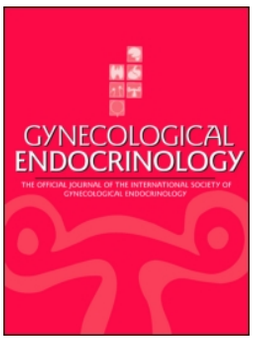

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ORIGINAL ARTICLE

Luteal estradiol pretreatment of poor and normal responders during GnRH antagonist protocol

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ABSTRACT

Luteal estradiol pretreatment (LEP) to IVF protocols designed to improve follicle synchronization and retrieval of mature oocytes. We conducted a retrospective study including women undergoing IVF program who were given a course of 4 mg oral estradiol-17β daily from day 20 of the same cycle until day 1 of their next cycle before starting an antagonist protocol, forming LEP-group but control-group started on day 3 a stimulation without pretreatment. A total is divided into 2 groups (poor (group 1, $n = 148$) and normal responders (group 2, $n = 244$)). Our findings show for group 1 a significant decrease in cancelation rate (3% vs 14%) and a significant improvement in clinical outcomes (clinical pregnancy per transfer and live birth rate respectively: 47% and 44% vs 12% and 11%). For group 2, this pretreatment could increase significantly the maturation rate (77% vs 68%). The rate of frozen embryos was improved in both groups: (group 1: 11% vs 2% and group 2: 53% vs 41%). LEP increases the frozen embryos rate whatever the nature of the ovarian response, but especially for normal responders it coordinates follicular recruitment increasing the maturation rate. In the case of poor responders, it affects positively clinical outcomes decreasing the canceled cycles.

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KEYWORDS
Luteal estradiol pretreatment; oocyte maturation; poor responders; normal responders; in vitro fertilization

Introduction

The introduction of GnRH antagonist protocols for controlled ovarian hyperstimulation (COH) has offered the great opportunity to reduce the duration of treatment but became more difficult to regulate the available program of IVF or ICSI cycles, depending on the activity of assisted reproduction treatment (ART) centers and for domestic or work organization of patients. In the other hand, GnRH antagonist protocols could not resolve the problem of size heterogeneities of early antral follicles during the early follicular phase [1], causing eventually the slight reduction in the number of retrieved oocytes and in the pregnancy rate [2].

The gonadotrophin-dependent exponential growth phase of the follicles starts with the regression of the corpus luteum of the previous cycle, which is associated with a decline in the serum levels of steroid hormones and inhibin, with a resultant increase in the FSH levels. This increase begins about two days before the onset of menstruation and continues throughout the following early follicular phase. The premature, gradual exposure of follicles to FSH may accelerate the development of more sensitive follicles and accentuate size discrepancies observed during the first days of the subsequent cycle [3]. So, most early antral follicles are required to grow coordinately in response to COH to undergo simultaneously functional and morphological maturation, but the FSH-sensitive follicles fail to accomplish this maturation decreasing a number of the viable oocytes and embryos [4] causing an asynchronous multi-follicular growth. This heterogeneity is just a direct consequence of some follicles which are able to respond earlier to lower FSH levels than others since the luteal-follicular transition phase by an intrinsic inconsistent sensitivity [3,5].

Therefore, more attention has been paid to the potential interest of steroid pretreatments to program cycles and to synchronize the follicular cohort before stimulation trying by negative control to modify the hormonal environment which is dependent on endogenous gonadotrophin secretion. However, estrogens are believed to primarily inhibit FSH secretion [6,7], then, other researches could have the initiation proposing a luteal synchronization of follicular growth to increase oocytes yield [1,8,9]. Despite low response to controlled ovarian stimulation (COH) is encountered in 9% to 26% of IVF cycles [10], E2 can be able to control and regulate the FSH luteal secretion and synchronizes early antral follicle growth [1,11] offering good pretreatment for ovarian response. Luteal estradiol pretreatment (LEP) is suitable to reach oocyte maturation at once in homogenous follicular cohort assuring eventually a good quality of embryos [12,13]. This hormonal pretreatment could be helpful in coordinating antral follicle growth and optimizing COH for IVF cycles for poor and normal responders. Whereas, this is the principal objective of this study with amelioration of embryological and clinical outcomes in IVF programs for poor and normal responders. Moreover, it is interesting to see if LEP is efficient to improve the rate of cycles with frozen embryos.

