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The impact of timing for estrogen supplementation in polycystic ovary syndrome patients undergoing primed in vitro maturation

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Abstract

Objective: This study aims to determine the effects of early and late onset estrogen supplementation on the immature oocyte retrieval, fertilization and clinical pregnancy rates in follicle stimulating hormone (FSH) and human chorionic hormone (hCG) primed in vitro maturation (IVM) cycles of the patients with polycystic ovary syndrome (PCOS).

Methods: This is a retrospective analysis of 161 patients with PCOS who underwent FSH and hCG primed IVM. Group 1 included 120 patients who received early onset estrogen supplementation while group 2 consisted of 41 patients who had late onset estrogen supplementation in primed IVM cycles. Immature oocyte (germinal vesicle and/or metaphase I) retrieval and fertilization rates were the primary outcomes, whereas clinical pregnancy and live rates were the secondary outcomes.

Results: Group 1 patients had significantly higher body mass index and more previous IVF attempts ($p = 0.001$ and $p = 0.008$, respectively). All of the retrieved oocytes from the PCOS patients were either germinal vesicle or metaphase I oocytes and there were no metaphase II oocytes among the retrieved oocytes. Both groups had statistically similar numbers of metaphase I and fertilized oocytes ($p > 0.05$ for both). However, group 1 patients had significantly lower number of germinal vesicle oocytes but significantly higher number of metaphase II oocytes ($p = 0.001$ for both). Both groups had statistically similar fertilization (85.0% vs 78.0%), clinical pregnancy (49.2% vs 43.9%) and live birth (37.5% vs 39.0%) rates ($p > 0.05$ for all).

Conclusion: Early onset estrogen supplementation appears to improve the quality of retrieved immature oocytes and contribute to the maturation of oocytes in stimulated IVM cycles.

Key words: estrogen, fertilization, in vitro maturation, polycystic ovary syndrome, pregnancy.

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Precis: An optimal IVM cycle might refer to the combination of FSH-hCG priming and early onset estrogen supplementation which would allow acceptable immature oocyte retrieval, fertilization and clinical pregnancy rates.

Introduction

In vitro maturation (IVM) is an assisted reproduction technology (ART) which refers to the extraction of immature oocytes from antral follicles and their maturation in the laboratory.[1]

Although this technique was first described in 1935, the first successful delivery by IVM was achieved with the donated oocytes in 1991 and patient's own oocytes in 1994.[2]

Nevertheless, there are still controversies related to this ART. These include discrepancies in nomenclature and clinical definitions as well as questions about the development of nuclear and cytoplasmic maturity under in vitro conditions.[3] Some centers prefer the sole collection of immature oocytes which subsequently undergo maturation in laboratory whereas other centers accept the collection of metaphase II oocytes together with immature oocytes.[4]

In IVM cycles, combined follicle stimulating hormone (FSH) and human chorionic gonadotropin (hCG) priming offers several benefits such as larger follicles for easier and faster oocyte pickup.[5,6] Since the duration of IVM cycles are shorter than that of in vitro fertilization (IVF) cycles, estrogen supplementation may be required to allow the endometrium to reach an adequate thickness.[7]

Late onset estrogen supplementation indicates the beginning of estrogen treatment at the day of oocyte retrieval while early onset estrogen supplementation refers to the beginning of estrogen treatment before the day of oocyte pickup.[8,9] Theoretically, early onset estrogen supplementation may exert negative feedback on endogenous FSH secretion and, thus, impair subsequent maturation of oocytes. However, it may be possible to ameliorate the impaired oocyte maturation with early onset estrogen supplementation in IVM cycles.[10]

This study aims to determine the effects of early and late onset estrogen supplementation on the immature oocyte retrieval, fertilization and clinical pregnancy rates in FSH and hCG primed IVM cycles of the patients with polycystic ovary syndrome (PCOS).

Materials and Methods

This is a retrospective analysis of 161 patients with PCOS who underwent primed IVM at a private assisted reproduction center between September 2007 and January 2015. All participants gave written informed consent for their treatment. The present study was approved by the institutional review board of Clinart International Hospital with an issue number of 000298/18.05.2015.

Inclusion criteria were the diagnosis of PCOS, resistance to clomiphene citrate, history of ovarian hyperstimulation syndrome (OHSS), refusal to use high dose gonadotropins, history of unsuccessful IVF attempts and the patient's desire to try IVM.

