ASSISTED REPRODUCTION TECHNOLOGIES

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Estrogen treatment in infertile women with premature ovarian insufficiency in transitional phase: a retrospective analysis

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Abstract

Purpose During the transitional phase of premature ovarian insufficiency (POI), sporadic resumption of ovulation is possible because of fluctuation of hormonal levels but the chance of spontaneous pregnancy is low, and the main perspective of childbearing in these women is egg donation or adoption. The purpose of the study was to verify whether treatment with estrogens in POI patients in transitional phase could reduce FSH levels and to evaluate if this pre-treatment could improve reproductive outcomes of in vitro fertilization (IVF).

Methods Study patients (26) were administered with valerate estradiol 2 mg daily adding dihydrogesterone 10 mg daily during luteal phase for 3 months before IVF. Control group (26 patients) did not receive any pre-treatment. Ovarian stimulation was conducted in both groups with the same short GnRH-antagonist protocol. Clinical and laboratory data of patients were retrospectively analyzed.

Results In the study group, 4/26 POI patients became spontaneously pregnant during pre-treatment. In the remaining patients, the mean level of FSH after the pre-treatment was significantly reduced compared with baseline. Levels of circulating estradiol on the day of hCG administration were significantly higher in the study group. The total number of MII oocytes retrieved and fertilized oocytes was significantly higher in the study group, as well as the number of embryos transferred for pickup and clinical pregnancy rate.

Conclusions Treatment with estrogens in infertile POI patients in transitional phase reduces circulating FSH levels, hence causing potential spontaneous conception. Moreover, in these patients, estrogen pre-treatment seems to improve IVF outcomes in a GnRH-antagonist short protocol compared to no pre-treatment.

Keywords Premature ovarian insufficiency · IVF · POF · Estrogens · Infertility

Introduction

Premature ovarian insufficiency (POI) is a condition characterized by oligomenorrhoea, hypoestrogenism, and elevated gonadotropin levels in women under 40 years of age [1, 2]. The term POI has replaced the previous "premature ovarian failure," as that definition suggested a sudden event rather than a gradual process [1, 2].

The disorder has been estimated to affect 1% of women under the age of 40 years [2–4].

POI can be either primary (spontaneous) or secondary (induced by radiation, chemotherapy, or surgery).

In most of spontaneous POI (90%), no iatrogenic cause is found [2–4]. Indeed, in these patients, the progressive reduction in ovarian reserve due to early loss of primordial follicles eventually leads to premature ovarian insufficiency.

European Society of Human Reproduction and Embryology (ESHRE) [5] recommends both of the following two diagnostic criteria for POI:

- oligo/amenorrhea for at least four months
- an elevated follicle-stimulating hormone (FSH) level > 25 mIU/ml confirmed twice > 4 weeks apart

However, there is a transitional phase of POI when menopausal symptoms and oligomenorrhoea are not yet established

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[2, 6–8], as the disease requires several years to establish its full-blown stage [1, 2, 7]. In fact, the disorder starts with subfertility ("occult" or "incipient" ovarian insufficiency) [8], then it progresses to biochemical insufficiency (elevated levels of FSH) also known as "transitional phase" of POI [6].

Despite the relatively low prevalence of women affected by this condition, POI represents a challenging issue for fertility clinics. In fact, the diagnosis of POI in western countries is often made in women desiring pregnancy. During the transitional phase of POI, sporadic resumption of ovulation is possible, because of potential fluctuation of hormonal levels due to intermittent recovery of ovarian function [9] but the chances of spontaneous pregnancy are very low (5–10%) [5]. According to ESHRE guidelines, the main perspective of childbearing in women with high levels of gonadotropins is egg donation or adoption [5]. Nonetheless, the occurrence of spontaneous pregnancies in POI patients during hormone replacement therapy has enhanced the hypothesis that a fall in endogenous FSH levels could improve ovarian response by the induction of FSH receptors in granulosa cells [10–12].

