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Evaluation of the ideal vaginal Progesterone effectiveness doses for luteal support in embryo thawing cycles after endometrial preparation without using the GnRh analogue

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Abstract. – OBJECTIVE: Frozen-thawed embryo transfer (FET) cycles require the use of luteal phase support (LPS) for supporting implantation, endometrial and embryo maturity. Individualized LPS should be chosen according to the used endometrial preparation protocol. The aim of the study was to analyze the effectiveness of two different vaginal Progesterone doses for women who underwent FET cycle and the same endometrial preparation without using the GnRh analogue.

PATIENTS AND METHODS: 607 women who underwent FET cycle were included in the study. 305 patients received luteal support with 600 mg/day vaginal Progesterone and 302 patients were treated with 800 mg/day of vaginal Progesterone.

RESULTS: In the 800 mg/day group, the mean serum Progesterone concentration on the day of embryo transfer was higher than in the 600 mg group (14.00±6.18 ng/mL and 12.22±5.39, respectively, $p < 0.001$). Moreover, human chorionic gonadotrophin (hCG) positive and ongoing pregnancy rates were higher in the group of patients who received LPS with 800 mg/day of Progesterone than in the group of patients treated with 600 mg/day of Progesterone.

CONCLUSIONS: In patients undergoing FET cycles following endometrial preparation made without previously using the GnRh analogue, 800 mg doses of vaginal Progesterone as LPS improve reproductive outcomes.

Key Words:
Assisted reproductive technologies, Frozen embryo transfer, Hormone replacement treatment cycle, Luteal phase support, Endometrium preparation, Progesterone.

Introduction

In the last decade, there has been a growing interest of the scientific community in the clinical applications of the luteal phase support (LPS) in IVF cycles and especially in programmed cycle of frozen-thawed embryo transfer (FET), since a worldwide increase has been recorded. LPS is fundamental in programmed FET cycles to compensate for endocrine defects in the luteal phase that can disturb embryo implantation[1,2]. Moreover, LPS can avoid hypertensive disorders and adverse perinatal outcomes related to the loss of angiogenesis molecules produced from the corpus luteum in women who underwent FET cycles[3,4]. It has been also showed that LPS can improve live birth rate and decrease miscarriage rate in FET cycles[5]. After estrogenic endometrial preparation and maturation, secretory endometrium and early pregnancy can be supported by stimulating corpus luteum to secrete endogenous estrogen and Progesterone prior to embryo transfer through administration of serial injection of human chorionic gonadotrophin (hCG) or with exogenous replacement of Progesterone[6,7]. Despite both may have beneficial effect on live birth rate and ongoing pregnancy[8-10], most endometrial preparations for FET cycle are performed without using hCG, mainly due to the difficulty in managing the timing of nat-

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ural ovulation. Moreover, hCG administration was associated with complication of ovarian hyperstimulation syndrome instead of Progesterone, that is a naturally occurring hormone during pregnancy[8].

Progesterone for LPS is administrated *via* a range of different routes such us oral, vaginal, intramuscular (i.m.), and subcutaneous. The oral Progesterone is degraded in the liver and, since its poor clinical efficacy, today has been almost entirely given up[11-13]. Transvaginal administration showed a comparable efficacy in terms of live birth and ongoing pregnancy rate to i.m. route[14-18]. However, there is evidence in the scientific literature that transvaginal administration was the preferred route for LPS[2,19]. Indeed, vaginal Progesterone is directly transported from the vaginal mucosa and lymphatics to the uterus, which results in an adequate endometrial tissue Progesterone concentration with lower circulating levels due to its local effect[20-22]. Moreover, transvaginal Progesterone administration is easier than i.m. for women[2] and it is able to avoid the risk of injection site pain/swelling and redness caused by i.m. administration for the oily preparation of vials[23]. Existing evidence suggests that different formulations of vaginal Progesterone used in LPS (Crinone, Cyclogest, Lutigest, e Ultrogestan Vaginal) are equal in terms of efficacy and safety[24]. The subcutaneous route of Progesterone administration is relatively new, and it shows a comparable efficacy to the i.m. and vaginal route with few side effects[25,26].