Material and methods

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with

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the Helsinki Declaration of 1975, as revised in 2008. All patients who participated in this study signed an informed consent after being informed about the terms and issues of study.

Patients' selection

This retrospective study included 392 infertile women (20–41 years of age) undergoing IVF cycles in Anfa Fertility Center from October 2015 to January 2018. Selected women received luteal estradiol pretreatment "LEP" or no pretreatment to serve as "control". LEP and the control groups were comparable. All of them had regular normo-ovulatory cycles (25–35 days) age ≤41 years, body mass index (BMI) 18–30 kg/m^2, and first or second IVF/ICSI attempt, both ovaries present, no current or past diseases affecting ovaries or gonadotrophin or sex steroid secretion, clearance or excretion, no current hormone therapy, and adequate visualization of ovaries in transvaginal ultrasound scans. The included patients were evaluated for serum basal hormone levels, as well as a hysterosalpingogram and hysteroscopy or laparoscopy as needed. Patients not meeting the aforementioned criteria as well as those with known history of polycystic ovarian syndrome, endometriosis, previous high ovarian response or repeated IVF failures were excluded from the analysis.

Patients of LEP group ($n = 196$) received micronized 17β-estradiol oral tablets (4 mg/day; Estrofem, Novo Nordisk Pharmaceuticals, Pakuranga, Auckland), from day 20 of the same cycle until day 1 of their next cycle. Luteal estradiol administration did not alter the expected onset of menstrual bleeding. Participants who were included in the control group ($n = 196$) remained untreated during the luteal phase (Figure 1). We were interested to divide the total lot into two groups; poor (group 1) and normal responders (group 2) to evaluate the effect of the protocol depending on the each group presenting nonsignificant differences between the patients' characteristics.

For group 1, we included 148 poor-responder patients ($n = 74$ for each group LEP/control). Those patients met the Bologna criteria presenting at least 2 of the following 3 features that must be present: (1) advanced maternal age (≥40 years) or any other risk factor for POR; (2) previous POR (≤3 oocytes with a conventional stimulation protocol); (3) an abnormal ovarian reserve test (i.e. antral follicle count, 5–7 follicles or anti-mullerian hormone (AMH) with value of 0.5–1.1 ng/mL).

For group 2, we included 244 normal responder patients ($n = 122$ for each group LEP/control). They were characterized by a regular menstrual cycle and having more than 5 oocytes in a previous IVF attempt or at least 5 follicles in a spontaneous cycle to exclude the poor responders. Indeed, they met the following features: (1) maternal age under 40 years; (2) FSH level <12 mIU/mL (day 2–3); (3) E2 level <75 pg/mL (day 2–3); and 4) AMH with value of 1.3–2.6 ng/mL [14].

Stimulation protocol in IVF

All patients were stimulated with fixed antagonist protocol using the r-FSH (Orgalutran 0.25 and Gonal-F). Further r-FSH administration (Gonal-F; Serono Laboratories, Saint Cloud, France) was started by daily subcutaneously injection (150–225 IU/day) for patients with normal ovarian response and more (mean= 300 IU/day) for patients with poor ovarian response. The FSH

