Annals of Clinical Case Reports

Case Report
Published: 15 Aug, 2023

# Efficacy of Co-Adjuvant Melatonin and Resveratrol Chronotherapy for Pregnancy Achievement in Premature Ovarian Failure (POF)

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## Abstract

Premature Ovarian Failure/Insufficiency (POF/POI) is a severe chronic female condition characterized by low peripheral $E_2$ and FSH levels before 40 years age. Available therapy did not demonstrate any significant improvement in fertility rate, delaying menopausal symptomatology, and long-term $E_2$ deprivation effects. To restore normal reproductive function, general health and well-being, we proposed to treat POF women with physiological Hormone Replacement Therapy (pHRT), plus Melatonin (MEL) and Resveratrol (RESV). A 39-year-old POF woman, diagnosed after meeting hormonal criteria, emotional distress, miscarriages, and history of familial early menopause and autoimmune disease, poorly tolerated oral contraceptive HRT. Later on, started on natural $E_2$ and micronized Progesterone (P4) treatment; however, no amelioration was achieved. We then started with a "physiological-like¨ schedule, consisting in mimicking a normal female cycle; i.e., hormone doses and timing profile similar to the natural cycle. Because patient was thin, the lowest $E_2$ dose to obtain physiological $E_2$ levels was chosen. After a 3 month-pHRT combined with MEL (50 mg/night) and RESV (100 mg/day) administration, peripheral hormone levels reached not only physiological values but also comparable to those seen on fertile stage, as well as a corresponding cervical mucus pattern and reported sense of “well-being". Five months later, peripheral hCG and abdominal ultrasound confirmed spontaneous pregnancy, on time a healthy baby was born at term by natural delivery. Thus, pHRT and adjusted endogenous rhythm, due to MEL+RESV, could result in novel and multiple health women’s benefits. Therefore pHRT+MEL+RESV therapy should be strongly considered for treating POF/POI and perimenopausal women.

**Keywords: Premature Ovarian Failure (POF); Physiological Hormone Replacement Therapy (PHRT); Melatonin; Resveratrol; Chronotherapy; Menopause; Antioxidants**

OPEN ACCESS

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**Received Date**: *28 Jul 2023*
**Accepted Date:** *08 Aug 2023*
**Published Date:** *15 Aug 2023*

***Citation:***
*Pagano ES, Peña J, Spinedi E. Efficacy of Co-Adjuvant Melatonin and Resveratrol Chronotherapy for Pregnancy Achievement in Premature Ovarian Failure (POF). Ann Clin Case Rep. 2023; 8: 2454.*

***ISSN: 2474-1655.***



## Introduction

Premature Ovarian Failure (POF), a complex, heterogeneous and chronic female pathology, is characterized by low E2 and high FSH peripheral levels before age 40 years [1] and ranges 1% to 3% at reproductive age [2]. Symptoms are irregular menstrual cycles, although some patients present any residual ovarian function (50%, approx.). Diagnosis should be confirmed by obtaining, one-month separated, two serum FSH determinations to (>40 mIU/mL), with/without amenorrhea. POF could be transient or progressive and usually leads premature menopause. Although related to infertility, it carries other health problems: Poor bone mineral-density, hypothyroidism, cardiovascular disease risk, and psychiatric disorders [3].

POF pertains to a condition known as Premature Ovarian Insufficiency (POI), characterized by transient impaired ovary function rather than cessation. Its pathogenic causes could be chromosomal, genetic, autoimmune, metabolic, infectious or iatrogenic. It is estimated that 20% to 25% of POF cases are genetic [4]; however, most cases are idiopathic. Ovarian follicular-dysfunction or follicle depletion, probably due to reduced recruitment [5,6], accelerate atresia and Oxidative Stress (OS) mechanisms [7].

