GENANTE™

Studio clinico 1 Urologia

Resveratrol Based Multivitamin Supplement Increases Sperm Concentration and Motility in Idiopathic Male Infertility: A Pilot Clinical Study.

ESTER ILLIANO[1], FRANCESCO TRAMA[1*], ALESSANDRO ZUCCHI[2], ROSSANA GIULIETTA IANNITTI[3], BERNARD FIORETTI[3] and ELISABETTA COSTANTINI[1]

[1] Andrologycal and Urogynecological Clinic, Santa Maria Terni Hospital, University of Perugia,05100 Terni, Italy
[2] Urological Clinic, University of Pisa, 56125 Pisa, Italy
[3] Department of Chemistry, Biology and Biotechnologies, University of Perugia, Via Elce di Sotto 8, 06132 Perugia, Italy

**Corrisponding author*

S&R FARMACEUTICI

Resveratrol-Based Multivitamin Supplement Increases Sperm Concentration and Motility in Idiopathic Male Infertility: A Pilot Clinical Study

INTRODUZIONE: L'infertilità maschile riflette una varietà di differenti meccanismi patogenetici, anche se nella maggior parte dei casi, la causa è idiopatica (circa 30%). Il resveratrolo è un polifenolo che promuove l'attività mitocondriale grazie a l'attivazione della SIRT1. In diversi modelli animali il resveratrolo ha mostrato effetti positivi sui mitocondri e sul potenziale di membrana. Dato che l'attività mitocondriale è importante per la funzionalità spermatica, il resveratrolo potrebbe essere una molecola utile nel migliorare i parametri seminali.

SCOPO: Valutare gli effetti di GENANTE®, un integratore a base di resveratrolo 150 mg in pazienti affetti da infertilità idiopatica.

DISEGNO DI STUDIO: Studio pilota prospettico a centro singolo. Venti pazienti hanno assunto GENANTE® (compressa orale ogni 12 ore), e sono stati seguiti fino a 6 mesi e valutati tramite; esame clinico, spermiogramma, determinazioni ormonali e ultrasuoni scrotali e prostatici.

RISULTATI: Il trattamento con GENANTE® migliora significativamente i parametri dello spermiogramma; motilità (48,3% ± 13,8 contro 59,0% ± 12,8, p = 0,0001) e concentrazione (22,6×106/ml ± 9,5 vs. 25,7×106/ml ± 8,1, p = 0,0001) dopo 3 e 6 mesi di trattamento.

CONCLUSIONE: I risultati suggeriscono che agire sul processo metabolico ed energetico con un preparato a base di resveratrolo (GENANTE®) migliora i parametri seminali e può pertanto contrastare condizioni di subfertilità/infertilità maschile.

AZIONE SU PARAMETRI SEMINALI:

S&R FARMACEUTICI

Journal of
Clinical Medicine

MDPI

Article

Resveratrol-Based Multivitamin Supplement Increases Sperm Concentration and Motility in Idiopathic Male Infertility: A Pilot Clinical Study

Ester Illiano [1], Francesco Trama [1,*], Alessandro Zucchi [2], Rossana Giulietta Iannitti [3], Bernard Fioretti [3] and Elisabetta Costantini [1]

[1] Andrologycal and Urogynecological Clinic, Santa Maria Terni Hospital, University of Perugia, 05100 Terni, Italy; ester.illiano@inwind.it (E.I.); elisabetta.costantin@unipg.it (E.C.)
[2] Urological Clinic, University of Pisa, 56125 Pisa, Italy; zucchi.urologia@gmail.com
[3] Department of Chemistry, Biology and Biotechnologies, University of Perugia, Via Elce di Sotto 8, 06132 Perugia, Italy; r.iannitti@srfarmaceutici.com (R.G.I.); bernard.fioretti@unipg.it (B.F.)
* Correspondence: francescotrama@gmail.com; Tel.: +39-07557883743

Received: 8 November 2020; Accepted: 7 December 2020; Published: 11 December 2020

