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Dual oocyte retrieval and embryo transfer in the same cycle for women with premature ovarian insufficiency

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Synopsis: For women with premature ovarian insufficiency, rescuing spontaneously growing ovarian follicles early in the follicular phase before commencing ovarian stimulation might improve reproductive outcomes.

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Keywords: Dual oocyte retrieval; Follicular waves; Ovarian stimulation; Premature ovarian insufficiency; Random start

Abstract

Objective: To compare dual oocyte retrieval with minimal ovarian stimulation and embryo transfer in the same menstrual cycle versus conventional ovarian stimulation among women with premature ovarian insufficiency (POI).

Methods: A retrospective study of 51 women with POI attending a reproductive center in Turkey between 2013 and 2015. Women with an ovarian follicle of 12 mm or larger early in the follicular phase who underwent oocyte retrieval followed by an immediate cycle of ovarian stimulation (group 1, n=14) were compared with those who received conventional ovarian stimulation (group 2, n=37). Both groups underwent subsequent ovarian stimulation cycles to obtain two embryos for transfer.

Results: The groups had similar baseline parameters. Serum estradiol was higher in group 1 (P<0.001); total number of oocyte retrievals was higher in group 2 (P<0.001); and total number of oocytes retrieved was similar (P=0.192). Group 1 had more higher-quality embryos (P=0.031). There was a non-significant trend toward higher live birth rates in the dual trigger group (28% vs 8%, P=0.08).

Conclusion: Rescuing growing follicles early in the follicular phase combined with subsequent ovarian stimulation and embryo transfer in the same cycle resulted in fewer oocyte retrieval cycles and might potentially improve reproductive outcomes.

1 INTRODUCTION

A clearer understanding of the dynamics of human ovarian folliculogenesis may have a considerable influence on ovarian stimulation protocols in assisted reproductive technology (ART). In particular, the theory of developing follicular waves, which considers that follicles are recruited in cohorts continuously throughout the menstrual cycle, might promote individualized stimulation protocols, especially for women with diminished ovarian reserves, such as in the case of premature ovarian insufficiency (POI) [1, 2]. POI is a severe form of ovarian dysfunction characterized by oligomenorrhea or amenorrhea, elevated gonadotropin levels, and a deficiency of sex steroids. It affects 1%–3% of women under the age of 40 years. These women often respond poorly to ovarian stimulation [3].

In ART, the main goal of ovarian stimulation is to develop multiple follicles [4]. Unfortunately, multi-follicular growth is rarely obtained in cases of POI, and there is no consensus on how to stimulate follicular growth for women affected by this disorder [5]. Strategies such as random start ovarian stimulation are based on the continuous waves of developing follicles during the menstrual cycle that can be rescued from atresia by commencing ovarian stimulation [6]. Taking advantage of these follicular waves can result in a higher yield of oocytes, which is a key factor for treatment success.

The study institute has developed a protocol of dual oocyte retrieval based on experience obtained from early follicular phase oocyte retrieval studies [7] and from random start ovarian stimulation protocol used for emergency fertility preservation in oncology patients [6]. The aim of the present study was to assess the effectiveness

of early follicular phase aspiration of larger follicles and initiation of minimal ovarian stimulation after oocyte collection to recruit multiple oocytes for women with POI, and to compare this “dual oocyte collection” approach with traditional ovarian stimulation in which follicles are aspirated in the late follicular phase. The hypothesis was that dual oocyte retrieval would result in higher numbers of oocytes, hence improving reproductive outcomes for women with POI.

2 MATERIALS AND METHODS

In a retrospective study, data were reviewed from women with POI who underwent ART at the Clinart IVF center, Trabzon, Turkey between January 1, 2013, and December 31, 2015. The study was approved by the local ethical committee of ClinArt International Hospital (study no. 000298/2015). Written informed consent was obtained from all participants.

All women included in the study were diagnosed with POI by the following criteria: elevated serum follicle-stimulating hormone (FSH) levels higher than 40 IU/L, up to two ovarian follicles (2–9 mm) at the baseline pelvic scan, presence of oligomenorrhea/amenorrhea, and low levels of anti-Müllerian hormone (<0.30 pg/mL).

For the present study, women with POI who had follicles of 12 mm early in the menstrual cycle and underwent a dual oocyte retrieval protocol were included in group 1, whereas women who underwent a classical ovarian stimulation protocol, including clomiphene citrate and gonadotropins, were included in group 2. For all women, efforts were made to obtain at least two embryos for the best chance of

clinical pregnancy; as a result, any available embryos were vitrified and subsequent ovarian stimulation cycles were performed as needed. If two embryos were not obtained, however, only one embryo was transferred.