Available POI therapies did not show significant improvement in fertility rate or delaying early menopause [8]. We now propose a treatment consisting in: Physiological HRT combined with

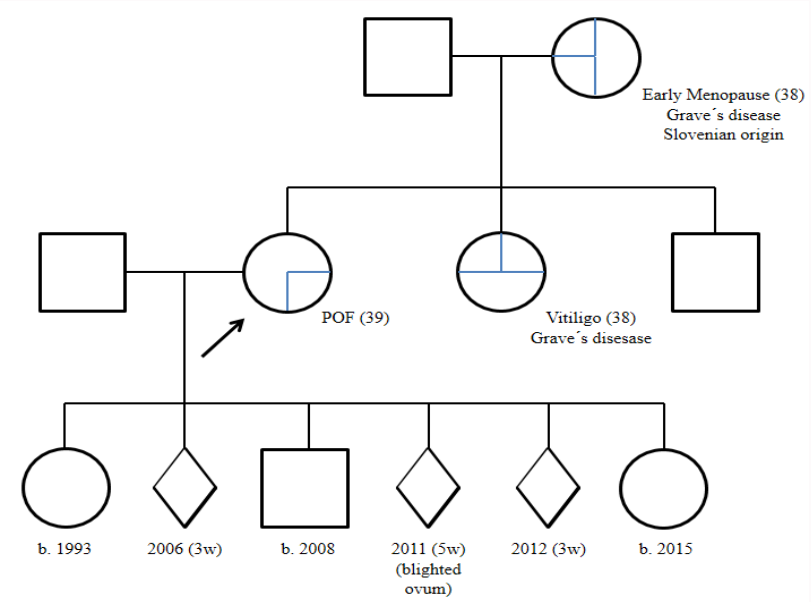


**Figure 1:** Family pedigree for the case-patient.
Clear evidence of a genetic background for POF and subfertility: Mother developed an early menopause (age 38 years), two direct relatives (mother and sister) with autoimmune disease and 3 miscarriages interspersed with 3 born children being the last one naturally conceived under the herein reported treatment, pHRT+MEL+RES.

Melatonin (MEL) plus Resveratrol (RESV) that could help for POF/POI women with limited options to restore reproductive function.

## Case Presentation

A 39-year-old patient, Caucasian, gravid 5 para 2 (Figure 1), diagnosed as POF accordingly to $E_2$, LH/FSH peripheral levels (DPC, RIA kits) (Table 1). She presented poor concentration, anxiety and mood swings, features that were previously misinterpreted. Family history: Early menopause, vitiligo and Graves' disease, no evidence of autoimmunity, no abdominal surgery (normal HSG). Because patient´s genetic background (50% Slovenian, maternal-line), whom 4.8% (approximately) display idiopathic POF in Slovenia, carrying a FRAXA pre-mutation [9], indicating higher incidence than the general women population (1%-3%) [2], genetic tests were performed ruling-out this possibility (normal karyotype and 23/36 CGG repeats in exon 1 of FMR- 1 gen). She referred short-lasting cycles (24 days) since menarche, with appropriate duration for 8 to 9 months after labor, but shorter cycles re-appeared.

Initially, patient was advised to follow a contraceptive HRT because of two miscarriages. One-year later, due to intolerance, patient adopted an HRT (natural E2; 50 µg patches) during 28 days, and micronized P4 (200 mg ovules) throughout days $14^{th}$ to $28^{th}$. Because fixed $E_2$ doses administration does not mimic $E_2$ blood levels seen throughout a physiological cycle, we chose to follow a more physiological schedule, meaning hormonal doses and elapsed time as in the natural cycle. Being she slim (1.65 m of height and 57 k of weight; BMI 20.94), the designed protocol was adequate to mimic her menstrual cycle by using the smallest $E_2$ dose rendering circulating steroid levels closest to her own before diagnosis. Hormonal blood levels and detailed physiological HRT (pHRT) design are depicted in Table 1&2, respectively. A 28 day-cycle was accomplished, beginning with patches in progressive $E_2$ doses: 25, 37.5, up to reach 50 µg (mimicking ovulation peak); thereafter, decreasing to 37.5 µg patch until cycle-ends. She received micronized P4 during the last 14 cycle-days, and one-week hormone deprivation between cycles allowed menses. Due to severe mood changes, this free week was discontinued. Based on a chronotherapeutic approach [10], melatonin treatment (2 h before bedtime) slowly increasing from 3 mg/night to 50 mg/night was indicated [11,12]. Potent OS inhibitor, Resveratrol (RESV) was also added to protocol (every morning, 100 mg) [13,14] to provide constant antioxidant milieu. After 3 months, peripheral hormonal levels achieved physiological values, comparable to those of a fertile stage (Table 1, lines 4-Dec-2014 and Apr-2007). Thereafter, patient reported an expected sense of well- being, no-headaches, no-mood swings, with a normal pattern of cervical mucus. Despite complete relief of symptoms, treatment was suspended to analyze hormonal profile, and when found no-detectable $E_2$ peripheral levels, treatment was restored. Five months later, blood hCG level (125,637 mIU/mL) and abdominal echography confirmed a 9 week-pregnancy, thus withdrawing MEL and RESV. Hormonal support continued by switching $E_2$ patches to pills (2 mg/day), while keeping P4 administration until reaching 12-weeks pregnancy (see hormones values in Table 1). Finally, a healthy baby-girl was spontaneously delivered at term (3.93 kg, 51 cm, Apgar $5^{th}$ min: 10).