While the routes of Progesterone administration have been extensively studied, the optimal dose of vaginal Progesterone has been the subject of little research. Previous studies[27] reported the use of vaginal Progesterone with concentrations ranging from 200 to 1,200 mg. The majority of the studies used 600 mg/day of micronized Progesterone as standard[28]. Relatively higher doses of vaginal Progesterone have been associated with more intensive LPS; therefore, they should be recommended in programmed FET cycles to contribute to good pregnancy outcomes, thus reducing the risk of pregnancy loss[29,30]. However, different Progesterone route and doses of administration are often compared with each other, which also follow different endometrial preparation, making data in literature still controversial[31,32]. Future prospective studies are needed to clarify the best individualized way of Progesterone replacement regimens for infertile women based on the treatment protocol.

The aim of the study was to evaluate the reproductive outcomes in two different groups of women who underwent LPS with 600 mg *vs.* 800 mg transvaginal Progesterone after the same endometrial preparation without using the GnRh analogue for FET cycle.

Patients and Methods

Patients

This study included 607 consecutive FET cycles, in which patients from southern Italy were treated with vaginal Progesterone supplementation for LPS at "Momò Fertilife – Center for Reproductive Medicine" in Bisceglie (Italy) between January 2017 and December 2021. The Cochran's sample size formula was used to assess the sample size. Briefly, the ideal sample size was first calculated from the total number of patients undergoing FET cycle in the geographic area of interest (about 741 cycles per year). Then, an ideal sample of 529 cycles, smaller than the total number of cycles included in this study, was obtained by adopting a 99% confidence interval level and a 3% margin of error. Patients underwent transfer after warming of a single blastocyst. The blastocysts were graded 3BB or higher. Each patient was included only once. Patients with Progesterone levels higher than 1.2 ng/mL on the day of switch were also included[33]. Patients with endometrium thickness $\leq$ 7.5 mm on the shift day or underwent natural cycle FET or triggered with GnRH analogue before FET or with Isthmocele[34] or with obesity [body mass index (BMI) > 30][35] were excluded. Considering the inclusion and exclusion criteria, patients were randomly included into two distinct groups using MedITEX – IVF software (CRITEX GmbH; Regensburg; Germany). 305 patients who underwent FET cycle were included in Group A and were treated with 600 mg/day vaginal Progesterone; 302 patients who received luteal support with 800 mg/day of vaginal Progesterone were included in Group B. The follow-up of 24 patients of Group A and 21 of Group B could not be performed. Therefore, the analysis was limited to 281 patients of each group with an allocation report in this study of 1:1 (Figure 1).

Oocyte Collection and Blastocyst Vitrification

The cumulus-oocytes complexes were retrieved by vaginal ovarian pick up (OPU) under ultrasound guidance (VOLUSON S8, GE Healthcare; Chicago, IL, USA) between 35 and 36 hours later after induced ovulation. After 3 hours from