Abstract: Background. It is known that a multitude of factors may lead to male factor infertility, but still, in the majority of cases, the cause remains largely idiopathic, reflecting poor understanding of the basic process of spermatogenesis and the mechanisms involved. Resveratrol is a polyphenol compound that displays several cellular aspects mainly associated with SIRT1-pathway activation and promotion of mitochondrial enhancer activities. In several animal models, resveratrol has shown positive effects on mitochondria and membrane potential. This could explain effects on sperm concentration and motility. The aim of this study is to evaluate the effects on the semen parameters of GENANTE®, a multivitamin supplement containing 150 mg of resveratrol/day, in patients with idiopathic infertility. Methods. This was a prospective single center clinical study. Twenty patients took a multivitamin supplement based on 150 mg of resveratrol (GENANTE®), in the form of an oral tablet every 12 h, and were followed up at 1, 3, and 6 months after treatment. Pre- and post-treatment evaluation included history, clinical examination, semen analysis, hormonal determinations, and scrotal and prostatic ultrasound. Results. Our preliminary pilot study demonstrated that the multivitamin supplement based on resveratrol improves sperm motility (48.3% ± 13.8 vs. 59.0% ± 12.8, p = 0.0001) and concentration (22.6×10^6/mL ± 9.5 vs. 25.7×10^6/mL ± 8.1, p = 0.0001) after 3 and 6 months of treatment in men with idiopathic infertility. Conclusion. Our data suggest that targeting the metabolic and energetic pathways involved in spermatogenesis and mitochondrial activity could lead to potential effects and counteract subfertility/infertility in men through a mitochondria dynamics mechanism. Trial registration number: ClinicalTrials.gov registration identifier: NCT03864198, registered on 1 January 2019.

Keywords: resveratrol; male infertility;-mitochondrial activity

1. Introduction

Infertility is defined as the inability to achieve spontaneous pregnancy after at least one year of regular, unprotected sex [1]. Infertility affects 15–20% of couples [2]. A male factor is estimated to be present in approximately 50% of cases, with sole responsibility in 30% of cases and a co-contributing female factor in 20% of cases [3]. Male infertility may include the abnormal semen parameters (oligozoospermia, asthenozoospermia, teratozoospermia) or a combination of all three (oligo-astheno-teratozoospermia), or azoospermia [4]. The causes of male infertility can be divided into four

main areas (endocrine and systemic disorders, primary testicular defects in spermatogenesis, sperm transport disorders), including idiopathic infertility which affects up to 25% of patients [4]. Idiopathic male infertility is clinically diagnosed after excluding all other known causes of infertility.

Semen quality has often been used as an indirect measure of male infertility. This includes examination of sperm count, motility, and morphology. The majority, 80%, of altered parameters accounting for low sperm concentration are associated with a decrease in sperm motility (asthenozoospermia) and spermatozoa with normal morphology [5]. Whether there is a deterioration of semen quantity or quality is controversial [6,7]. However, there seems to be a clear trend toward a decline in sperm quality in our society [8]. There have been several explanations for this phenomenon. They include environmental stress, a modern lifestyle, infection, and/or chemicals that may alter the endocrine system. The result is a steady decline in male reproductive potential [9].

It is also known that in most infertile men who have abnormalities in sperm count, morphology, and/or motility, there is no identifiable cause [4]. Numerous nutritional [10–14] and medical interventions (hormonal therapies that modulate the hypothalamic–pituitary–testicular axis) have been used to treat male idiopathic infertility [2]. However, the management of these patients remains challenging. This is primarily due to the large numbers of various products and the conflicting evidence from individual trials. Most of the studies on infertility treatment, both in vitro and in vivo, have focused on oxidative stress mechanisms [15–17]. The oxidative stress mechanism could cause: lipid peroxidation, with alteration of membrane fluidity and permeability, which results in a decrease of sperm motility and in a reduction of sperm interaction with the oocyte; protein modification which causes a reduction of ATP production; or sperm DNA fragmentation [16].

In particular, the focus has been on increasing seminal antioxidant capacity, reducing the production of reactive oxygen species (ROS), stabilizing sperm chromatin (through zinc-based molecules), and inducing sperm capacitation (functional maturation of the spermatozoa). By contrast, few studies have centered on other mechanisms involved in metabolism, mitochondrial energy, and metabolism/mitochondrial function, which has recently emerged as one of the important factors in infertility physiopathology [18,19].

Resveratrol, trans-3,5,4′-trihydroxystilbene, is a polyphenol compound present in grapes, peanuts, berries, and wine [13]. It is a phytoalexin whose biological function is to protect the plant in case of parasitic attack or environmental stress [14]. Scientific reports have identified a wide variety of characteristics of this molecule. This includes anti-inflammatory, cardioprotective, anticancer, antimicrobial, antiaging, and antioxidant effects [20]. Resveratrol is also the most potent natural compound that activates sirtuin 1 (SIRT 1), the most-conserved mammalian NAD+-dependent protein, and a member of the family of sirtuins, which may account for its many metabolic benefits in humans [20].