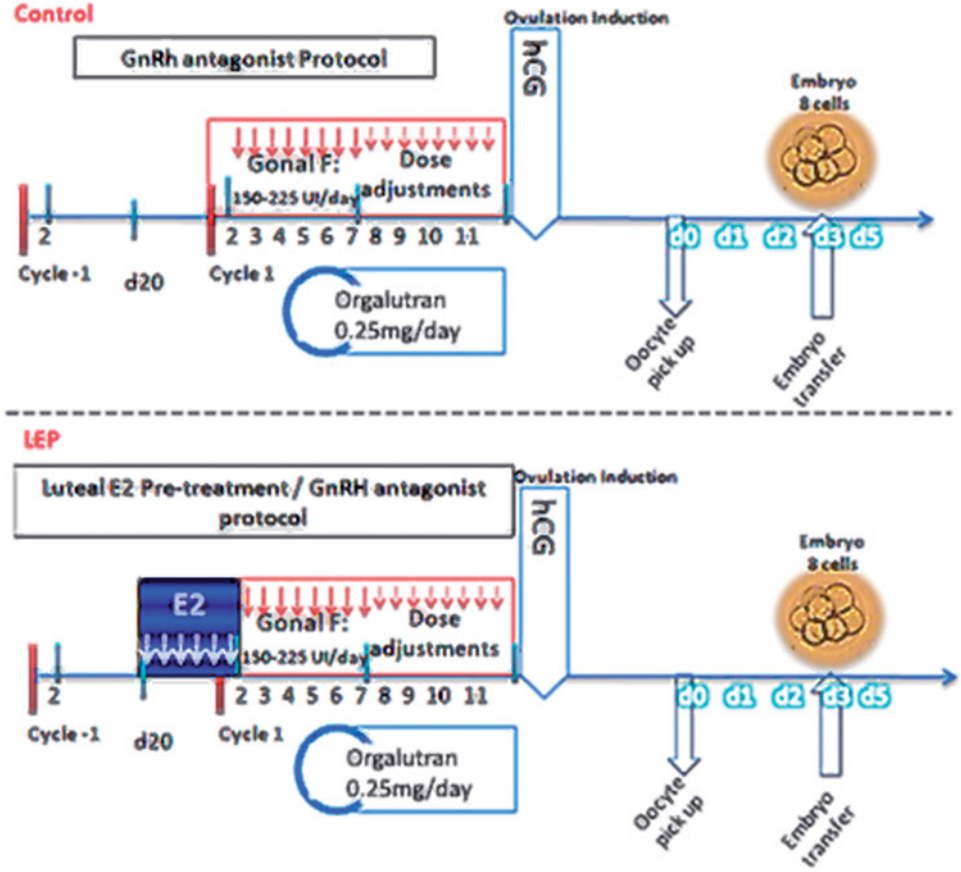

Figure 1. Protocol design. Note: LEP: luteal estradiol pretreatment; E2: estradiol; hCG: human chorionic gonadotropin for ovulation induction; GnRH: gonadotropin releasing hormone. At the top is the stimulation protocol for control group while at bottom is the stimulation with estradiol pretreatment for LEP group.

dose was based on the woman's age and AMH concentration that was maintained constant for 5 days and it was adjusted according to usual parameters of follicle growth determined by serum estradiol concentrations and ultrasound monitoring. A potent, third-generation GnRH antagonist, Ganirelix (Orgalutran®, MSD Schering-Plough, France) injected subcutaneously once daily starting on day 5 or day 6 of FSH administration. An intramuscular injection of 10,000 IU of human chorionic gonadotrophin (HCG, Gonadotrophines Chorioniques Endo®, Organon) was performed after obtaining follicles ≥17 mm. Embryos produced by ICSI were cultured up to day 3. Adequate embryo quality (good quality embryos; A + B) was defined based on the presence of uniformly sized and shaped blastomeres and fragmentation lower or equal to 10%. One or two good quality embryos were transferred *in utero* using a Frydman catheter (CCD Laboratories, Paris, France). Luteal phase was supported by vaginal administration of micronized progesterone 600 mg/day (Utrogestan®, Besins International, Montrouge, France) from the day of oocyte pick-up to the day of pregnancy test. If a pregnancy occurred, progesterone administration was extended up to the evidence of fetal heart activity at ultrasound.

Statistical analysis

Data are presented as mean ± standard deviation (SD) or standard number representing the total. Thus, these data are analyzed by the Student's *t*-test for comparison of mean values or chi squared test for comparison of percentages using the Statistical Package, Statistica (version 6.0) to compare a significantly different populations: $p < .05$ shows the significant difference.