For dual oocyte retrieval with minimal stimulation in the same cycle, women underwent transvaginal ultrasound on day 2 or 3 of the menstrual cycle, and those with at least one follicle of 12 mm or larger were offered oocyte collection if their serum estrogen level was at least 150 pg/mL. In this case, a single dose of recombinant gonadotropin (225 IU; Gonal-f, Merck, Modugno, BA, Italy) was administered, followed by recombinant human chorionic gonadotropin (hCG; 10 000 IU; Pregnyl, Schering-Plough, Kenilworth, NJ, USA) the same evening. Oocyte retrieval was performed 35 hours after hCG injection.

The collected oocytes were fertilized and vitrified at the cleavage stage. Letrozole (Femara, Novartis Pharmaceuticals, Schaffhabserstrasse/Tein, Switzerland) was administered at a dose of 2.5 mg/day twice daily starting on day 3 of the menstrual cycle for minimal ovarian stimulation in the same cycle. Follow-up scans after oocyte collection were carried out on day 5 of letrozole administration (i.e., day 8 of the cycle), and every 2–3 days thereafter. When a follicle measuring 16 mm or more was observed, a single dose of 225 IU of Gonal-f and a second hCG injection were administered, and a second oocyte collection was performed 35 hours later. If the second oocyte collection was successful and the retrieved mature oocyte was fertilized, then both fresh and vitrified-warmed embryos were transferred. Otherwise, either a fresh or vitrified-warmed embryo was transferred, depending on when it was obtained. The dual oocyte pick-up cycle is summarized in Box 1.

For the classical ovarian stimulation protocol, women underwent transvaginal ultrasound on day 2 or 3 of the menstrual cycle, and clomiphene citrate combined with 150–225 IU of Gonal-f was begun in the absence of an ovarian cyst. hCG was administered when the follicles reached 17 mm in size, and oocytes were collected and fertilized. If the first collection failed to achieve at least two embryos, another ovarian stimulation cycle was initiated immediately after, until two embryos were obtained.

Oocyte collection was performed under mild general sedation (no intubation) using Propofol (Fresenius Kabi, Bad Homberg, Germany) and Sevofluorane (Aesica Queenborough, Queenborough, UK). All women received information about the sedation and possible adverse effects.

Intra cytoplasmic sperm injection (ICSI) was used to fertilize the oocytes. Fertilization was confirmed by the presence of two pronuclei and two polar bodies 18–24 hours after injection. Embryos were cultured until day 3 and were classified as grade 1, those with even blastomeres and no cytoplasmic fragments; grade 2, those with even blastomeres and minor cytoplasmic fragments or blebs; or grade 3, those with uneven blastomeres and no or few cytoplasmic fragments [8]. Embryos were transferred as either fresh, vitrified-warmed, or both fresh and vitrified-warmed when two embryos were available.

The luteal phase was supported with 8 mg/day of oral estrogen (Estrafem, Novo Nordisk, Bagsværd, Denmark) and twice daily intramuscular injections of 50 mg of progesterone in oil (Progestan 50 mg; Koçak Farma, Istanbul, Turkey). Serum β-hCG

was measured 12 days after embryo transfer. Clinical pregnancy was considered as the presence of an intra-uterine gestational sac. An ongoing pregnancy was defined as a pregnancy beyond 12 weeks of gestation. Live birth was defined as delivery at or after 24 weeks of gestation.

Data were analyzed by using SPSS version 18 (IBM, Armonk, NY, USA). The distribution of continuous variables was assessed by Shapiro–Wilk test and the data were presented as mean ± SD or median (range) as appropriate. Student *t* and Mann–Whitney *U* test were used to compare continuous variables. Pearson χ^2 test and Fisher exact test were used to compare categoric variables. A *P* value of less than 0.05 was considered statistically significant.

3 RESULTS

During the study period, 71 women with POI were treated at the study center. Of these, 21 had a follicle of 12 mm early in the menstrual period and underwent dual oocyte retrieval, and 50 underwent classical ovarian stimulation. Overall, 20 women (7 and 13, respectively) were excluded from the final analysis owing to a failure to retrieve oocytes or achieve viable embryos.