## Discussion

It is known that POF patients eventually ovulate and conceive (from 24% idiopathic POF patients, 4.4% achieve spontaneous pregnancy [15]). Although few positive cases under HRT suggest that our proposal is more effective: Adjusted pHRT, plus chronotherapy could highly benefit fertility and pregnancy in POF patients wishing to conceive.

The evidence indicates that HRT is superior than contraceptive pills, for many reasons [16], thus supporting pHRT [3]. In fact, HRT has been indicated for years at fixed $E_2$ doses; conversely, following our protocol an average of normal hormonal female natural cycle is mimic. There are only few data using a physiological approach in POF patients, although only evaluating cardiovascular protection, bone

**Table 1:** Patient´s peripheral blood hormonal levels collected over a 10-year period to assess hormonal status and progression of HRT.

| | Hormonal serum levels | | | | | | Hormonal Replacement Treatment | | Observations |
|---|---|---|---|---|---|---|---|---|---|
| Date of follow up | Age | FSH (mIU/ml) | LH (mIU/ml) | E2 (pg/ml) | P4 (ng/ml) | Prolactin (ng/ml) | E2 (Patch, µg) | P4 (Ovule, mg) | |
| Apr-07 | 34 | 7.6 | 8.5 | 64 | 0.5 | 14.6 | - | - | Hormonal values of the last period before pregnancy |
| Jan-08 | 35 | | | | | | - | - | Natural delivery of healthy baby |
| Oct-10 | 37 | 28.5 | 12.6 | 23 | 0.6 | 10.3 | - | - | |
| Mar-12 | 38 | 52.2 | 21.6 | <12 | 0.3 | 5 | - | - | |
| May-12 | 39 | 17.6 | 10.4 | 28 | 0.6 | 20.7 | - | - | |
| Mar-13 | | 66.8 | 32.8 | <18 | 0.4 | 11.6 | - | - | POF Diagnosis |
| Jun-13 | 40 | | | | | | | | CCT start |
| Dec-13 | | 10.7 | 4.9 | 68 | 0.3 | 12.3 | | | |
| Jul-14 | 41 | | | | | | | | pHRT start |
| 11-Sep-14 | | 12.7 | 9.6 | 101 | 0.3 | 12.5 | 37.5 | | pHRT levels on 10th day of treatment (2nd week, first parch of 37.5 ug E2; last day of usage) |
| 12-Nov-14 | | 39.7 | 14.4 | <18 | 0.3 | | 25 | - | pHRT resumed after a one-month withdrawal period (first day, 1st week) |
| 19-Nov-14 | | 20.3 | 11.9 | 49 | 0.3 | | 37.5 | - | pHRT (first day, 2nd week) |
| 26-Nov-14 | | 21.5 | 23.9 | 92 | 0.5 | | 50 | - | pHRT (first day, 3rd week) |
| 4-Dec-14 | | 6.7 | 5.4 | 39 | 19.7 | | 37.5 | 200 | pHRT (first day, 4th week) |
| Apr-15 | 42 | | | | | | 2000* | 200 | Pregnancy diagnosis |
| Nov-15 | 43 | | | | | | - | - | Natural delivery of healthy baby |
| Jul-17 | 44 | 54.5 | 26.2 | <15 | <0.1 | 10.1 | - | - | Breastfeeding |

CCT: Contraceptive Treatment; pHRT: Physiological Hormone Replacement Treatment. Estradiol support was changed from patches to pills when pregnancy was confirmed (04/2015)*.