Results

Embryological and clinical outcomes data in both groups (LEP and Control) for the first lot (Group 1, $n = 148$) including poor responders are presented in Table 1. Therefore, the number of oocyte retrieved was approximately similar between the two groups (mean = 4) with no significant difference, either the maturation rate with 61% vs 53% for LEP and Control. In the other hand, the pretreated patients could have a higher frozen embryos rate with a huge difference (11% vs 2%, $p = .03$). Using this approach, patients had on the one hand a significantly higher pregnancy and live birth rate per transfer (47% and 44% vs 12% and 11% respectively), and on the other hand, a significant decrease in the cancelation rate of IVF-cycle (3% vs 14%).

Concerning the second lot (Group 2, $n = 244$) treating a normal responders, their data are presented in Table 2. No significant difference was observed in the number of oocyte retrieved (11.46 ± 5.05 vs 12.02 ± 3.31). In the opposite, our findings showed an important increase about embryological outcomes; maturation, fertilization and frozen embryos rate (77% vs 68% (0.04); 73% vs 67% ($p = .03$); 53% vs 41% ($p = .04$) respectively) but without a significant effect on the cancelation rate and on clinical outcomes.

Discussion

Before the first step in IVF or ICSI cycles (hormone therapy), a pretreatment with estrogen can be given, to suppress the woman's own hormone production as conducted by different authors [1,15,16]. This might improve the woman's response to the hormone therapy in IVF/ICSI cycles. In this way, adverse events such as cyst formation and the number of pregnancy losses might be reduced and pregnancy outcomes might be improved.

A combined OCP pretreatment in GnRH antagonist cycles is associated with fewer clinical pregnancies affecting negatively the implantation by lowering endometrial thickness [17], or by

Table 1. Comparison of IVF outcomes between LEP and control of poor responders of Group 1.

Characteristic-Group 1	LEP-test ($n = 74$)	Control ($n = 74$)	*p* Value
Age of the partner	46.4 ± 11.36	42.39 ± 5.94	.12 (ns)
Age of the patient	38.35 ± 2.49	37.56 ± 3.60	.22 (ns)
Number of IVF attempts	2.65 ± 1.09	2.38 ± 1.56	.35 (ns)
AMH (ng/mL)	0.7 ± 0.48	0.61 ± 0.24	.20 (ns)
Estradiol (pg/mL)	12.92 ± 13.29	12.06 ± 9.69	.87 (ns)
Total dose of gonadotropins (IU)	3242.93 ± 431.24	2998.85 ± 694.82	.73 (ns)
Endometrial thickness (mm)	8.7 ± 1.34	9 ± 0.67	.42 (ns)
Number of oocytes per patient	2.5 ± 1.15	2.01 ± 1.25	.26 (ns)
Maturation rate	**61%**	**53%**	**.04 (s)**
Fertilization rate	79%	77%	.50 (ns)
Cleavage rate	87%	84%	.10 (ns)
Rate of good quality embryos (A + B)	76%	75%	.42 (ns)
Rate of cycles with frozen embryos	**11%**	**2%**	**.03 (s)**
Canceled cycle rate	**3%**	**14%**	**.01 (s)**
Clinical pregnancy rate/ transfer	**47%**	**12%**	**.01 (s)**
Live birth rate/ transfer	**32%**	**11%**	**.01 (s)**

Results are expressed as *n*, *n*(%) or mean ± standard deviation (SD). A statistic significant difference is considered when $p < .05$ (n). $p \geq .05$ is not significant (ns). AMH, estradiol were measured on day 2 of the cycle and the endometrial thickness was evaluated in day of oocyte retrieval.
The bold values are indicating that the difference is statistically significant between the LEP-test and Control groups.

Table 2. Comparison of IVF outcomes between LEP and control of normal responders of Group 2.