Demographic characteristics and baseline hormonal values of the 51 women included in the analysis are summarized in Table 1. The two groups were similar in age, duration of infertility, body mass index, basal antral follicle count, basal luteinizing hormone, anti-Müllerian hormone, progesterone, prolactin, and thyroid function tests. As expected, basal estrogen was significantly higher in the dual oocyte

retrieval group owing to the presence of growing ovarian follicles at the beginning of the menstrual cycle.

The characteristics of the ovarian stimulation cycles are presented in Table 2. The dual retrieval group had a lower number of oocyte retrievals as compared with the classical ovarian stimulation group (2 vs 4, $P<0.001$), and used a significantly lower amount of gonadotropins (450 ± 75 vs 2730 ± 720, $P<0.001$). Both groups had a similar number of total oocytes retrieved and a similar number of embryos per cycle. However, the dual oocyte retrieval group had more embryos of better quality (Table 2).

The reproductive outcomes are summarized in Table 3. Although there was a trend toward higher live birth rate in the dual retrieval group, the difference did not reach statistical significance (29% vs 8%, $P=0.08$).

4 DISCUSSION

The aim of the present study was to examine whether early follicular phase oocyte retrieval, followed by late follicular phase oocyte retrieval might be convenient and result in good outcomes as compared with classical ovarian stimulation for women diagnosed with POI. In the study, oocytes obtained from early follicular phase follicles were fertilized by ICSI, embryos were vitrified at the cleavage stage, and subsequent ovarian stimulation was provided by minimal stimulation to obtain more oocytes and embryos in order to transfer at least two embryos in the same cycle. The aim of this approach was to rescue already growing ovarian follicles in the early follicular phase.

The present study showed that women in the dual retrieval group needed fewer ovarian stimulation cycles, used lower amounts of gonadotropins, and had better embryo quality. Although there was a trend toward a higher live birth rate in the dual retrieval group, the difference was not significant, probably owing to the small sample size. However, it should be emphasized that the study group comprised only women with POI who presented with growing ovarian follicles (i.e., a minority of women with POI), and other women with diminished ovarian reserve who did not meet the POI criteria were excluded.

Premature ovarian insufficiency should be evaluated in a multidisciplinary manner because of the different health aspects experienced by affected women, such as osteoporosis and increased risk of cardiovascular disease [9]. Infertility is an important issue for these women, and many of them seek fertility treatments. Many clinicians use a classical approach of ovarian stimulation, starting stimulation in the early follicular phase and using high doses of gonadotropins [10]. As found in the present study, however, flexibility in the treatment approach by attempting to rescue already developing ovarian follicles can achieve a good number of oocytes with fewer oocyte retrieval cycles. Despite their severely diminished ovarian reserve, these women have a reasonable chance of achieving pregnancy, mainly because of their young age, if embryos can be obtained [11].

In a normal menstrual cycle, usually one follicle becomes dominant and the others undergo atresia [2]. However, follicles are recruited in cohorts continuously throughout the menstrual cycle, in a process called follicular wave development. Thus, it is not surprising that more than one follicle can reach dominancy owing to the

high levels of FSH circulating early in the follicular phase. This has been observed among both older women and those with diminished ovarian reserves [12]. During fertility treatments, this phenomenon can be used to increase the total number of oocytes retrieved by performing early follicular phase retrieval.

The high levels of FSH observed among these women may prevent the 2–5-mm follicles of the next cohort from atresia, resulting in the emergence of larger follicles during the inter-ovulatory interval [13]. Although elevated estradiol in the early follicular phase can be a sign of subtle ovarian insufficiency, functional cysts observed at this stage may contain competent oocytes. We previously reported a case of POI with dual oocyte retrieval and same-cycle vitrified-warmed embryo transfer that resulted in live birth [7].

Early follicular phase oocyte retrieval does not seem to impair endometrial receptivity, probably because of the short duration of estradiol exposure [14]. Without estradiol priming, progesterone has minimal effects on the endometrium. Furthermore, by starting letrozole immediately after the first oocyte retrieval, estradiol priming is suppressed and the window of implantation is shifted to after the second oocyte retrieval. This enables a fresh embryo transfer to be performed in the same cycle.

The current study has limitations, the main one being the small number of women in each group. It should be noted, however, that the diagnosis of POI is not common. In addition, the dual oocyte retrieval group included women with leading follicles that were aspirated in the early follicular phase, which is also uncommon among women with POI.