**Table 2:** Physiological hormone replacement therapy + melatonin & resveratrol antioxidant-chronotherapy.

| | Physiological Hormone Replacement Therapy | | Antioxidative & Chronotherapeutic Daily Interventions | |
|---|---|---|---|---|
| Treatment (Week) | Estradiol (mg) | Progesterone (mg) | Morning Resveratrol (mg) | Night Melatonin (mg) |
| 1 | 25 | --- | 100 | 50 |
| 2 | 37.5 | --- | 100 | 50 |
| 3 | 50/37.5 | 200 | 100 | 50 |
| 4 | 37.5 | 200 | 100 | 50 |

The physiological HRT proposed is based on mimicking the female cycling of ovarian hormones. As a consequence, the scheme begins with a low dose of $E_2$* during the first week (1 patch of 25 µg), continuing with a higher $E_2$ dose during the second week (37.5 µg) up to reach a maximum dose of 50 µg of $E_2$ at the beginning of the 3rd week (one 50 µg-patch), thereafter decreasing to 37.5 µg-patch until the end of the 4th week. All patches were replaced every 3.5 days, for a total of 2 patches per week. Micronized Progesterone ovules (vaginal pessaries) were added every night beginning on the 14th (maximum $E_2$ blood level = the fake "ovulation peak¨). In this way, a 28 day-cycle was accomplished. Adopting a week of rest (no $E_2$ neither $P_4$) may be necessary or not depending on patient´s response to treatment.
In this case, the lowest dose commercially available for $E_2$ transdermal patches, which delivers 25 µg/day or 50 µg/day; although there are no reports on patch fractionation it was done in order to achieve the intermediate 37.5 µg dose by adding ½ patch to a full 25 µg patch. Still, it does not result clear from literature though whether the patch fractionations (e.g. administering 0.25 or 0.5 patch) or different administration times (daily, weekly) are equivalent.

health [17] and uterine characteristics [18]. The so-called physiological Sex Steroid Replacement (pSSR) therapy resulted more effective than standard HRT (sSSR) in improving endometrial thickness and uterine volume, two important parameters to achieve and maintain pregnancy [19], but do not reproduce physiological female cycle, a ¨golden- goal¨ of our applied treatment. In fact, it differs from ours in that neither takes into account women´s body type for $E_2$ dose nor in reproducing middle-cycle $E_2$ peak. Mimicking ovulation $E_2$ peak helps for developing a next period oocyte and sending correct feed-back signals within the HPO axis.

For reproductive medicine applications, this therapy may represent no risk, non-invasive first alternative for young women with POF willing to conceive, instead of getting hyper-ovulation and ovary exhaustion. Considering economic costs and the personal burden to avoid, because most patients will not accept egg/embryo donation, a first choice of treatment like ours could be appreciated.

As far as short cycling, a previous study showed that women displaying early ovulation presented lower pregnancy rates than women with similar fertility problems but normal/late ovulation (28.1% *vs.* 59.4%, respectively) [20]. Accordingly, although further research is needed, it is possible that our pHRT could:

1) Adjust cycle duration;