Characteristic-Group 1	LEP-test ($n = 122$)	Control ($n = 122$)	*p* Value
Age of the partner	39.38 ± 6.85	41.11 ± 7.14	.21 (ns)
Age of the patient	33.6 ± 5.27	33.73 ± 4.98	.96 (ns)
Number of IVF attempts	2.65 ± 1.98	2.23 ± 1.29	.10 (ns)
AMH (ng/mL)	2 ± 0.09	2.05 ± 0.08	.81 (ns)
Estradiol (pg/mL)	36.65 ± 18.98	32.46 ± 19.41	.19 (ns)
Total dose of gonadotropins (IU)	2432.53 ± 834.1	2875.15 ± 1012.92	.82 (ns)
Endometrial thickness (mm)	9.29 ± 1.01	9.06 ± 0.67	.13 (ns)
Number of oocytes per patient	11.46 ± 5.05	12.02 ± 3.31	.33 (ns)
Maturation rate	**77%**	**68%**	**.04 (s)**
Fertilization rate	**73%**	**67%**	**.03 (s)**
Cleavage rate	98%	97%	.30 (ns)
Rate of good quality embryos (A + B)	78%	75%	.34 (ns)
Rate of cycles with frozen embryos	**53%**	**41%**	**.04 (s)**
Canceled cycle rate	1%	3%	.10 (ns)
Clinical pregnancy rate / transfer	48%	38%	.14 (ns)
Live birth rate / transfer	45%	33%	.09 (ns)

Results are expressed as *n*, *n*(%) or mean ± standard deviation (SD). A statistic significant difference is considered when $p < .05$ (n). $p \geq .05$ is not significant (ns). AMH, estradiol were measured on day 2 of the cycle and the endometrial thickness was evaluated in day of oocyte retrieval.
The bold values are indicating that the difference is statistically significant between the LEP-test and Control groups.

altering E2 and P endometrial receptors [18]. However, LEP compared to control could to improve the number of retrieved oocytes [19]. Indeed, luteal FSH suppression by LEP improves the homogeneity of early antral follicles during the early follicular phase optimizing ovarian response to GnRH antagonist protocol increasing the retrieval of mature oocytes especially for poor responders increasing maturation rate and even for normal responders. These results were in accordance of Fanchin et al. studies [1,11,20] and Dragisic et al. results [21] with an optimization of embryo selection for embryo transfer. However, others did not show any efficiency of LEP [22] especially in poor responders [23].

Poor response to COH is associated with a low follicular response to gonadotropins as a consequence of shortened follicular phase limiting ability to recruit a sizable cohort [24,25]. Therefore, poor responders are suffering of a limited of retrieved oocytes and a reduced number of available embryos for transfer. According to our study, LEP increased the clinical outcomes aproximatively four times more for poor responders confirming Dragisic et al. [21] findings. In the other hand, fertilization, cleavage rate and embryo quality were not improved for LEP group compared to control. This issue could be due to the mean age of patients at 38 years. Contrariwise, treating younger poor responders with LEP, Dragisic et al. [21] obtained an interesting increase of oocytes retrieved and mature oocytes number and improving however the ovarian responsiveness during COH for IVF. Nevertheless, our study could to show the effectiveness of LEP to improve the rate of cycles with frozen embryos for poor responders.

On the other side, our findings about normal responders support the hypothesis of Fanchin et al. [1,11] that LEP leads to an increased number of follicles synchronously attaining maturities. So, this study showed an important improving about embryological outcomes but without a significant increase in clinical outcomes joining the findings of other studies [22,26]. But, a recent study found that patients treated with luteal estrogen resulted in an increased number of oocytes retrieved and prevalence of good quality embryos about 51% compared with the rate resulted (47%), and tendencies for a higher pregnancy rate [27]. Noting minimal improvement about embryological outcomes from maturation and fertilization rates for pretreated normal responders by E2, they could benefice from better frozen embryo yield for eventual frozen-thawed IVF transfer cycles with a probable improvement of clinical outcomes compared to those of fresh IVF cycles.

Conclusion

Our study could to confirm the positive effect of LEP on the embryological and/or the clinical outcomes for the poor and normal responders, and improving the frozen embryos rate which could serve for an eventual IVF cycles. The current study presents reasonable data showing that LEP may improve clinical pregnancy and live birth rate for poor responders. Indeed, these candidates are generally considered as some of the most challenging patient's profile to treat especially for those with advanced maternal age. For normal responders, we need more randomized controlled trials to prove the lack of effectiveness of LEP for them while embryological outcomes could be improved.

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Disclosure statement

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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