In view of the poor reproductive outcome of these patients, we tried to find a treatment option to improve their chances to conceive with their own gametes. The rationale of our study was that a treatment with estrogens may establish an estrogenic follicular environment which could promote oocyte growth, maturation, and survival [13, 14]. Moreover, the reduction in circulating FSH could induce granulosa cell FSH receptor expression. Finally, the increased number of FSH receptors could enhance the response to exogenous FSH [15].

The study aimed to verify whether a pre-treatment constituted of continuous estrogen therapy adding progestins during luteal phase for 3 months could reduce FSH levels previous to a cycle of controlled ovarian hyperstimulation (COH) for in vitro fertilization (IVF) in POI patients in transitional phase. The secondary aim of the study was to evaluate if this pre-treatment could enhance follicular response and improve reproductive outcomes of IVF compared with women not submitted to this pre-treatment.

Material and methods

We retrospectively analyzed women in transitional phase of POI referred for infertility to the Assisted Reproduction Center of Pisa University from January 2013 to January 2017. The study was approved by the institutional Review Board of Pisa University Hospital and was conducted according to Helsinki Declaration.

Inclusion criteria for the study were the following: age < 40 years and FSH blood levels > 14 mIU/ml on the second day of menstrual cycle.

In order to analyze only spontaneous POI in transitional phase, patients were excluded from the analysis if there was at least one of the following exclusion criteria: oligo/amenorrhea for at least four months, anti-mullerian hormone (AMH) serum levels > 1.2 ng/ml, ovarian antral follicle count (AFC) > 7 on the second day of the menstrual cycle, any grade of pelvic endometriosis on transvaginal ultrasound (US) scan, any previous ovarian surgery or previous gonadotoxic therapies, potentially influencing ovarian response to COH, age ≥ 40 years, and severe male factor infertility according to World Health Organization criteria [16].

In June 2015 at our center, we began treating POI patients in transitional phase with a continuous estrogen therapy of valerate estradiol 2 mg daily (Progynova, Bayer) adding dihydrogesterone 10 mg daily (Dufaston, Abbott) during luteal phase (16th to 25th day of cycle) for 3 months before standard GnRH-antagonist short protocol for IVF. Patients undergoing this protocol constituted our study group (26 patients) (Fig. 1). The goal for this group of patients was to get a FSH level of < 12 mUI/ml at COH start.

Our control group (26 patients, Fig. 2) was constituted by POI patients in transitional phase undergoing IVF at our fertility center before June 2015. Patients included in the control group did not receive any pre-treatment before IVF (Table 2).

Patients in both study and control groups were administered with 400 mcg folic acid oral daily supplementation at the time they were referred to our center.

Because of limits imposed by Italian legislation, only patients with FSH serum levels of < 30 mUI/ml at start were allowed to undergo COH [17].

COH was conducted in both groups with the same short GnRH-antagonist protocol: 300 IU of recombinant FSH daily (rFSH) (Gonal F pen® Merck-Serono) and 150 IU of highly purified urinary gonadotropin (hMG) daily (Meropur® fl, Ferring) from the day 2 of the cycle. When the leading follicle reached 14 mm, daily injections of GnRH-antagonist (Cetrotide® 0.25 mg sc, Merck-Serono) were administered to prevent premature ovulation by using the flexible antagonist protocol, according to a personalized regimen, until the day of ovulation induction. Recombinant hCG (Ovitrelle® 250 mcg/0.5 ml, Merck-Serono) single dose administration was used to trigger final oocyte maturation, and US-guided follicle puncture was performed approximately 36 h after induction of ovulation. Patients in both groups were monitored with serum estradiol (E_2) and progesterone assay and pelvic US. Measurements were performed with the Elecsys immunoanalyzer (Roche Diagnostics, Mannheim, Germany). With this method, intraassay and interassay coefficients of variation were < 3 and 6% for FSH, < 5 and 10% for E2, and < 3 and 5% for progesterone, respectively [18, 19]. A single expert embryologist evaluated the maturity and potential fertilization of the inseminated oocytes as well as the quality of the deriving embryos according to the method described by Veeck 24 and 48 h after insemination [20]. US-guided embryo transfer (ET) was performed on day 2 or 3 using a