Recent studies in animal models have demonstrated that resveratrol has a positive effect on the hypothalamic–pituitary–gonad axis, as well as blood testosterone levels, sperm production, and sperm motility [21,22]. Furthermore, resveratrol may decrease germ cell apoptosis [18,23]. An animal study with resveratrol and lycopene in post-thaw bull sperm demonstrated that resveratrol offered high mitochondrial activity, sperm motility, and DNA integrity [24]. It improved DNA integrity and sperm parameters in streptozotocin-nicotinamide-induced type 2 diabetic rats [25]. In vitro treatment with 15μM/mL of resveratrol on human sperm revealed that it has the capability to counteract the detrimental effects of benzo-α-pyrene exposure on sperm motility, abnormal chromatin compactness, lipid peroxidation, and mitochondrial superoxide [26]. Resveratrol can protect the quality of the mitochondria and increase its membrane potential [27], an effect that possibly accounts for the positive effect on sperm motility and explains the improvement of total and progressive sperm motility.

Despite these insights, the effect of resveratrol supplementation on male infertility has not yet been explored.

The aim of this study was to evaluate the effects of a nutraceutical based on resveratrol, (GENANTE®, a twice-a-day multivitamin supplement containing 150 mg of resveratrol, vitamin D,

B6, B12, and folic acid) on the semen parameters of patients with idiopathic infertility. The primary outcome was to evaluate the semen parameters before and after 1, 3, and 6 months of treatment.

2. Material and Methods

This was a prospective single center study. The project was accepted by the local ethics committee and registered on clinicaltrials.gov (NCT03864198). We included idiopathic infertile male patients. The inclusion criteria were as follows: age 18–50 years and patients with oligozoospermia (<5 million spermatozoa/mL) and/or with asthenozoospermia (<32% progressive motile spermatozoa) e/o in accordance with WHO criteria [28]. The following patients were excluded: patients with azoospermia; patients who smoked and/or used drugs, or had taken drugs with proven fertility toxicity; patients with a history of consumption of alcohol; those who were exposed to any environmental or occupational toxic substances, including radiation, intensive cell-phone use or heat (patients who claimed to have a mobile phone in their front pocket for at least 5 h, for at least 10 min/h); patients who had epididymitis, epididymo-orchitis or orchitis secondary to mumps, bacterial infections, or sexually transmitted diseases; patients with a history of cryptorchidism, previous testicular torsion, genitourinary anomalies, alterations of the epididymis or deferens, and/or inguinal surgery; patients with hormonal alterations. The reason for exclusion was the causal relationship of these conditions with the deterioration of fertility [29–31].

The pretreatment evaluation included a patient history; clinical examination; semen analysis; hormonal determination (follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, estradiol, prolactin, and 25-OH-Vitamin D3); a scrotal ultrasound to exclude signs of obstruction (e.g., dilatation of rete testis, enlarged epididymis with cystic lesions, or absent vas deferens), signs of testicular dysgenesis (e.g., non-homogeneous testicular architecture and microcalcifications), or testis tumors; and a prostatic transrectal ultrasound to exclude distal obstruction and any male accessory gland infection [32,33].

Hormonal assessments were performed in the same laboratory, and their evaluation was based on reference ranges for normal men provided by the laboratory measuring the samples.

In our laboratory, normal ranges were: prolactin 3.46–19.40 ng/mL; FSH 0.95–11.95 mUI/mL, LH 1.14–8.75 mUI/mL; total testosterone 10–20 years: 18.5–48.3 pg /mL, 20–30 years: 19.5–51.7 pg/mL, 30–50 years: 16.1–47.9 pg/mL, >50 years 12.1–39.6 pg/mL; estradiol 11–44 pg/mL; 25-OH-Vitamin D3 < 20 ng/mL deficiency, 20–29 ng/mL insufficient, 30–100 ng/mL sufficient. All assessments were certified (certified quality system UNI EN ISO 9001:2015).