In conclusion, the approach to treating women with POI should be flexible; in particular, developing follicles should be rescued when detected by sonography, and conventional ovarian stimulation should be deferred until later in the cycle or performed in subsequent cycles. This approach may result in good oocyte and embryo yields that are comparable to those of classical ovarian stimulation, and might result in improved reproductive outcomes.

Author contributions

SH conceived, planned, and performed the study; analyzed the data; and drafted the manuscript. BA designed the study, and drafted and revised the manuscript. EH performed the study; acquired, analyzed, and interpreted the data; and drafted the manuscript. AB performed the study; carried out statistical analysis; and drafted the manuscript. ST planned and performed the study; and drafted and revised the manuscript. All authors approved the final version of the manuscript for publication.

Conflicts of interest

The authors have no conflicts of interest

References

1. Yang DZ, Yang W, Li Y, He Z. Progress in understanding human ovarian folliculogenesis and its implications in assisted reproduction. Journal of assisted reproduction and genetics. 2013;30(2):213-9.

2. Baerwald AR, Adams GP, Pierson RA. Ovarian antral folliculogenesis during the human menstrual cycle: a review. Human reproduction update. 2012;18(1):73-91.

3. Nelson LM. Clinical practice. Primary ovarian insufficiency. The New England journal of medicine. 2009;360(6):606-14.

4. La Marca A, Sunkara SK. Individualization of controlled ovarian stimulation in IVF using ovarian reserve markers: from theory to practice. Human reproduction update. 2014;20(1):124-40.

5. Awwad J, Farra C, Hannoun A, Abou-Abdallah M, Isaacson K, Ghazeeri G. Idiopathic premature ovarian failure: what is the most suitable ovarian stimulation protocol? Clinical and experimental obstetrics & gynecology. 2013;40(3):327-30.

6. Cakmak H, Rosen MP. Random-start ovarian stimulation in patients with cancer. Current opinion in obstetrics & gynecology. 2015;27(3):215-21.

7. Hatirnaz S, Hatirnaz E, Ata B. Live birth following early follicular phase oocyte collection and vitrified-warmed embryo transfer 8 days later. Reproductive biomedicine online. 2015;31(6):819-22.

8. Hsu MI, Mayer J, Aronshon M, Lanzendorf S, Muasher S, Kolm P, et al. Embryo implantation in in vitro fertilization and intracytoplasmic sperm injection: impact of cleavage status, morphology grade, and number of embryos transferred. Fertility and sterility. 1999;72(4):679-85.

9. Luisi S, Orlandini C, Regini C, Pizzo A, Vellucci F, Petraglia F. Premature ovarian insufficiency: from pathogenesis to clinical management. Journal of endocrinological investigation. 2015;38(6):597-603.

10. Youssef MA, van Wely M, Al-Inany H, Madani T, Jahangiri N, Khodabakhshi S, et al. A mild ovarian stimulation strategy in women with poor ovarian reserve undergoing IVF: a multicenter randomized non-inferiority trial. Human reproduction (Oxford, England). 2017;32(1):112-8.

11. Morin SJ, Patounakis G, Juneau CR, Neal SA, Scott RT, Jr., Seli E. Diminished ovarian reserve and poor response to stimulation in patients <38 years old: a quantitative but not qualitative reduction in performance. Human reproduction (Oxford, England). 2018.

12. Sighinolfi G, Sunkara SK, La Marca A. New strategies of ovarian stimulation based on the concept of ovarian follicular waves: From conventional to random and double stimulation. Reproductive biomedicine online. 2018.

13. Vanden Brink H, Chizen D, Hale G, Baerwald A. Age-related changes in major ovarian follicular wave dynamics during the human menstrual cycle. Menopause (New York, NY). 2013;20(12):1243-54.

14. Dinh A, Sriprasert I, Williams AR, Archer DF. A review of the endometrial histologic effects of progestins and progesterone receptor modulators in reproductive age women. Contraception. 2015;91(5):360-7.

Box 1 Cycle management in dual oocyte pick-up with minimal stimulation and same-cycle embryo transfer.