Fig. 1 Flow chart of the study group. POI premature ovarian insufficiency, FSH follicle-stimulating hormone, COH controlled ovarian hyperstimulation, IVF in vitro fertilization, ET embryo transfer
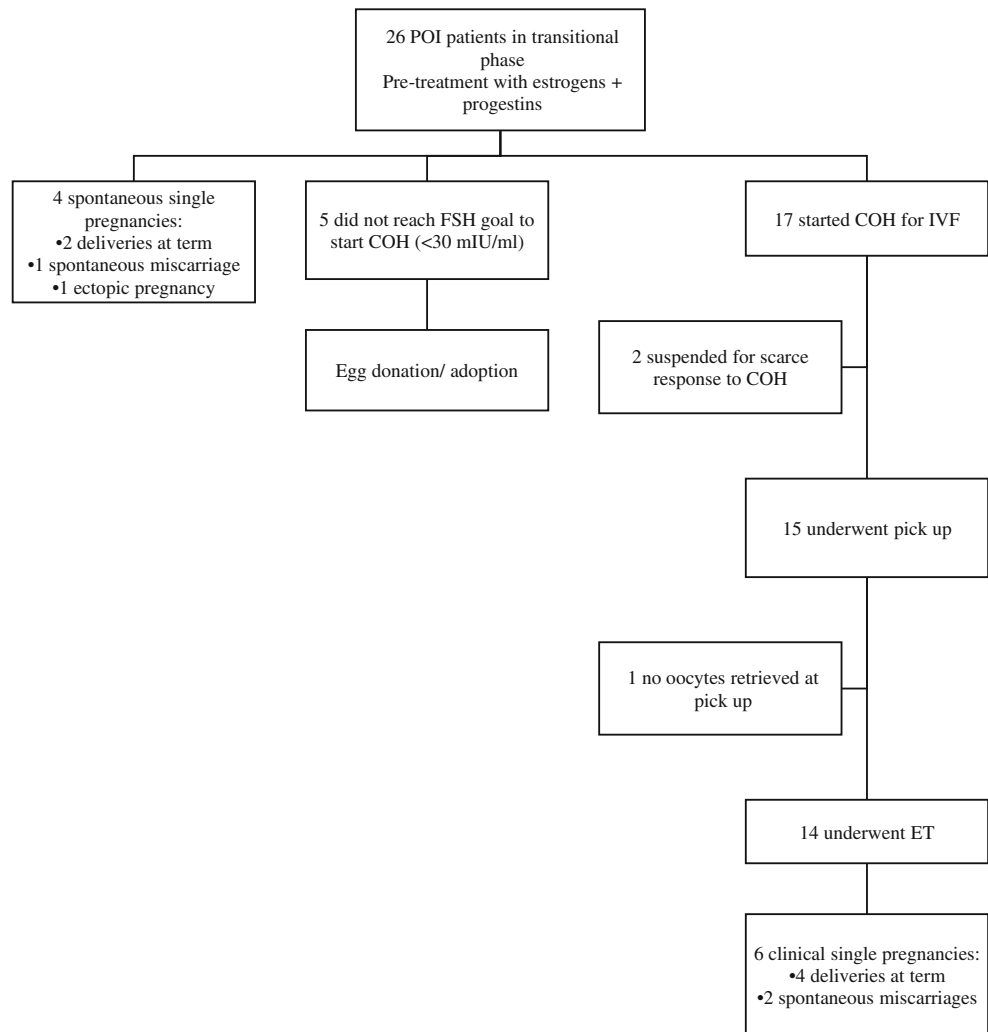
K-Soft 500 Embryo Transfer Catheter® (Cook, Ireland Ltd.) [18, 19]. Serum β-hCG concentration was determined 14 days after ET, and if positive (> 5 mUI/ml), the test was repeated 1 week later. Clinical pregnancy was diagnosed if a fetal heartbeat was observed by transvaginal US. From the day of the ET, all patients had luteal phase support with vaginal micronized progesterone 200 mg three times a day (t.i.d.) (Prometrium®, Rottapharm S.p.A.) and intramuscular progesterone every 72 h (Lentogest®, IBSA). The therapy was continued until either a negative serum pregnancy test result or clinical pregnancy was confirmed on US. Patients with a positive pregnancy test continued luteal support until the 12th week of pregnancy.