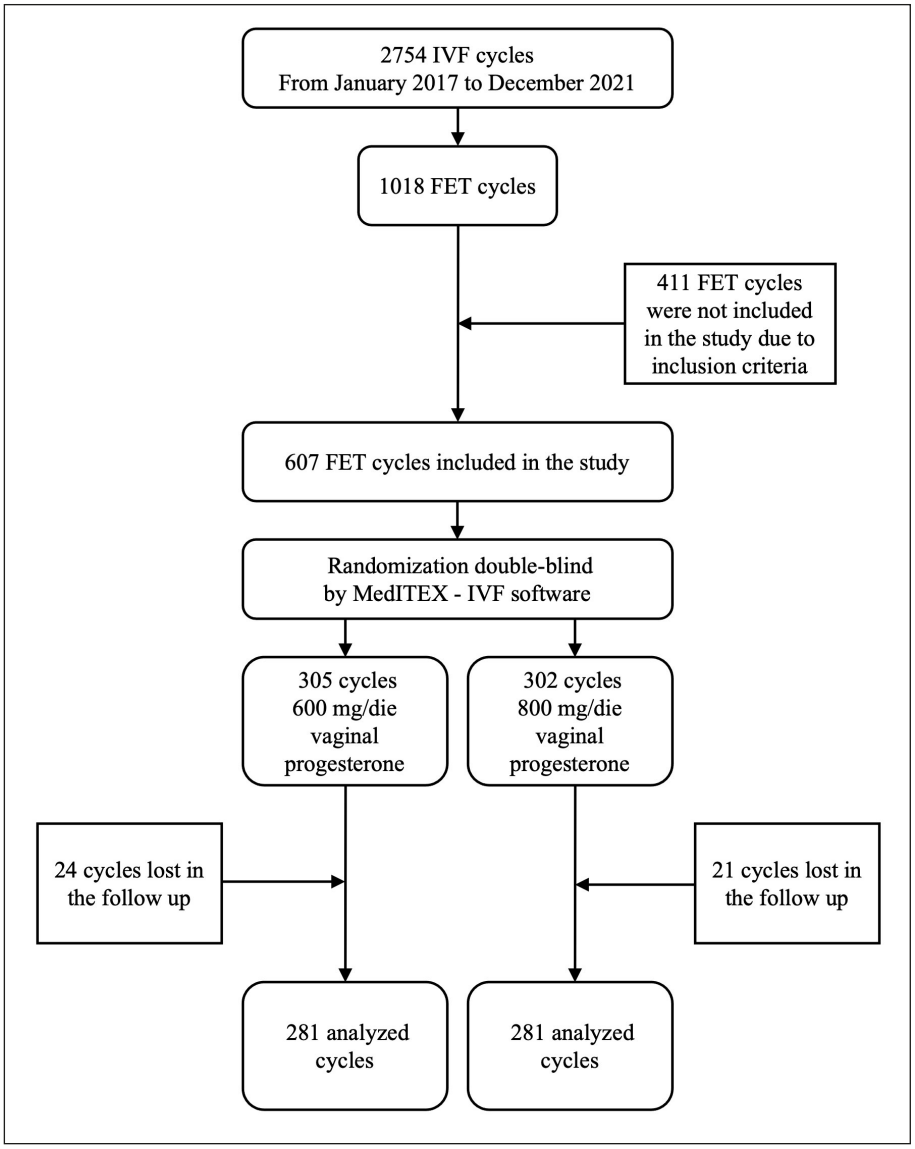

Figure 1. Flowchart of study participation. FET, Frozen embryo transfer.

the cumulus-oocyte retrieval, they were denuded from the corona radiata by repeated pipetting in 25 IU/ml hyaluronidase solution (LifeGlobal Group, Guildfort, CT, USA). The denuded oocytes were then analyzed under a stereomicroscope (Nikon SMZ 1500, Tokyo, Japan) to select the metaphase II (MII)-stage[36], the mature eggs, to be injected by using ICSI procedure[37,38]. The Geri-time Lapse system (Genea Biomedx, Sydney, Australia) was used to check fertilization and cleavage rates. Day 5 or day 6 blastocysts graded 3BB or higher were vitrified by using vitrification Cryotop Method for Embryo (Kitazato, Japan). Blastocysts were classified according to Gardner's staging.