2) Support follicular maturation and ovulation, and

3) Collaborate with endometrial thickness during luteal phase

By including MEL during pHRT could help to regain patient's normal circadian rhythm. Female hormonal levels oscillations through the menstrual cycle ensure fertility and proper functioning of several hormonal controlled systems [21]. On this basis, it is accepted that around 40 years-age melatonin synthesis declines thus lowering peripheral MEL levels which affects circadian rhythm and sleep; moreover, adding MEL-therapy increases antioxidant defense thus ameliorating POF, a state characterized by a pro-oxidative ovary environment [7] and, in turn contributing with oocyte quality improvement. Previous data indicated that decreased ovarian reserve and premature ovarian aging, due to maternal undernutrition during pregnancy and lactation, increased endogenous OS and decreased antioxidant defense mechanism [22]. Otherwise, MEL offers anti-oxidant properties at the ovary level and upon a wide range of organs/tissues [23,24], even at higher doses [12] in humans [23]. Melatonin´s antioxidant actions include non-genomic and genomic effects. Regarding the former, cattle oocytes cultured with melatonin show decreased cell ROS levels [25]; and immature mice GCs incubated with $H_2O_2$ demonstrated that melatonin protected luteinized GCs from OS in pre-ovulatory follicles [26]. Regarding melatonin´s genomic effects, melatonin significantly increased implantation site number, brood size and endometrium thickness in mice at early gestation [27] by down-regulating $E_2$ but not P4 blood levels. This work showed that MEL up-regulated mRNA expression of HB-EGF (a growth factor involved in implantation) and its Receptor (EGFR) in blastocysts and, also uterus mRNA expression of Aanat, the melatonin's rate-limiting enzyme, increased while MT2 was expressed on gestation day 2 [27]. As a result, MEL treatment is a good candidate for protecting ovarian function and to improve fertility in enhanced OS-dependent reproductive pathologies [28]. In humans, ovary-produced melatonin and MT2 contribute to follicular maturation and to preserve ovum integrity [29].

It is accepted that midterm life's ovary aging occurs while melatonin synthesis declines before FSH levels rise; indeed, melatonin treatment reduces FSH/LH peripheral levels in POF patients correlating with OS blood levels [30]. Melatonin benefits delays ovary aging [31], supporting that melatonin antioxidant-chronoregulator effects contribute to ovary protection. Additionally, melatonin treatment in peri-/post-menopausal women improves bone mineral density and turnover, and life quality in osteopenic postmenopausal women [32].

Normal aging implies free radicals' accumulation producing DNA mutations, protein damage, telomere shortening and apoptosis. Increasing evidence demonstrate that resveratrol possesses anti-aging effects by influencing several signaling pathways, increasing anti-OS defense, and activating telomerase and SIRT1 [14,33]. There are data documenting RESV antioxidant properties, including on OS-dysmetabolism, circadian clock, folliculogenesis, GCs luteinization [34] and ovary damage by increasing AMH production and diminishing inflammation [35]. In a DIO model, resveratrol alone and combined with melatonin counteracted increased body weight, glycemia, cholesterolemia, triglyceridemia, FFAs and hypertrophic adipocytes [36]. Given that SIRTs are markers of ovary aging [37], ovary reserve could increase by resveratrol. Because melatonin and resveratrol both modulate SIRTs in different cell types, it is plausible that sirtuins could play a key function in resveratrol and melatonin effects [38].

## Conclusions

Our data support that this therapeutic approach, pHRT-MEL-RESV, could benefit POF/POI patients wishing to recover normal reproductive function.

This work highlights the relevance of patient's medical history, including genetic assessment, for early and accurate POF/POI diagnosis. This combined therapy reproduces physiological hormonal levels and improves circadian rhythms by including antioxidant-chronotherapy, offering novel noninvasive treatment with multiple benefits for women´s health, even on different life-span stages.

In line with general recommendations on POI and menopause management, pHRT- MEL-RES could be applied in: 1) POF patients in order to give the hormonal axes a chance to be synchronized, and try to conceive avoiding major interventions, 2) elder POF/POI women (non-menopause) because pHRT+MEL could help to diminish peripheral FSH/LH levels, rise those $E_2$ and preserve endometrium´s architecture, protecting from OS by melatonin-resveratrol treatment should leave oocyte donation in unresponsive cases only, 3) women at peri-menopause/early menopause onset provided that minimal hormonal dose, resembling their last physiological levels before decline. Moreover, the hormonal plus antioxidant protection could improve bone health, metabolism, psychological wellness and life-quality.

Summarizing, pHRT+MEL+RESV therapy seems effective when applied in POF, sub-fertile, women desiring pregnancy and in peri-menopause/early menopause women. However, further clinical trials are needed to largely validate this innovative therapeutic approach.

## Acknowledgement

Author wishes to thank Dr. DP Cardinali and Dr. A Genaro for critical reading of the manuscript; and Dr J. Tresguerres for helpful discussion. ESP and ES are members of the National Research Career (CONICET, Argentina).

## Funding

This study received a partial financial support from Foundation Rolf C. Gaillard (Grant: FPREDM 2022-2024, addressed to ES), Lausanne, Switzerland.

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