The clinical and laboratory data of all patients included in the study were retrospectively analyzed.

Main outcomes were the variation in FSH levels between baseline and start of COH in study group and the comparison of clinical pregnancy rate between the two groups. Secondary outcomes were the comparison of estradiol level on the day of ovulation induction between the two groups; the comparison

of the number of oocytes retrieved, of the number of metaphase II (MII) oocytes retrieved, of the number of transferred embryos, and of the number of good quality embryos between the two groups.

This study was performed by retrospective analysis of our database of women referring our center for infertility from January 2013 to January 2017. All consecutive patients fulfilling the previous reported selection criteria were included in the analysis.

Statistical analysis was performed using Student's t test paired or not, as appropriate. Association between categorical variables was assessed with chi-square test. A $p < 0.05$ value was considered statistically significant.

Results

The two groups of the study resulted similar in baseline characteristics. In fact, women in the two groups did not differ
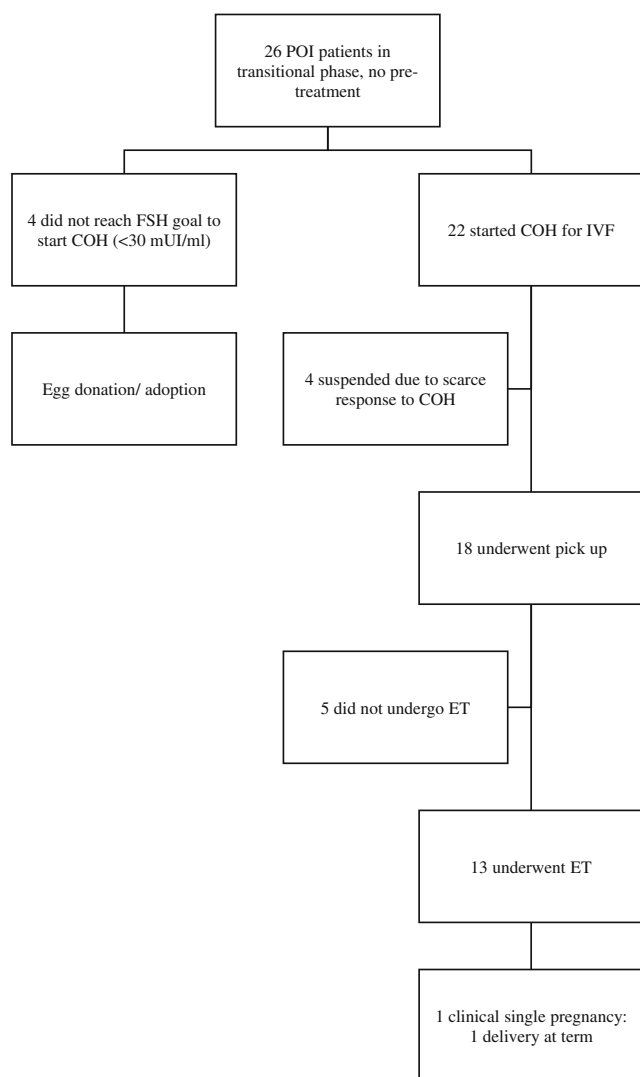
Fig. 2 Flow chart of the control group. POI premature ovarian insufficiency, FSH follicle-stimulating hormone, COH controlled ovarian hyperstimulation, IVF in vitro fertilization, ET embryo transfer

significantly for age, body mass index (BMI), baseline serum levels of FSH and AMH, and baseline AFC (Table 1).

In the study group, four out of 26 patients became spontaneously pregnant during pre-treatment, so they did not undergo a second evaluation of FSH after pre-treatment nor COH for IVF (Fig. 1). Two of them had single delivery at term, one had a spontaneous miscarriage, and one was diagnosed with ectopic pregnancy.

