Repeated Application of Luteal Phase Estradiol / GnRH Antagonist Priming Increases IVF Success for Poor Ovarion Reserve Patients

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Abstract

Background: The aim of this study is to compare the LPP [luteal phase estradiol LPP/GnRH antagonists protocol] treatment of patients with poor ovarian response [POR] in terms of the results of in vitro fertilization [IVF] treatment who underwent LPP in the first IVF treatment or in the use of LPP in the second treatment of patients with a different protocol.

Materials and methods: 293 cycles recorded in 226 patients with poor ovarian reserve who underwent LPP treatment, microdose flare-up protocols, and antagonist protocols between 2013 and 2019 were included in the study. Of these, 38 patients received LPP treatment in the first cycle and LPP treatment in the second cycle. After the microdose or antagonist protocols were applied in the first cycle, LPP treatment was applied to 29 patients in the second cycle. There were 128 patients who received LPP only once and 31 patients who received microdose flare-up only once, and these patients were included in the study based on LPP and microdose application results in the patient group with low ovarian response.

Results: In our second cycle results of LPP application in patients with low ovarian response that resulted in one failed IVF, the clinical pregnancy rate was higher than the patients who received only LPP and patients who received LPP after different protocols [$p=0.035$]. In unsuccessful IVF patients who had LPP applied in the first cycle, β -hCG positivity per embryo and clinical pregnancy rates were found to be significantly higher in patients who received the LPP application in the second protocol [$p=0.000$, $p=0.001$].

Conclusion: Repeated LPP may be the first choice protocol for low ovarian reserve patients.

Introduction

Poor Ovarian Reserve is observed at a rate of approximately 9–24% in the entire infertile population. Increasing FSH levels in patients with poor ovarian reserve causes the formation of larger follicles by increasing the sensitization of follicles in the late luteal phase. Due to this stimulation, asynchronization develops. Using estrogen and antagonists in the luteal phase, pregnancy rates can be increased by preventing asynchronization in priming follicles.

The first study on this subject was conducted by Dragisic et al. and was initially based on the studies conducted by Fanchin et al. in 2003 on adding antagonists in the luteal phase after the first estrogen priming application. Dragisic et al. thought that the success could be increased by adding both estrogen and antagonists to suppress FSH levels in the luteal phase and increase the number of mature oocytes [1, 2]. In their results, they found that the number of mature oocytes and the rate of embryo transfer were significantly higher [3].

Other commonly used ovarian stimulation protocols in patients with low ovarian response are the initiation of gonadotropins and GnRH-a in the follicular phase or the use of a microdose flare-up protocol after treatment with oral contraceptives [4].

Treatment with COCs in one cycle of microdose flare-up protocols results in lower FSH levels at the beginning of the next menstrual cycle, potentially enabling synchronization of early antral follicles and an increase in the number of oocytes present during oocyte retrieval.

GnRH antagonist treatment regimens, on the other hand, allow a more natural retrieval of follicles during the follicular phase without suppressing FSH and LH levels caused by GnRH-a [4].

We planned this study considering that the second application of LPP treatment—after the applications of the LPP protocol, microdose flare-up protocol, and antagonist protocol—in patients with poor ovarian reserve and resulting in unsuccessful IVF may increase success rates. Our study is the first in this field.

Materials And Methods

Our study was designed retrospectively. 293 cycles were recorded in 226 patients in the 24–46 age group who applied between January 2013 and March 2019 and had a bad ovarian response [less than 4 oocytes] in the previous cycle, had a bad ovarian reserve test [a low AMH less than or equal to 1.1, a low AFC less than 5–7], or an FSH level of 10 with poor ovarian response and underwent LPP treatment, microdose flare-up protocols, and antagonist protocols. 128 patients with low ovarian reserve received LPP treatment only once, and 31 patients received a microdose flare-up only once. Apart from these patients, 38 patients who underwent the LPP procedure in the first cycle received it again in the second cycle. The LPP protocol was applied to 29 patients in the second cycle who received microdose or antagonist protocols in the first cycle. Our study revealed the results of treatment protocols in patients with low ovarian response. The LPP + LPP protocol application results and different protocol + LPP application results were compared. Patients between 23 and 46 years of age were not made fresh embryo transfers and cycles with frozen embryo transfers were not included in the study. The study received approval from the ethics committee of the University of Health Sciences Gulhane Training and Research Hospital.