Exclusion criteria were being aged >38 years, having an endocrinopathy (such as thyroid dysfunction and diabetes mellitus) and undergoing fertility preservation for malignancy. In order to provide homogenicity, the patients who had partners with azoospermia, cryptozoospermia and oligoasthenoteratospermia (sperm count<15 million/mL, progressive motility <32% and morphology <4%) were also excluded.

The diagnosis of PCOS was made if at least two of the revised Rotterdam criteria were specified, which were as follows[1]: oligo- and/or anovulation,[2] clinical and/or biochemical hyperandrogenism[3] and polycystic ovaries determined by ultrasonography. The ultrasonography criteria for polycystic ovary were the presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter, and/or increased ovarian volume (>10 mL).[11]

A total of 161 patients with PCOS who underwent FSH and hCG primed IVM were allocated into two groups according to the timing of estrogen supplementation. Group 1 included 120 patients who received early onset estrogen supplementation and group 2 consisted of 41 patients who had late onset estrogen supplementation. The protocols are depicted in Figure 1.

Early onset estrogen supplementation referred to the beginning of estrogen treatment on day 3 of menstrual cycle. Early onset estrogen support was given to the PCOS patients who had previous implantation failure and preferred to receive estrogen beginning from day 3 of menstrual cycle. Late onset estrogen supplementation corresponded to the beginning of estrogen treatment on the day of oocyte pick up. Late onset estrogen support was given to the PCOS patients who had no previous implantation failure and refused to receive early onset estrogen treatment. Estrogen supplementation was provided by oral administration of estradiol tablet twice a day (Estrofem®, 2 mg, Nova Nordisk, Turkey). None of the participating women in these groups underwent IVM treatment previously.

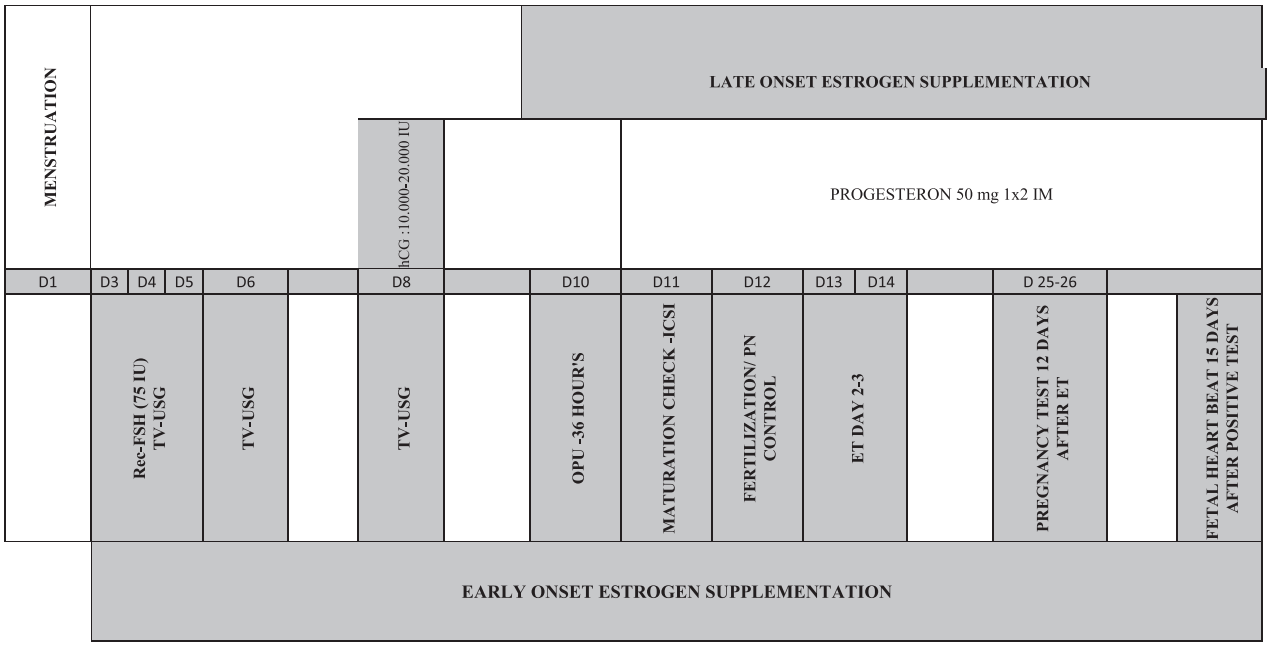

FIGURE 1 Cycle dynamics of in vitro maturation (IVM) protocol with early and late onset follicular phase estrogen supplementation