Luteal Phase Support, Transfer, and Reproductive Outcomes

After excluding confounding medical issue, the endometrium of all patients was prepared for transfer by using the hormone replacement therapy (HRT) without gonadotropin-releasing hormone agonist suppression. All women were treated with 6 mg per day (3 capsules three times daily) oral Estradiol (Proginova, 2 mg, Bayer, Leverkusen, Germany) from the second day of the menstrual cycle. After 14 days of estradiol administration, the endometrial thickness was estimated by transvaginal ultrasonographic examination[39-41]. Vaginal Progesterone supplemen-

tation was added at the doses described above in patients with 7,5 mm or greater bilayer endometrial thickness[42,43]. Group A was treated by using 600 mg/day (Progeffik, 200 mg, Effik, Italy three capsules daily) and group B 800 mg/day (Amelgen, 400 mg, Gedeon Richter, Milano, Italy S.r.l., two capsules daily) of micronized Progesterone. Embryo transfer was performed five days after the initiation of Progesterone supplementation[44]. Frozen embryos were warmed following the manufactural instruction of thawing protocol (Kitazato, Japan). Only one blastocyst per patient with the best morphological grade was transferred. Serum Progesterone concentration was measured the day of the start of progestogen therapy and the day of FET[45]. Pregnancy was confirmed by serum levels of beta-human chorionic gonadotrophin (beta- hCG) exceeding 15 mIu/mL on 9 days after transfer and intrauterine gestational sac identification *via* transvaginal ultrasonography.

Statistical Analysis

An accurate statistical assessment of the samples was obtained by analyzing general characteristics such as age, reasons, and length of infertility, and clinic data of the patients (Table I). Data were presented as mean and standard deviation (SD) for continuous parametric variables or as percentage for categorical variables. Statistical evaluations of the clinic parameters were compared between groups by the Student's *t*-test or Chi-square test. Differences with p-value < 0.05 were considered to be statically significant in the present study.

Results

The population consisted of 562 women who underwent FET cycles and showed comparable baseline characteristics (Table I). Cycle characteristics are presented in Table II.

The mean serum Progesterone levels before FET were significantly higher in group B treated with 800 mg/day Progesterone, compared to the patients of group A treated with 600 mg/day Progesterone (14.00±6.18 *vs.* 12.22±5.39, $p < 0.001$, Table II and Figure 2).

Similarly, a correlation between the amount of vaginal Progesterone supplementation and the rate for positive hCG pregnancy test was observed, since the hCG positive rate was statistical-

Table I. General and clinical characteristic of the two analyzed groups of patients.

	Group A (n=281)	Group B (n=281)	*p*-value
Female age (years)	36.98±4.99	37.71±5.62	0.06
Duration of infertility (years)	3.28±1.79	3.18±1.81	0.27
Maternal BMI (Kg/m^2)	23.29±3.35	23.94±3.49	0.12
AFC (n)	11.60±4.57	11.32±5.76	0.25
AMH (ng/mL)	2.50±1.64	2.41±2.53	0.31
Basal FSH (IU)	7.56±8.187	8.34±4.68	0.08
Basal LH (IU)	6.21±4.77	6.11±2.85	0.38
Endometrium thickness (mm)	9.45±1.43	9.30±1.65	0.11
Evaluated serum Progesterone on the day of switch (ng/mL)	0.84±0.46	0.82±0.88	0.31

Data are presented as mean ± SD. BMI = body mass index. p-value of > 0.05 were not considered statically significant.

Table II. Descriptive data of reproductive clinical outcomes between different Progesterone groups.

	Group A (n=281)	Group B (n=281)	*p*-value
Evaluated serum Progesterone on the day of transfer (ng/mL)	12.22±5.39	14.00±6.18	0.0002
hCG positive (n, %)	101 (35.9%)	124 (44.1%)	0.047
Miscarriage (n, %)	22 (21.8%)	23 (18.55%)	0.546
Ongoing pregnancy (n, %)	79 (28.1%)	101 (35.9%)	0.0467

p-value of < 0.05 were considered to be statically significant.

