the oocytes retrieved were cryopreserved, we were not able to compare fertilization, implantation and pregnancy rates.

Distribution of variables was evaluated visually with histograms. Continuous variables were defined with mean (standard deviation) or median (25^{th}–75^{th} percentile) depending on distribution characteristics. Categorical variables were defined with numbers and percentages. Continuous variables were compared between the groups with paired samples t-test or Wilcoxon signed rank test as appropriate. Categorical variables were compared with chi-square test and its derivatives as appropriate. A two-sided P value < 0.05 was considered statistically significant.

Sample size calculation was not done for this retrospective analysis, but all cycles meeting the inclusion criteria since the start of using FES in Dunya IVF Center were included in the analysis.

3. Results

Fifty-nine oocyte donors with an average age of 23 years and BMI of 21.6 kg/m^2 were included in the study with 59 FES and 59 unscheduled GnRH antagonist cycles. Characteristics of donors are shown in Table 1.

Median duration of estradiol administration was 3 days (25^{th}–75^{th} percentile, 2–4) in FES cycles. The longest duration of estradiol use was 7 days.

Median gonadotropin starting dosage was similar at 225 IU/day in FES and unscheduled cycles. In the FES group, stimulation lasted significantly longer by one day (11 vs 10 days, $P = 0.03$) and total gonadotropin consumption (2497 vs 2404 IU, $P = 0.03$) was statistically significantly higher, albeit with a minimal absolute difference, which is probably short of clinical significance. Number of GnRH antagonist injections were similar between FES and unscheduled cycles (5 vs 5). Numbers of COC (21 vs 20) and metaphase two oocytes (17 vs 17) were similar between the two groups. Results are shown in Table 2.

4. Discussion

We compared FES and unscheduled GnRH antagonist cycles of young oocyte donors. Despite small differences between total gonadotropin consumption and duration of stimulation, numbers of COC and metaphase two oocytes were similar in FES and unscheduled cycles, suggesting FES is a feasible method to schedule GnRH antagonist cycles.

Table 2. Stimulation cycle characteristics and outcomes.

Outcome Measure	Estradiol scheduled cycles (n = 59)	Control cycles (n = 59)	*P* value
Days of estradiol priming*	3 (2–4)	0	
Starting gonadotropin dose*	225 (225–225)	225 (225–225)	1
Duration of stimulation in days*	11 (10–11)	10 (10–11)	0.03
Total gonadotropin dosage in units**	2497 (338)	2404 (290)	0.03
Total number of GnRH antagonist injections*	5 (4–6)	5 (5–6)	0.28
Number of cumulus-oocyte complexes*	21 (18–29)	20 (17–27)	0.93
Number of metaphase-two stage oocytes*	17 (16–21)	17 (16–20)	0.98

GnRH, Gonadotropin releasing hormone.

* Values are median (25^{th}–75^{th} percentile).

**Values are mean (standard deviation).

GnRH antagonists have replaced the long protocol due to advantages such as less injections, less gonadotropin consumption, the opportunity of GnRH agonist triggering, and decreased risk of ovarian hyperstimulation syndrome (OHSS) [8]. However, an important disadvantage of GnRH antagonist cycles is they are less flexible for scheduling oocyte retrieval as compared with the long GnRH agonist protocol.

Several strategies have been used to increase the flexibility of GnRH antagonist cycles. The simplest modification is starting gonadotropin injections on the second or the third day of menstrual cycle, which might allow advancing or delaying ovulation trigger by only one day, without compromising clinical outcome [2]. Another simple alternative is advancing or delaying the ovulation trigger by one day [4]. A well-designed retrospective study convincingly suggests that clinical outcome is unaffected by this approach [4]. However, a randomized controlled trial (RCT) of 413 patients who underwent GnRH antagonist protocol showed that delaying the hCG injection for two days after reaching the criterion of 3 follicles $\geq$ 17 mm, resulted in significantly lower ongoing pregnancy rates compared to cycles triggered without delay [1]. Thus, this method can only provide one day flexibility.

Scheduling gonadotropin start by using oral contraceptives (OCP) in the preceding cycle is another option. However, whether OCP scheduling adversely effects pregnancy rates in GnRH antagonist cycles is controversial [3, 9, 10]. Moreover, the patients have to take OCPs for at least 14 days and advance planning is required.

GnRH antagonists can be used for suppressing endogenous FSH during the early follicular phase and to schedule GnRH antagonist cycles [11]. An RCT including 33 women who were given three daily GnRH antagonist injections from the second day of menstrual cycle before starting gonadotropins and 36 women who underwent unscheduled GnRH antagonist cycles reported similar numbers of retrieved oocytes (12.8 vs 9.9) and ongoing pregnancy rates (42% vs 36%) [11]. However, this method requires more GnRH antagonist injections, increasing both cost and discomfort for patients.