LPP group patients; 10 days after ovulation confirmation with LH peak and transvaginal ultrasonography [TVUSG] in patients whose LH peak occurred on the 8th, 10th, or 12th days of the cycle before starting treatment, Estrofem TB [2 mg estradiol hemihydrate, Novo Nordisk, Istanbul, Turkey] was administered. One day later, Cetrotide [Cetrorelix 0.25 mg. s.c., Merck Serono, Istanbul, Turkey] 0.25 mg/day was applied for 3 days. A total of 300 IU of FSH [Gonal-F, Merck Serono, Istanbul, Turkey] and 300 IU of hMG [Merional, IBSA, Turkey] was started on the second day of menstruation. Follicular development was followed by serial TVUSG and serum E2 levels. Cetrorelix [Cetrotide, Merck Serono] was administered until the day of injection at 0.25 mg/day subcutaneously with leading follicle levels greater than 12 mm or E2 levels less than 300 pg/mL of human chorionic gonadotropin [hCG].

On the first and second day of the cycle, 5 mg/ml subcutaneous leuprolide acetate [Lucrin, Abbott, France] was administered once a day to patients who underwent the microdose flare-up protocol. Leuprolide acid was then administered at 5 mg/ml per day. On the second day of the cycle, gonadotropin stimulation 200–450 IU [randomized by Gonal F, Merck Serono, Switzerland or Puregon, MSD,

Netherlands] and/or 75–150 IU hMG [Menogon, Ferring, Germany] was administered simultaneously with the leuprolide.

Gonadotropin stimulation 200–450 IU and/or 75–150 IU hMG was applied on the second day of spontaneous menstruation to patients who underwent the antagonist protocol. When the dominant follicle reached 14 mm in size or on the 6th day of the cycle, randomized subcutaneous cetorelix/ganirelix 0.25 mg/day [Cetrotide, Merck Serono, Switzerland or Orgalutron, MSD, Netherlands] was administered.

The cycle was canceled in patients who did not show a follicular growth response to ovarian induction and had peak E2 levels less than 100 pg/ml.

When one or more follicles grew to 17 mm or more, hCG [Ovitrelle 250µg, Merck Serono] HCG [10,000IU IM, Profasi 5000 IU, Serono, Istanbul, Turkey] was given for final oocyte maturation. Endometrial thickness was recorded by TVUSG on the day of hCG administration. Oocytes were collected with a Cook Echotip Double Lumen Aspiration Needle with a TVUSG guide 36 hours after hCG administration. ICSI was performed on all patients. According to the quality of the embryos formed after ICSI, one or two embryos were transferred according to the age of the patient with an abdominal USG after 48–120 hours [one embryo transfer for under 35 years of age, two embryo transfers for over 35 years old].

For luteal support, after oocyte collection, one Prolutan depot of 250 mg/ml per week [Bayer, Istanbul, Turkey] and a 600 mg daily dose of Progestan capsule [Koçak Farma, Istanbul, Turkey] was given intravaginally. A 6 mg daily dose of Estrodiol hemihydrate [Estrofem 2 mg, Novo Nordisk, Istanbul, Turkey] was administered orally. A pregnancy test was performed 12 days after the embryo transfer, and if it was positive, it was accepted as β -hCG positivity. Gestational sac positivity was accepted as the clinical pregnancy rate. Pregnancies over 24 weeks were accepted as live births.

Included in the study were 38 patients who received the LPP protocol a second time after being unsuccessful the first time. Along with the demographic data of those 38 patients, the duration of stimulation, the gonadotropin dose used, the number of oocytes collected, the MII number, and the cycle cancellation rates were evaluated. The number of embryos in each of the 38 patients, the embryo implantation rate per patient and embryo transfer [ET], the clinical pregnancy rate per patient and embryo transfer [ET], and the live birth rate per patient and embryo transfer [ET] were evaluated.

Our first LPP results and our second LPP results were compared. The LPP + LPP applications and different protocols + LPP application results were compared. In our study, the Kolmogorov-Smirnov test was used to check whether the data conformed to the normal distribution. An Independent t-test was applied to patients with normal distribution. The Mann-Whitney U test was applied to patients who did not conform with normal distribution. The Chi-square test was used for categorical data. Results of P less than 0.05 were considered to be significant.

Results

According to the data of our study, patients with low ovarian response were divided into groups according to their first cycle, with the second cycle being LPP. Patients who received only one cycle of treatment were divided into two groups: LPP protocol recipients and microdose flare-up recipients.

After the first LPP protocol, 38 patients were given LPP a second time. The first LPP results were compared with the second LPP results. No difference was observed between the groups in terms of the number of oocytes collected, the metaphase II oocyte count, the number of embryos obtained, and the embryo quality [Table 1].

Table 1
The first LPP results were compared with the second LPP results.