Immature oocyte (germinal vesicle and/or metaphase I) retrieval and fertilization rates were the primary outcomes whereas clinical pregnancy and live rates were the secondary outcomes. Fertilization rate was computed when the number of fertilized oocytes is divided by the number of metaphase II oocytes.

IVM protocol

FSH priming was made by the administration of recombinant FSH (Gonal-f®, Merck Serono, Germany) which was started on day 3 of spontaneous or progesterone induced menstrual cycles and then given subcutaneously at a daily dose of 75 IU for 3 days. The patients were evaluated on day 6 and 2 days later. When endometrial thickness was at least 8 mm and the leading follicle size ≤10 mm on transvaginal ultrasonography, hCG priming was performed with the subcutanous injection of 10 000–20 000 IU hCG (Pregnyl®, Merck Sharp & Dohme, New Jersey, USA). If the endometrial thickness was less than 8 mm, FSH priming was continued and the patient was evaluated 2 days later. In case the leading follicle size was >10 mm, the leading follicle was spared and retrieval was performed for the remaining follicles. None of the IVM cycles were canceled.

Oocyte retrieval and laboratory procedures

Oocyte retrieval was performed by single physician (S. Hatirnaz) in all IVM cycles. Oocytes were retrieved from small antral follicles using a 17 gauge double lumen aspiration needle (Swemed, Vitrolife, Switzerland) in combination with transvaginal ultrasonography, 36-h after hCG administration. An intra-follicular flushing pressure of 80–100 mmHg was routinely applied during oocyte pick up in all IVM cycles. Retrieved oocytes were first flushed and then filtered by cell strainer (Falcon 70 micrometers, Corning Incorporated, Massachusetts, USA). Following filtration, the number of retrieved oocytes was counted. We look for PBI presence or GV in the oocyte to remove MII oocytes if present. For checking the maturation, we use either sliding method or spreading method to see the oocytes within cumulus oocyte complexes (COCs). In case of difficulty, we can use inverted microscope to see GV or PBI. We add this information in the text and highlighted in the text.

The filtered COCs were rinsed with heparinized flush medium in a falcon petri dish (Falcon, cat. no.353803, Corning Incorporated). Then, rinsed COCs were deposited in a center-well dish containing non heparinized flushing medium. After that, the COCs were transferred to LAG medium (Vial 1 IVM system,

cat.no.82214010; Medicult, Cooper Surgical, USA) and incubated for 2–3 h. A stock solution of 0.5 mL human serum albumin, 0.75 IU FSH and 0.75 IU LH were added to the final IVM medium (as recommended by medicult IVM protocol). Nondenuded oocytes were incubated for 26–28 h and cumulus cells surrounding the oocytes were removed by hyaluronidase denudation, using Pasteur pipettes. From that time on, oocytes were accepted as mature and transferred to conventional IVF culture medium (60 mL, cat.no.10310060; Medicult, Cooper Surgical). Intracytoplasmic sperm injection (ICSI) was performed in the falcon petri dish and following this procedure, injected oocytes were placed in another culture medium (ISM 1 medium, 60 mL, cat.no.10500060; Medicult, Cooper Surgical). Sixteen to 18 h after ICSI, fertilization was checked by the existence of two pronuclear bodies and polar body formation. Fertilized embryos were followed until day 2 or day 3 and graded as follows: Grade 1 embryos have even blastomeres without fragmentation. Grade 2 embryos have even blastomeres with minor fragmentations, whereas grade 3 embryos have uneven blastomeres and few or none fragmentation. Grade 4 embryos were defined as unsusceptible for viable embryo transfer and, thus, deleted.[12] In accordance with the legal regulations of Turkish government, the patients who were <35 years old or who had less than two IVF attempts had 1 embryo transfer while the patients over 35 years old and the patients who had more than two IVF attempts had 2 embryo transfers on day 2 or day 3.[13]