Five patients in study group showed a persistently high level of FSH (> 30 mIU/mL) so they were not allowed to undergo COH for IVF according to the Italian legislation and were referred to egg donation or adoption [17].

In the study group, the mean level of FSH after 3 months of pre-treatment in the remaining 17 women was significantly reduced compared with mean FSH at baseline (Table 2). Only three patients (A, B, C) in the study group had FSH level ≥ 12 mIU/ml at start. Even if we suggested addressing them to

egg donation, they preferred to try COH and IVF regardless of the scarce chances with homologous IVF. Patient A had a baseline FSH level of 25.5 mIU/ml, and the level reduced until 17 mIU/ml at COH start. Despite the considerable decrease in FSH circulating level, this patient was suspended because of unsatisfactory response to COH. Patient B had a FSH level of 17.8 mIU/ml at baseline. She had no response to pre-treatment, as her FSH level at start was 19.2 mIU/ml. Remarkably, this was the only patient who did not retrieve any oocyte at pickup. Patient C had a baseline level of 30 mIU/ml. She had a substantial reduction in circulating FSH, as it was 19.1 mIU/ml after pre-treatment, but as expected, she was suspended before pickup due to insufficient response to COH.

Among the 17 patients who began COH, two had their cycle suspended due to scarce response at ovarian stimulation. Among the 15 patients submitted to oocyte pickup, one did not retrieve any oocyte, while 14 underwent ET. Six patients obtained clinical pregnancy (four term single deliveries, two spontaneous miscarriages).

In the control group, four patients had a persistently high level of FSH (> 30 mIU/ml), so they were not allowed to undergo COH for IVF and were addressed to egg donation or adoption (Fig. 2). Among the remaining 22, four had their cycle suspended because of unsatisfactory response to COH, while 18 were submitted to oocyte pickup. Remarkably, only 13 of them underwent ET. Only one patient in this group obtained a clinical pregnancy (one term single delivery).

IVF results in study group and controls are shown in Table 3. Total dose of gonadotropins administered was similar in study group and controls.

Serum levels of circulating estradiol on the day of hCG administration was significantly higher in study group (mean 1005.9 vs 503.6, $p = 0.01$), while progesterone level was similar in the two groups.

The total number of oocytes retrieved was higher in the study group, but the result was not statistically significant.

On the contrary, the number of MII oocytes retrieved (mean 2.40 vs 1.33, $p = 0.04$) and the number of fertilized oocytes (mean 2 vs 1.06, $p = 0.02$) were significantly higher in the study group compared to controls.

Interestingly, also the number of embryos transferred for pickup was significantly higher in the study group (mean 1.73 vs 1, $p = 0.02$). Similarly, clinical pregnancy rates were significantly higher in the estrogen pre-treatment group compared with controls (6/17 vs 1/22, $p = 0.01$). We did not find any difference in good-quality embryo rate between the two groups.

Cancelation rate before pickup was comparable between the two groups, but three patients in control group did not transfer any embryo after pickup, while only one patient in the study group did not undergo ET after pickup.

Table 1 Epidemiologic and laboratory data at baseline

	Study group [mean (SD)]	Control group [mean (SD)]	Normal range	p value
Age of female partner (years)	37.3 (2.3)	36.9 (3.2)		0.67
Age of male partner (years)	40.0 (4.6)	39.3 (5.7)		0.64
Body mass index (kg/m^2)	21.20 (1.73)	20.59 (1.96)	18.5 – 24.9	0.31
Basal FSH (mUI/ml)	20.0 (5.3)	18.5 (5.5)	2.0–12	0.37
Basal AMH (μg/ml)	0.53 (0.63)	0.40 (0.62)	1.2–3.6	0.51
Basal AFC	4.76 (2.36)	4.36 (1.62)	> 7	0.53
Mild male factor infertility (no., %) [16]	5/26 (19.23)	4/26 (15.38)		0.99
Tubal factor (no.)	0/26	0/26		