	LPP	LPP + LPP	p
	Ort ± SD	Ort ± SD	
Age	35.7 ± 4.7	36 ± 4.7	0.709
fsh	11 ± 4.2	11.2 ± 4.6	0.971
amh	0.6 ± 0.7	0.6 ± 0.7	0.819
Stimulation time	10.1 ± 2.1	10.5 ± 2	0.435
dose	5784.6 ± 1291.1	6000 ± 1210	0.342
number of oocytes collected	3.5 ± 2.9	4.5 ± 3.3	0.149
MII oocytes count	2.4 ± 2.1	3.1 ± 2.5	0.181
number of embryo	1.7 ± 1.7	2.0 ± 1.7	0.335

Patients with unsuccessful IVF application, those who underwent the LPP + LPP protocol, and those who underwent different protocols + LPP treatment were compared. β -hCG positivity per embryo and clinical pregnancy rates were found to be significantly higher in the group of patients that received the LPP + LPP protocol. There was no significant difference between the two groups in terms of live birth rate. No significant difference was observed between the groups in terms of the number of oocytes collected, the metaphase II oocyte count, the number of embryos obtained, the embryo quality, and the number of transferred embryos [Table 2].

Table 2
LPP + LPP and those who underwent different protocols + LPP were compared

	LPP + LPP	Other protocols + LPP	p
	Ort ± SD	Ort ± SD	
age	36 ± 4.7	35.4 ± 5.4	0.666
fsh	11.2 ± 4.6	9.2 ± 3.7	0.510
amh	0.6 ± 0.7	0.6 ± 0.5	0.664
Stimulation time	10.5 ± 2	10.3 ± 2.3	0.699
dose	6000 ± 1210.3	5710.3 ± 1575.7	0.363
number of oocytes collected	4.5 ± 3.3	6.5 ± 4.6	0.080
MII oocytes count	3.1 ± 2.5	4.6 ± 3.5	0.090
number of embryo	2.0 ± 1.7	2.7 ± 2	0.137
On going pregnancy per embryo transferred	3	2	0.296
B-HCG positivity per embryo transferred	7	2	0.000
Clinical pregnancy per embryo transferred	6	2	0.001

The 128 patients who received only one LPP protocol and the 38 patients who received the LPP + LPP protocol took the LPP protocol the first time [a total of 166 LPP-receiving patients] and were compared with 31 patients who only received the microdose flare-up and 19 patients who received microdose flare-up for the first time in the different protocol + LPP group [50 microdose flare-up recipients in total]. While the collected oocyte count, MII oocyte count, and embryo count were significantly higher in the microdose flare-up group, the live birth rate was significantly higher in the LPP group. [NOTE: The FSH value of the patients in the microdose flare-up group was significantly lower; this difference may be due to the better FSH values in the microdose flare-up group.] The live birth rate was 12% in the LPP group and 2% in the microdose flare-up group [Table 3].

Table 3
LPP for the first time were compared with patients who received only microdose flare up

		LPP [166]	Microdose flare up[50]	p
		Ort ± SD	Ort ± SD	
age		36.4 ± 4.6	36 ± 4.4	0.444
fsh		11.4 ± 5.2	9.3 ± 3.3	0.029
amh		0.6 ± 0.6	0.6 ± 0.4	0.593
Stimulation time		10.7 ± 1.9	9.1 ± 1.6	0.265
number of oocytes collected		4.1 ± 3.3	5.8 ± 3.9	0.034
MII oocytes count		2.9 ± 2.4	4.1 ± 3.2	0.000
number of embryo		1.9 ± 1.7	3.0 ± 2.5	0.000
On going pregnancy per embryo transferred		20[%12]	1[%2]	0.020
Clinical pregnancy per embryo transferred		29[%29.3]	11[%33.3]	0.663
B-HCG positivity per embryo transferred		30[%30.3]	11[%33.3]	0.746
Embryo quality	Grade I	70[%42.2]	30[%60]	0.085
	Grade II	36[%21.7]	5[%10]	
	Grade III	3[%1.8]	0	
	TFF	35[%22]	10[%20]	
	EKK	22[%13.2]	5[%10]	
number of transferred embryo	0	67[%40.4]	17[%34]	0.112
	1	61[%36.8]	14[%28]	
	2	38[%22.8]	19[%38]	

Our results of LPP protocol application in the second cycle in patients with low ovarian response, which resulted in a failed IVF once, and clinical pregnancy rates were found to be significantly higher than the patients who only received an LPP protocol and patients who received an LPP protocol after different protocols [p = 0.035].

Discussion

There is no standard treatment protocol applied to patients with low ovarian reserve. In this patient group, the rate of cycle cancellation and lack of response to stimulation is high. In this study, we compared the

results of second cycle LPP application in patients with low ovarian reserve who resulted in a failed IVF after having taken the commonly used LPP, microdose flare-up protocols, and antagonist protocols. β -hCG positivity and clinical pregnancy rates were found to be significantly higher in the LPP + LPP group. There was no significant difference between the two groups in terms of live birth rate. No significant difference was observed between the groups in terms of the number of oocytes collected, metaphase II oocyte count, number of embryos obtained, embryo quality, and number of transferred embryos. Similarly, an increase in the number of oocytes collected was not shown with LPA when compared to MD in the study performed by Ata et al. however, a tendency towards higher embryo implantation and clinical pregnancy rates has been observed, but the differences are not statistically significant [5].