Luteal estradiol priming (LEP) has been also proposed for cycle scheduling. The rationale behind LEP is to inhibit the luteo-follicular FSH increase until the desired day of gonadotropin start. An RCT including 472 women undergoing ovarian stimulation showed that cycles following LEP required higher total FSH dose and longer duration of stimulation without affecting live birth rates (26.6% vs 30%) [12]. Likewise, in an RCT including 86 women, LEP with oral estradiol valerate resulted in comparable clinical pregnancy rates (CPR) with unscheduled cycles (38.6% vs 38.1%, respectively) [13]. In another study, transdermal estradiol patches and GnRH antagonist injections starting from the preceding luteal phase were given to poor responders in an attempt to synchronize the follicular cohort and improve cycle outcome [14, 15]. Women receiving LEP with transdermal estradiol had similar CPR when retrospectively compared with similar women stimulated with microdose flare-up protocol (36.8% vs 23.7%, respectively, P = 0.3) [15]. Clearly, LEP allows scheduling GnRH antagonist cycles without impairing cycle outcome [12, 13, 16].

With FES, we suggest another option using oral estradiol valerate. We first described FES of GnRH antagonist cycles with 6 mg/day estradiol administered orally from cycle day 2 until (including) one day before the scheduled start of stimulation [6]. In a retrospective analysis of 70 women, we demonstrated similar numbers of oocytes collected (10 vs 10) with similar gonadotropin consumption and CPRs (48.6% vs 37.1%, in FES and control cycles, respectively, P = 0.33) with FES [6]. Whether suppressing the already increased endogenous FSH levels during the early follicular phase results in a decreased oocyte yield or pregnancy rates by causing atresia or impairment of the developmental competence of follicles that have become FSH dependent can be questioned. While designing this new scheduling protocol, we thought that the continuous nature of follicle recruitment in humans would allow compensation for such possible loss of FSH dependent follicles, by the FSH independent recruitment of other follicles during the suppression period [6]. Our present and previous findings, as well as other studies involving FSH suppression during the follicular phase support this idea [6, 11, 17]. FES requires less estradiol exposure and tablets as compared with LEP.

Retrospective rather than randomized controlled design can be regarded as a limitation. However, the sole purpose of randomization is to ensure that the study arms are similar for important baseline characteristics, and each oocyte donor serving as her own control also provides such similarity in our study. The period between the two cycles of the same donor was shorter than six months to prevent a negative effect of time for the second cycle. Neither the physician nor the oocyte donors were blinded to the intervention; however, since the intervention was given as part of routine clinical practice in order to meet the scheduling needs of both parties, we do not think their management was biased by the knowledge of treatment. Furthermore, at the time of oocyte retrieval, the embryologists were not aware of the scheduling status of donors and the medical team aimed to collect the maximum number of oocytes in each cycle. Finally, the absolute differences between numbers of oocytes and metaphase two oocytes in the two groups are minimal to absent, relieving concerns regarding sample size to an extent.

We are unable to report pregnancy or live birth rates because (i) in some cycles oocytes were shared between recipients, (ii) embryos derived from these oocytes were not transferred immediately and a substantial proportion of the oocytes have been banked for future use. However, previous studies including RCTs of estradiol pretreatment starting even in the luteal phase did not suggest impaired cycle outcomes [12, 13, 15, 16]. Likewise, in our previous study assessing the clinical outcome of FES in women undergoing Assisted reproductive technologies (ART) with own oocytes, CPR was higher in the FES group, however, the difference was not significant (49% versus 37%) [6]. Overall, available evidence suggests estradiol exposure, starting even in the preceding luteal phase, do not seem to impair clinical pregnancy/live birth rates. Thus, we can expect recipients of the oocytes from FES cycles or women undergoing ART with own oocytes following FES can expect similar success with using oocytes from unscheduled cycles. Still, it should be noted that our study involves healthy women with high ovarian reserve and efficacy of FES should be validated in other populations such as women with low ovarian reserve or women with various infertility etiologies.

Last of all, although oral estradiol dose suggested in FES is expected to increase serum estradiol levels only mildly compared to ovarian stimulation, it will prolong the estrogen exposure duration and it may be safer to avoid FES in patients with estrogen-dependent diseases such as breast or endometrial cancer.

5. Conclusions

In conclusion, FES seems to be an efficient, easy, and inexpensive method for scheduling GnRH antagonist cycles. RCTs would provide more convincing evidence if they report similar results.

Author contributions

ET contributed to analysis and interpretation of data, drafting the manuscript, and final approval. SY contributed to study design, drafting of the manuscript, and final approval. BA contributed to data acquisition, critical revision of the manuscript and final approval. BU contributed to interpretation of data, critical revision of the manuscript and final approval. BA contributed to study design, analysis and interpretation of data, critical revision of the manuscript, and final approval. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The protocol of this retrospective cohort study was approved by the Near East University School of Medicine Research Ethics Committee (Reference number: YDU-2019/66-751).

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Conflict of interest

The authors declare no conflict of interest.

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