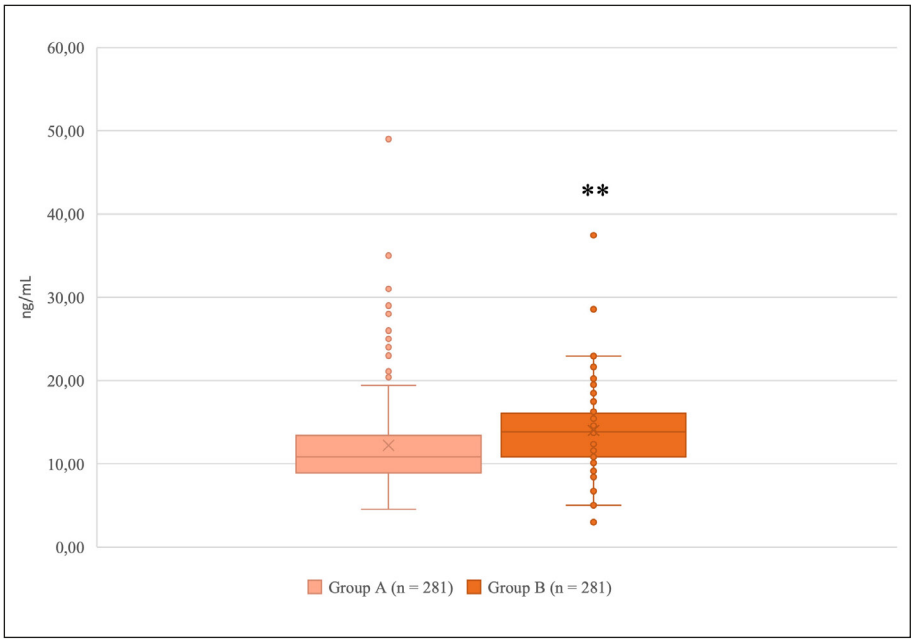

Figure 2. Evaluated serum Progesterone on the day of transfer in the two different examined groups. **p<0.001.

ly significant higher in group B (124/281, 44.1%) compared to group A (101/281, 35.9%) ($p < 0.05$, Table II and Figure 3). Moreover, ongoing pregnancy was better in the group of patients underwent LPS with 800 mg/day of Progesterone than in the group treated with 600 mg/day of Progesterone (101/281, 28.1% *vs.* 79/281, 35.9%, $p < 0.05$, Table II and Figure 3). Comparable miscarriage pregnancy rate was observed in both treatment groups (not significant, Table II and Figure 3).

Discussion

Frozen-thawed embryo transfer (FET) cycle need LPS to prevent luteal phase insufficiency caused by missed corpus lutea and altered secretion of late luteal Progesterone and Estradiol. LPS by vaginal Progesterone administration is the most widely used route since it significantly increased the pregnancy rate with fewer side effects compared to other existing administration routes[6,21]. Dosages between 200 and 1,200 mg Progesterone for LPS in FET cycles have been reported with different outcomes associated with different endometrial preparation[27,31,32,46-48]. For the first time in this study, effects of specific vaginal Progesterone dosages (600 and 800 mg) are evaluated in a homogeneous sample of women for general and clinical characteristics and all equally subjected to the same estrogenic endometrial preparation made without previously using the GnRh analogue.