SD standard deviation, *FSH* follicle-stimulating hormone, *AMH* anti-müllerian hormone, *AFC* antral follicle count

Discussion

POI, as defined as the triad of oligo-amenorrhoea, hypoestrogenism, and elevated gonadotropin levels, requires several years to develop [1, 2, 7]. Before the full-blown stage has established, the disease can progress at a subclinical stage for years. In fact, according to some authors [2, 7] and as confirmed by our experience, the disorder starts with subfertility ("occult" or "incipient" ovarian insufficiency) [8], then progressing to biochemical insufficiency (elevated levels of FSH) also known as "transitional phase" of POI [6]. Only at the end of the process, the disease appears with overt clinical presentation, including irregular cycles and/or vasomotor symptoms.

A further complication is that there are three conditions with partial overlap in definition: POI, reduced ovarian reserve (ROR), and "poor ovarian response" (POR).

According to ESHRE consensus [21], to define a "poor responder," a woman under 40 years of age must have undergone at least one cycle of COH [22]. Concerning ROR, there is no consensus in literature concerning its proper definition. Published research on the topic has been conducted with different cutoff of instrumental and laboratory parameters of ovarian reserve evaluation [22].

As a consequence, in our study, we analyzed patients with high circulating levels of FSH and infertility, and we decided to define them as POI patients in the "transitional phase" or "biochemical POI" [2, 7, 8] thus before amenorrhea and menopausal symptoms had occurred.

There are no guidelines on medical treatment of infertility in POI patients, and according to ESHRE guidelines on the subject, there are no interventions that have proven to increase the chances of natural conception, while egg donation is considered a reliable opportunity to achieve pregnancy [5].

It is generally accepted that a serum FSH level of > 40 mIU/mL is associated with sterility and that induction of ovulation in these patients is ineffective [23]. On the other hand, the Italian legislation allows couples to undergo assisted reproduction techniques only if the female partner has a FSH level on the third day of cycle < 30 mIU/ml [17]. For this reason, it was hypothesized that the hypergonadotropic condition alone may reduce ovarian responsiveness [24, 25].

Nevertheless, some studies in literature analyzed the effect of estrogen treatment in patients with high level of circulating gonadotropins. They suggested that a fall in endogenous FSH levels improves ovarian response by the induction of an up-regulation of FSH receptors in granulosa cells [12, 26]. The occurrence (although rare) of a spontaneous pregnancy during cyclic estrogen and progestin therapy [27, 28] enhanced the mentioned hypothesis. In these cases, the estrogen-induced decrease in serum gonadotropins seems to improve the responsiveness of remnant ovarian follicles.

First in 1996, Taylor showed a spontaneous ovulation in 46% of POI patients treated with estrogens [29]. Then, in 2007, Tartagni and colleagues [30] evaluated in a randomized placebo-controlled trial the effect of estrogen pre-treatment with 0.05 mg ethinyl-E2 (EE) t.i.d. for 2 weeks on COH in two groups of POI patients. The researchers observed that levels of FSH before stimulation were significantly lower in the study group than in controls, and the rate of ovulation in the study group was significantly higher. Particularly, ovulation induction was satisfactory only in women whose FSH

Table 2 Variation in follicle-stimulating hormone levels before and after estrogen pre-treatment in the study group

	Baseline [mean (SD)]	Start of COH [mean (SD)]	Normal range	p value
FSH (mUI/ml)	20.0 (5.34)	11.2 (4.41)	2.0–12	< 0.0001