Luteal phase support

Luteal phase support was provided by oral ingestion of progesterone capsules three times a day (Progestan®, 100 mg, Koçak Farma, Turkey) and estradiol tablets twice a day (Estrofem® tablet, 2 mg, Nova Nordisk, Turkey) as well as intramuscular injection of progesterone ampule once a day (Progestan®, 50 mg/mL). Luteal phase support was maintained until the fetal heart beat is detected by ultrasonography. Serum hCG concentration was measured 12 days after the embryo transfer and if the test was positive, ultrasonography was performed 10 to 15 days later. The detection of fetal heart beat was accepted as clinical pregnancy.

Statistical analysis

Collected data were analyzed using the Statistical Package for Social Sciences version 20.0 (SPSS, Armonk IBM, New York, USA). Continuous variables were expressed as mean ± standard deviation (range: minimum–maximum) whereas categorical variables were denoted as numbers or percentages where appropriate. Student's *t*-test, chi-square test and Mann–Whitney *U* test were used for the comparisons. Logistic regression analysis was used to overcome the confounding effect of body mass index (BMI) and previous IVF attempt. The odds ratios were denoted within 95% confidence intervals. Two-tailed p values less than 0.05 were accepted to be statistically significant. A post hoc power analysis was revealed a power of 80% and an alpha error of 5% with 161 PCOS patients to detect an effect size of 22%.

TABLE 1 Baseline characteristics of the patients

	Group 1	Group 2	p
Age (years)	28.6 ± 5.1	29.2 ± 3.4	0.336
Body mass index (kg/m^2)	26.19 ± 5.87	22.51 ± 1.64	0.001*
Duration of infertility (years)	6.9 ± 4.8	7.8 ± 4.2	0.297
Previous IVF attempts (n)	1.48 ± 0.76	0.54 ± 0.12	0.008*
Antral follicle count (n)	12.1 ± 3.5	13.6 ± 2.8	0.433

Note: Values are given as mean ± SD. and *Significant difference.

Results

Table 1 demonstrates the demographic characteristics of the patients. Both groups were statistically similar with respect to age and antral follicle count ($p > 0.05$ for all). However, the PCOS patients in group 1 had significantly higher BMI and number of previous IVF attempts ($p = 0.001$ and $p = 0.008$, respectively).

Table 2 displays the clinical characteristics of the primed IVM cycles. All of the retrieved oocytes from the PCOS patients were either germinal vesicle or metaphase I oocytes and all of the metaphase II oocytes in this study were matured in vitro. There were no metaphase II oocytes among the retrieved oocytes. The PCOS patients in group 1 had significantly lower number of germinal vesicle oocytes but significantly higher number of metaphase II oocytes ($p = 0.001$ for both). Both groups had statistically similar numbers of metaphase I and fertilized oocytes as well as fertilization rates (85.0% vs 78.0%) ($p > 0.05$ for all).

TABLE 2 Laboratory findings of the patients

	Group 1	Group 2	*p*
Endometrial thickness at transfer time (mm)	8.2 ± 2.1	8.5 ± 1.7	0.686
Retrieved oocytes (n)	21 ± 8.2	20.9 ± 4.8	0.94
Germinal vesicle oocytes (n)	6.3 ± 4.3	9 ± 2.6	0.001*
Metaphase I oocytes (n)	6.6 ± 2.5	6.9 ± 1.7	0.521
Metaphase II oocytes (n)	8.0 ± 3.2	5 ± 2.1	0.001*
Oocyte maturity rate (%)	38.1 ± 3.9	23.9 ± 4.4	0.001*
Fertilized oocytes (n)	6.8 ± 2.7	6.9 ± 1.5	0.506
Fertilized/collected oocytes (%)	32.4 ± 3.3	33 ± 3.1	0.758
Embryo transfer (n, %)			0.72
Single embryo		60 (50%)	19 (46.3%)
Double embryo		60 (50%)	22 (53.7%)
Embryo grade (n, %)			0.431
Grade 1		3 (1.7%)	2 (3.2%)
Grade 2		134 (74.4%)	52 (82.5%)
Grade 3		43 (23.9%)	9 (14.3%)
Embryo quality (n, %)			0.178
Two-cell embryos		12 (6.7%)	3 (4.8%)
Three-cell embryos		11 (6.1%)	6 (9.5%)
Four-cell embryos		122 (67.8%)	38 (60.3%)
>Four-cell embryos		35 (19.4%)	16 (25.4%)

Note: Values are given as mean ± SD or number (percentage) as indicated. and *Significant difference.