In the study conducted in 2005 by Schmidt et al., the GnRH antagonist protocol [ganirelix protocol] was compared with the microdose leuprolide protocol in weak ovarian responders. As a result of the clinical study in poor responders, no significant difference was observed in terms of mature follicle count, mean oocyte count, mature oocyte count, fertilization rates, implantation rates, or clinical pregnancy rates. The use of ganirelix for GnRH antagonist protocols has proven to be as effective as the traditional microdose protocol in treating poor responders [6].

In our study, the group where patients received only LPP and the group that received only microdose flare-up was examined. The collected oocyte count, MII oocyte count, and embryo count were significantly higher in the microdose flare-up group, while the live birth rate was higher in the LPP group [12% and 2%, respectively].

Also, in the study of DiLuigi et al. in 2011, the LPP protocol was applied to 26 patients, the microdose flare-up protocol was applied to 28 patients, and it was shown that the LPG protocol was as effective as the microdose flare-up protocol in patients with poor response to IVF [7].

As a result of their study in 2008, A. Demirel and T. Gürgan suggested that the microdose flare-up protocol was superior to the multiple dose antagonist protocol in terms of the total gonadotropin dose used, the number of mature oocytes collected, and the implantation rate [4].

In a study in which 45 patients in the LPP group and 76 patients in the microdose flare-up group were compared, no difference was observed between implantation rate, clinical pregnancy rate, and spontaneous abortion rate. However, an important difference between the two protocols is that gonadotropin was administered in the LPG protocol for a longer time [days]. This is due to increased pituitary suppression caused by increased E2 levels in the LPG protocol and delayed FSH level elevation with exposure to a GnRH antagonist in the late luteal phase, which further delays FSH level elevation [8].

Controlled ovarian hyperstimulation [COH] protocols for patients with poor ovarian response are designed to prevent early follicle selection in the luteal phase and to optimize the follicular hormonal environment and antral follicle response. The use of the E2 LPP/GnRH antagonist protocol appears to increase ovarian response during COH for IVF and may result in smoother follicular development, more oocytes, more embryo transfers, and a better pregnancy rate [3].

In our study, we found that repeated LPP applications in low ovarian reserve were associated with higher β -hCG positivity and clinical pregnancy rates.

Chang et al. retrospectively examined the PORs stimulated with the luteal estradiol protocol and the standard GnRH antagonist protocol and found that the luteal estradiol protocol had significantly higher peak estradiol levels, oocyte counts, pregnancy rates, and lower cancellation rates [9].

These results may be a result of the heterogeneity in the luteal estradiol group, where most patients used estradiol during the day of hCG administration. Estrogen is known to induce FSH receptor proliferation in granulosa cells and stimulate follicular growth and granulosa cell proliferation. Long-term use of estradiol may result in higher peak estradiol levels and obtained oocyte counts [10].

In our study, the collected oocyte count, MII oocyte count, and embryo count were found to be significantly lower in patients who received the LPP protocol compared to the microdose flare-up group, while the live birth rate was found to be higher [12% as opposed to 2%].

In a study involving 180 patients less than 35 years of age with poor ovarian response, the effects of the luteal phase estrogen LPP/GnRH antagonist protocol and the microdose flare-up protocol were compared. Patients undergoing stimulation with the LPP protocol showed a tendency to have an increase in the implantation rate and the pregnancy rate per cycle. The cycle cancellation rate was found to be higher in the OCP-MDL group, but no statistically significant difference was found [11].

In our literature review, there is no previous study on this subject, and our study is the first of its kind to be done. The low number of our cases is a limitation of our study, and larger studies are needed on this subject.

Conclusion

Luteal phase estrogen LPP/GnRH antagonist may be the first choice in patients with DOR. In unsuccessful cases, the LPP protocol should be considered in a second treatment.

Declarations

Acknowledgements

Not applicable.

Informed consent

All the procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Ethical Review Board: Sağlık Bilimleri Üniversitesi Girişimsel Olmayan Etik Kurul 19/223.

Conflict of Interest Statement

There are no conflicts of interest in this paper.

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Author Contributions

STC, MÖ conceived and designed the study and was responsible for the data acquisition, analysis, and interpretation and drafting the manuscript. STC, MÖ,CK participated in the conception and design, acquisition of the data, data analysis and interpretation, and drafting the manuscript. UY,UF and EA participated in drafting the manuscript and revising it critically for important intellectual content. All the authors read and approved the final manuscript.

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