SD standard deviation, *FSH* follicle-stimulating hormone

Table 3 Study and control results

	Study group ($n = 17$)* [mean (SD)]	Control group ($n = 22$)* [mean (SD)]	p value
Estradiol on hCG day (pg/ml)	1005.9 (644.7)	503.6 (235.7)	0.01
Progesterone on hCG day (pg/ml)	1.55 (0.96)	1.38 (0.57)	0.55
Total dose of rFSH (UI)	2532.7 (1060.1)	2775.0 (841.2)	0.55
Total dose of hMG (UI)	1821.4 (1131.1)	2451.5 (1589.7)	0.21
Number of oocytes retrieved per pickup	2.67 (2.06)	1.72 (1.18)	0.11
Number of metaphase II oocytes retrieved per pickup	2.40 (1.76)	1.33 (1.14)	0.04
Number of oocytes fertilized	2.00 (1.25)	1.06 (0.87)	0.02
IVF	9/15[a]	12/17[a]	0.53
ICSI	5/15[a]	5/17[a]	0.81
Number of embryos transferred per pickup	1.73 (0.96)	1.00 (0.77)	0.02
Good quality embryo (blastocyst, I–II) rate	0.87 (0.83)	0.78 (0.88)	0.77
Number of patients with ovum retrieval	14/17 (82.35%)	16/22 (72.73%)	0.70
Number of patients with ET	14/17 (82.35%)	13/22 (59.09%)	0.17
Clinical pregnancies per cycle initiated	6/17 (35.29%)	1/22 (6.25%)	0.01
Number of cycles canceled due to scarce response to controlled ovarian stimulation	2/17	4/22	0.58

SD standard deviation, *hCG* human chorionic gonadotropin, *rFSH* recombinant follicle-stimulating hormone, *hMG* human menopausal gonadotropin, *IVF* in vitro fertilization, *ICSI* intracytoplasmic sperm injection, *ET* embryo transfer

*Patients who started COH

[a] Results among women achieving pick up (two women with cycle canceled in the study group and four women with cycle canceled in the control group)

levels at the beginning of COH were lower than 15 mIU/mL, as confirmed by our study. Later, in the study by Popat in 2008, the administration of 100 mcg transdermal estradiol normalized circulating levels of LH in 50% of POI patients [31, 32]. Recently, Check et al. evaluated the IVF outcome in women in overt menopause pre-treated with estrogens. They observed that the live pregnancy rate per transfer was 20% with generally one or occasionally two embryos transferred. The pregnancy rate per retrieval was 9.3% [33].

Interestingly, the results of our study show that a pre-treatment with estrogens plus progestins in patients with elevated circulating FSH and infertility can have different outcomes in different POI patients.

Some patients do not respond to the treatment, maintaining a persistently higher level of FSH (Fig. 1). These patients should not be allowed to undergo COH due to the expected scarce response, and they should take advantage of egg donation to obtain pregnancy. The poor response to pre-treatment could be explained by a reduced sensitivity to estrogen-induced feedback on pituitary FSH release. Inhibin is considered a potential candidate involved in pathogenesis of POI due to its negative feedback control of FSH. It was suggested that a functional mutation in the inhibin gene could determine a subsequent loss in the amount of bioactive inhibin thus removing the negative feedback on the pituitary release of FSH [1].

On the contrary, another group of patients demonstrated an amazing effect, becoming spontaneously pregnant (Fig. 1),

even if some of them evolved in spontaneous miscarriage (2/4: one spontaneous miscarriage, one ectopic pregnancy). These women obtained the most favorable effect as they could conceive spontaneously.

The third group of patients was constituted by women who underwent a significant reduction in FSH levels from baseline, hence obtaining satisfactory results in subsequent IVF outcomes. Based on our data, the goal of the pre-treatment should be obtaining a level of circulating FSH lower than 12 mIU/ml to maximize the chances of success of IVF. In fact, the three patients with the highest levels of FSH were the patients with the worse outcome of IVF. The result is in accordance with data reported in literature showing that a level of FSH higher than 13–15 mIU/ml is a negative prognostic factor for response to COH [30, 34].

On the other hand, some patients in the study group had their COH cycle canceled due to scarce response to ovarian stimulation, notwithstanding the reduction in FSH level. This could be caused by a polymorphism of FSH receptors in the ovary, which could determine an altered response to gonadotropins, even with many receptors available. In these women, follicles may be available, but are unresponsive to hormonal stimulation [1].