Both groups were also statistically similar in aspect of endometrial thickness at transfer time, embryo grade, embryo quality, number of single and double embryo transfer ($p > 0.05$ for all). Logistic regression analysis revealed that both patient groups had statistically similar number of germinal vesicle oocytes and metaphase II oocytes when adjusted for BMI groups and per previous IVF attempt (Table 3).

Table 4 shows that PCOS patients in the early and late onset estrogen supplementation groups had statistically similar clinical pregnancy (49.2% vs 43.9%), miscarriage (8.3% vs 4.9%), perinatal death (3.3% vs 0.0%), live birth (37.5% vs 39.0%) and twinning (6.7% vs 7.3%) rates ($p > 0.05$ for all).

Discussion

Steroidogenesis within the ovary is sine qua non for follicular growth and in vivo maturation of oocytes.[14] Steroidogenesis initially causes the synthesis of androgens which trigger the promotion of antral follicles and the differentiation of granulosa cells. Subsequently, the androgens are aromatized and converted to estrogens.[15] Estradiol is the most potent estrogen which regulates gonadotropin release, oocyte maturation, fertilization, early embryonic development and implantation.[16] It is well known that estradiol is crucial for the maturation of oocytes as it exerts paracrine effects on the oocytes.[16,17]

It has been well established that FSH induces follicular estrogen secretion which exerts negative feedback on pituitary FSH secretion in turn.[18] This negative feedback effect reduces FSH release and causes an arrest in the development of follicles. Thus, early suppression by estrogen could alter the dominance of follicles and viability of oocytes.[18,19] FSH priming in IVM usually enhances the retrieval of immature and mature oocytes simply by increasing the size of follicles and making them more easily accessible for pickup.[20] FSH priming also induces the secretion of estrogen by granulosa cells and this estrogen protects oocytes from undergoing premature nuclear maturation by providing sufficient cytoplasmic maturation.[21,22] Moreover, FSH priming might expand the volume of cumulus cells and exert beneficial effects on their morphology. These cumulus cells may contribute to the quality of oocytes and increase the chance of pregnancy.[23,24]

Russell et al. randomly assigned 14 women who underwent unstimulated IVM cycles into two groups. Early follicular endometrial priming was initiated on cycle day 3 with 2 mg 17β-estradiol twice per day in the first group, whereas mid-follicular endometrial priming was initiated with 1–2 mg of 17β-estradiol between cycle days 5 and 7 in the second group. Both

TABLE 3 Logistic regression analysis for clinical characteristics

	Categories	Group 1	Group 2
GV oocytes			
BMI (kg/m^2)	<18.5 vs 18.5–24.9	0.74 (0.5–1.08)	0.91 (0.52–1.58)
	25–29.9 vs 18.5–24.9	1.16 (0.66–1.69)	0.96 (0.60–1.54)
	≥30 vs 18.5–24.9	0.9 (0.57–1.42)	0.77 (0.45–1.29)
Previous IVF attempts	Per attempt	1.12 (0.82–1.54)	1.33 (0.95–1.78)
Metaphase II oocytes			
BMI (kg/m^2)	<18.5 vs 18.5–24.9	0.59 (0.35–0.99)	0.58 (0.34–0.97)
	25–29.9 vs 18.5–24.9	0.86 (0.55–1.36)	0.99 (0.65–1.52)
	≥30 vs 18.5–24.9	1.11 (0.63–1.7)	1.15 (0.82–1.49)
Previous IVF attempts	Per attempt	0.72 (0.4–1.26)	0.7 (0.4–1.2)

Note: Values are given as odds ratio (confidence interval).

groups had statistically similar oocyte retrieval and fertilization rates but the maturation rate was significantly lower and the cleavage arrest rate was significantly higher in early follicular endometrial priming group. The authors found that mid-follicular estrogen supplementation combined with unstimulated retrieval of immature oocytes was able to achieve a maturation rate of 60% and a fertilization rate of 75%.[25]