Protocol step	**Follicle observation**		**1st OPU**	**TVUS**	**TVUS**	**TVUS**	**2nd OPU**	**Embryo transfer**	**Luteal phase support**
Stage of cycle	Day 2–3	Day 3	Day 4–5	Day 6–7	Day 8–9	Day 10–11	Day 12–13	Day 15–16	Day 16–17 onward
Treatment	Gn+ hCG	Letrozole 2.5 mg twice daily for 5 days	35 h			Gn+ hCG	35 h	Fresh; Fresh/vitrified-warmed; Vitrified-warmed	Estrogen 8 mg daily; progesterone in oil, 50 mg 1 × 2 i.m.; LMWH 4000 IU s.c. daily

Abbreviations: Gn, gonadotropin; hCG, human chorionic gonadotropin; i.m., intramuscular; LMWH, low molecular weight heparin; OPU, oocyte pick-up; s.c., subcutaneous; TVUS, transvaginal ultrasound.

Table 1 Demographic characteristics and basal hormonal parameters of the study women [a].

Variable	Dual oocyte retrieval (n=14)	CC + gonadotropin (n=37)	*P* value
Age, y	28.86 ± 4.75	30.32 ± 4.64	0.242 [d]
Duration of infertility, y	2 (1–7)	4 (1–7)	0.081 [d]
BMI	28.18 ± 3.91	27.78 ± 3.97	0.758 [e]
Basal AFC [b]	0	1 (1–2)	–
Basal LH, mIU/mL	17.44 ± 3.85	16.93 ± 3.92	0.724 [d]
AMH, ng/mL	0.13 ± 0.04	0.13 ± 0.05	0.445 [e]
Basal FSH, mIU/mL	41.38 ± 3.68	44.99 ± 6.44	0.032 [d]
Progesterone, ng/mL	1.00 ± 0.22	1.06 ± 0.21	0.392 [e]
Estrogen, pg/mL [c]	258.43 ± 84.92	25.89 ± 9.54	<0.001 [d]
Prolactin, ng/mL	23.05 ± 4.61	25.59 ± 5.92	0.123 [d]
Thyroxine hormone, ng/dL	92.50 ± 12.67	98.82 ± 13.09	0.128 [d]

Abbreviations: AFC, antral follicle count; AMH, anti-Müllerian hormone; BMI, (calculated as weight in kilograms divided by the square of height in meters); CC, clomiphene citrate; FSH, follicle-stimulating hormone; LH, luteinizing hormone.
[a] Values are given as mean ± SD, median (range), or number (percentage) unless stated otherwise.
[b] Measured on patient referral.
[c] Measured on cycle start day.
[d] By Mann–Whitney *U* test.
[e] By independent samples t test.

Table 2 Results of oocyte pick-up, endometrial thickness, embryo transfer, and embryo quality [a].

Variable	Dual oocyte retrieval (n=14)	CC+ gonadotropin (n=37)	*P* value
Number of OPUs	2	4 (3-4)	<0.001 [b]
Total gonadotropin dose, IU	450 ± 75	2730 ± 720	<0.001 [b]
Number of collected oocytes	3 (2–3)	2 (2–3)	0.192 [b]
Number of MII oocytes	2 (1–3)	2 (1–3)	0.534 [b]
Number of oocytes fertilized	2 (1–3)	2 (1–2)	0.899 [b]
Number of formed embryos	2 (1–2)	2 (1–2)	0.583
Embryo grade			0.031
I	6 (27)	20 (34)	
II	14 (64)	20 (34)	
III	2 (9)	19 (33)	
Endometrial thickness at ET, mm	10.79 ± 1.12	9.91 ± 1.20	0.024 [b]
Fresh + frozen ET	8 (57)	10 (27)	0.057 [c]
Frozen ET	3 (21)	27 (73)	0.001 [d]
Fresh ET	3 (21)	0	0.017 [c]

Abbreviations: CC, clomiphene citrate; ET, embryo transfer; MII, metaphase II; OPU, oocyte pick-up.
[a] Values are given as mean ± SD, median (range), or number (percentage) unless stated otherwise.
[b] By Mann–Whitney *U* test.
[c] By Fisher exact test.
[d] By χ^2 test.

Table 3 Clinical outcomes among the study women [a].

Outcome	Dual oocyte retrieval (n=14)	CC + gonadotropin (n=37)	*P* value [b]
Positive β-hCG test	6 (43)	11 (30)	0.510
Spontaneous abortion	0 (0)	3 (8)	0.552
Clinical pregnancy	4 (29)	3 (8)	0.080
Live birth	4 (29)	3 (8)	0.080

Abbreviations: CC, clomiphene citrate; hCG, human chorionic gonadotropin.
[a] Values are given as number (percentage) unless stated otherwise.
[b] By Fisher exact test.