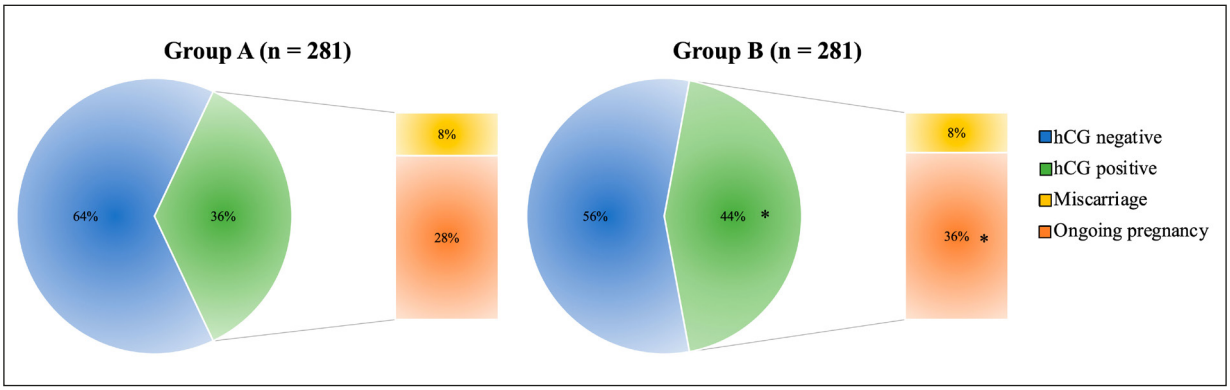

Figure 3. Reproductive outcome in different luteal Progesterone groups. *p<0.05.

Increased levels of serum Progesterone, higher than 10 ng/mL (the minimum dose needed in programmed FET cycles to optimize the outcomes[49-51]), were found on the day of warmed blastocyst transfer, in patients treated with both 600 and 800 mg/day of vaginal Progesterone. However, an 800 mg dose of vaginal Progesterone allowed to reach higher levels of serum Progesterone compared to a 600 mg dose. The serum Progesterone levels on the day of transfer were comparable with those obtained in the study by Enatsu et al[30] in the Asian population by using, however, higher doses (900 and 1,200 mg/day) of Progesterone administration. Moreover, the results obtained in the study by Enatsu et al[30], might have been influenced from the additional Progesterone supplementation administrated when the serum Progesterone concentrations on luteal day 5 were < 9 ng/mL. The obtained results in our study comply with the fundamental concept of the "minimum effective dose", saving costs associated with drugs and making treatment more convenient and practical for patients.

LPS with 800 mg doses of vaginal Progesterone allowed more patients to become pregnant without any serious adverse events, thus indicating a correlation between high doses of Progesterone and pregnancy rate. Currently, no data regarding the effects of LPS with Progesterone administration < 1,200 mg/day have been reported. However, a reverse effect could be supposed since, in a very recent study[52], Progesterone serum levels greater than 32.5 ng/mL on the day of embryo transfer significantly reduced the possibility of live birth after blastocyst transfer.

Bias and potential differences between the groups have been optimally controlled. Moreover, the proposed protocols can be easily applied to the real-life practice of IVF.

Limitations

However, some limitations of the study should be taken into consideration in the interpretation of the results. One of the limitations of the study is that it only focused on Italian populations, mostly from southern Italy. Therefore, the applied protocols need to be replicated in populations of different ethnicity. Moreover, repeating analysis multiple times on large sample sizes could add power to the results.

Conclusions

This study suggests that, in FET cycles following estrogenic endometrial preparation made without previously using the GnRh analogue, 800 mg doses of vaginal Progesterone as LPS improve reproductive outcomes. Subsequent studies are needed to identify the individualized treatment and the ideal Progesterone dose for each type of endometrial preparation protocol.

Conflict of Interest

The Authors declare no conflict of interest.

Funding

This research received no external funding.

Ethical Statement

Written informed consents were obtained, at the first clinical evaluation, from all patients enrolled voluntarily in the study after being well informed about the hypothetical risks of the procedure. This study was regularly approved by the Local Ethical Board of the Momò Fertilife Institute (approval number 07/2018) according to the Declaration of Helsinki ethical principles.

Authors' Contributions

Conceptualization, G.M.B. and D.B.; methodology, D.B.; software, G.M.B; validation, G.M.B. and D.B.; formal analysis, A.M. and M.D.; investigation, A.M. and S.H.; resources, A.M..; data curation, E.C. and G.C.; writing—original draft preparation, G.M.B. and D.B.; writing—review and editing, E.C., G.C., M.D., S.H. and A.M.; supervision, A.M.; project administration, G.M.B. and D.B.; funding acquisition, G.M.B. and D.B. All authors have read and agreed to the published version of the manuscript.

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