In a clinical study, impaired endometrial thickening was treated with either human menopausal gonadotropin (hMG) or 17β-estradiol in IVM cycles. Although estrogen treatment between days 6 and 10 of menstrual cycle significantly thickened endometrium, the number and size of ovarian follicles were reduced significantly. However, embryonic development and clinical pregnancy rates were similar in both groups.[26]

Contrary to this study, Vitek et al. administered early onset estrogen supplementation beginning from day 3 of menstrual cycle in unstimulated IVM cycles. They found that early onset estrogen supplementation resulted in statistically similar number of oocytes retrieved, fertilization rates, embryo quality at transfer, implantation and clinical pregnancy rates as did mild FSH stimulation. The clinical pregnancy and live birth rates were respectively 40% and 40% in unstimulated IVM cycles with early onset estrogen supplementation.[10]

TABLE 4 Comparison of pregnancy outcomes of two groups

	Group 1	Group 2	p
Clinical pregnancies	59 (49.2%)	18 (43.9%)	0.56
Singleton	51 (42.5%)	15 (36.6%)	
Twin	8 (6.7%)	3 (7.3%)	
Miscarriages			0.467
Singleton	6 (5%)	-	
Twin	4 (3.4%)	2 (4.9%)	
Perinatal deaths			0.307
Singleton	2 (1.7%)	-	
Twin	2 (1.7%)	-	
Live births			0.862
Singleton	43 (35.8%)	15 (36.6%)	
Twin	2 (1.7%)	1 (2.4%)	

Note: Values are given as number (percentage).

Such discrepancy in literature might be primarily attributed to the differences in the definition of IVM and priming protocols adopted for IVM cycles. For instance, hCG use does not comply with the definition of IVM and IVM cycles with hCG use should be defined "natural cycle IVF" or "truncated IVF."[3,27] Another reason might be the variations in the clinical characteristics and phenotypes of PCOS patients.

A study reported that metaphase II oocytes retrieved from the cohort of follicles (<10 mm) can produce the same quality of embryos as that from large follicles, likely contributing to the clinical outcomes.[28] It was also demonstrated that in vivo matured oocytes obtained from hCG primed IVM cycles give a good pregnancy rate, probably due to the better developmental capacity of embryos derived from in vivo matured oocytes than those of in vitro matured oocytes.[29]

In this study, PCOS patients who received early and late onset estrogen supplementation were statistically similar with respect to the number of metaphase I and fertilized oocytes. However, the number of germinal vesicle oocytes was significantly lower and the number of metaphase II oocytes was significantly higher in PCOS patients who had late onset estrogen supplementation. Despite this finding, both groups had statistically similar fertilization (85.0% vs 78.0%), clinical pregnancy (49.2% vs 43.9%) and live birth (37.5% vs 39%) rates.

The rationale behind the early onset estrogen supplementation was to balance the follicular growth induced by FSH-hCG priming and, thus, to prevent the premature arrest during folliculogenesis and rescue the

immature oocytes with high developmental capacity and maturation chance. Therefore, a significantly higher number of metaphase II oocytes could be acquired from a significantly smaller number of germinal vesicle oocytes and this improvement in structural quality of the immature oocytes resulted in statistically similar clinical pregnancy and live birth rates.

To the best of our knowledge, this is the first study to compare the efficiency of early and late onset estrogen priming in a relatively large cohort of PCOS patients undergoing stimulated IVM cycles. However, the power of the present study is limited by its retrospective design, lack of randomization and absence of data related with anti-Müllerian hormone and estradiol levels. FSH stimulation can also be considered as a power limiting factor as it may raise endogenous estradiol levels. Another power limiting factor is the significantly shorter exposure of the immature oocytes to the estrogen administration in patients receiving late onset estrogen supplementation. That is, it would be prudent to expect that late onset estrogen supplementation would affect implantation and early embryonic development rather than the oocyte quality and maturation.

In conclusion, early onset estrogen supplementation appears to improve the quality of retrieved immature oocytes and have beneficial effects on the maturation of oocytes in FSH-hCG primed IVM cycles. Further research is warranted to specify the efficiency of early onset estrogen priming in stimulated IVM cycles.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

Conflict of Interest

The authors declare no conflict of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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