The reason why different POI patients have a different response to the treatment should be investigated in different gene mutations determining the disease. Only a minimal part of these mutations have been elucidated, and research is warranted to enlighten the topic.

Regarding the effect of the evaluated pre-treatment on IVF outcome, the higher level of estradiol on the day of induction obtained in the study group could be linked to a more successful folliculogenesis, as estradiol is produced by granulosa cells of growing follicles [13]. Even if women in study group did not obtain a significant increase in the number of retrieved oocytes at pickup, the significantly higher number of retrieved MII oocytes, of fertilized oocytes, and of transferred embryos obtained in the study group compared to controls indicate an improvement of oocytes maturation process, which represents a key factor for fertilization. Indeed, the significantly higher number of embryos transferred in study group resulted in a higher clinical pregnancy rate.

The levels of progesterone measured on the day of hCG administration were comparable between the two groups. Both mean values were relatively high, but this could be explained with the peculiar hormonal status of POI patients [35]. Nevertheless, it is generally accepted that a level of 1.5 ng/ml may be acceptable [36]. Moreover, in literature, there is a heated debate on the role of raising of progesterone level during late follicular phase in assisted reproduction cycles, so in consideration of the lack of significant differences between the two groups regarding this parameter, we did not consider this issue crucial.

Even if some differences in the outcome of IVF between the two groups of the study were not significant, it seems clear that there is a trend of better outcomes in the study group compared to controls, perhaps related to the relatively small population of the study.

Remarkably, at the age of our population, the reported pregnancy rate is lower than 25%, and in poor responder patients, that rate is even lower, approximately 10%, so our results in terms of pregnancy rate seem much more important [37].

Furthermore, the number of cycles canceled after pickup due to lack of fertilization was higher in control group, as all but one woman in the study group underwent ET after pick up. This represents a fundamental result both for the chance of success for our infertile couples and in the economic and psychological aspects. Notably, the patient who did not undergo ET after pickup in study group had a level of FSH at start (19.2 mIU/ml) higher than the target.

Our study has some limitations which should be noted. Firstly, the number of patients analyzed is limited. However, this is due to the low prevalence of the disease.

Secondly, the retrospective nature of the study does not allow us to draw definitive conclusions concerning the practical applications of the exposed therapy in different types of POI patients.

Thirdly, the different evaluation of FSH serum level in the two groups (only at study enrollment in the control group while also after 3 months of estrogen pre-treatment in the study group) could suggest a selection bias in the characteristics of the population enrolled in the two groups. Moreover, the fact that we selected patients on the basis of FSH levels could also

be argued, as FSH has fluctuating levels, especially in transitional phase of POI. For that reason, some authors consider FSH a less reliable parameter of ovarian reserve than AMH and AFC [2, 38–41]. Nonetheless, ESHRE diagnostic criteria for POI do not consider AMH and AFC. Moreover, all the women involved in our retrospective analysis had AFC and AMH values suggestive for scarce ovarian reserve, thus confirming the diagnosis of POI in transitional phase (Table 1).

Finally, the comparable clinical features of women in the two groups at enrollment as well as the different estradiol on hCG day and the dramatic reduction of FSH serum level in the study group and their significant better pregnancy outcomes compared to controls suggest an evident efficacy of the investigated estrogen pre-treatment.

Conclusions

Treatment with estrogens in POI patients reduces circulating FSH levels, hence causing potential spontaneous conception. Moreover, an estrogen pre-treatment seems to improve IVF outcomes in a standard GnRH-antagonist short protocol when compared to no pre-treatment in POI patients in transitional phase. Randomized trials with a larger cohort of patients are needed to confirm the results of our retrospective study. In the future, we should investigate potential mutations in several genes involved in POI pathogenesis, thus clarifying what kind of POI patients could benefit mostly from this pre-treatment. Our results underline the feasibility of a pre-treatment with estrogens and progestins before IVF in POI patients, at least in those with specific gene mutations. This is a safe and cost-effective option in transitional phase POI patients who wish to try to conceive with their own gametes, as these patients should otherwise be addressed to egg donation or adoption.

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