Laboratory testing of testosterone to determine diurnal variation was carried out with two morning samples (7.00 a.m. and 11.00 a.m.). Prolactin levels are influenced by several factors, such as pick-up time (the prolactin secretion has a circadian rhythm, with high levels in the night and low during the day). However, since it was not possible to do so during sleep, dosage was carried out during the day, with three samples taken at intervals of 10–30 min. Estradiol, FSH, and LH were assayed by one sample.

Scrotal and transrectal ultrasounds were performed by the same urologist.

Patients were prescribed a multivitamin supplement (trademark GENANTE®, S&R Farmaceutici S.p.A. Bastia Italy). It consisted of REVIFAST® (160 mg), trans-resveratrol (102 mg), Vitamin B6 (1.4 mg), Vitamin B 12 (2.5 mg), Vitamin D (25 mcg), and Extrafolate S® (400 mcg). They received an oral tablet every 12 h, for a total of 2 tablets a day, for a daily consumption of 150 mg of resveratrol.

REVIFAST® is the trade name of a new ingredient based on resveratrol that is supported by a magnesium hydroxide matrix at the concentration of 30% w/w [34]. Resveratrol is known to have low solubility in water and good membrane permeability and accordingly is a class 2 molecule by pharmaceutical classification, and therefore, it is poorly bioavailable [34]. Furthermore, it is known to have a fast metabolism that converts it to glucuronide and sulfate compounds.

Extrafolate S® is the biologically active form of folic acid. This allows it to bypass any polymorphisms of the tetrahydrofolate methylene (MTHFR) gene reductases responsible for reduced enzyme activity of MTHFR.

All patients were followed at 1, 3, and 6 months after treatment with Genante© using the same pretreatment flow chart. Scrotal and transrectal ultrasounds were performed during follow up to rule out de novo pathologies.

All patients signed an informed consent form explaining the nature of the study and the possibility of treatment failure.

Statistical Analysis

With the enrolment of 20 patients, $p = 0.05$, and the use of the $\chi 2$ test, the study was estimated to have an 80% power rejection of the null hypothesis that Genante® does not change the seminal parameters in infertile patients. The power of the study was calculated using PS Power and Sample Size ver. 3.0, 2009. Continuous variables were presented as median values, and categoric data were presented as absolute or relative frequencies. Statistical analysis was performed using the Wilcoxon Signed Rank test to compare the variables, and the $\chi 2$ test and McNemar test for categorical data. All calculations were performed using IBM-SPSS® version 22.0 (IBM Corp., Armonk, New York, NY, USA, 2013). A two-sided p-value < 0.05 was considered significant.

3. Results

Between January 2019 and June 2019, 20 patients, with idiopathic infertility according to WHO criteria, underwent treatment with Genante® in our tertiary urological center. The demographic and clinical characteristics of the included patients are shown in. Table 1.

Table 1. The demographic and clinical characteristics of patients.

Patients	20
Age (mean ± SD)	30.9 ± 3.28
BMI (mean ± SD)	27.9 ± 1.4
Married *n* (%)	10 (50)
Erectile Dysfunction *n* (%)	0 (0)
Male hyopogonadism *n* (%)	0 (0)
Oligozoospermia *n* (%)	0 (0)
Asthenozoospermia *n* (%)	6 (30)
Teratozoospermia *n* (%)	7 (35)
Oligoasthenozoospermia *n* (%)	7 (95)
Oligoteratozoospermia *n* (%)	0 (0)
Asthenoteratozoospermia *n* (%)	0 (0)
Oligosthenoteratozoospermia *n* (%)	0 (0)
Normal scrotal ultrasound	20 (100)
Normal prostatic transrectal ultrasound	20 (100)
FSH mlU/mL (mean ± SD)	4.60 ± 1.3
LH mlU/mL (mean ± SD)	3.87 ± 1.8
Total Testosterone nmol/L (mean ± SD)	14.89 ± 0.3
Estradiol pg/mL (mean ± SD)	25.2 ± 2.1
Prolactin ng/mL (mean ± SD)	11.17 ± 1.9
25-OH-Vitamin D3 ng/mL (mean ± SD)	55.8 ± 2.8

FSH: follicle-stimulating hormone. LH: luteinizing hormone.

Half of the patients were married; the other half had stable relationships, but they were not married. The most frequent sperm abnormality among the included patients was oligoasthenozoospermia (95%). At pretreatment, all patients had normal transrectal prostatic ultrasounds and scrotal ultrasounds.

After six months of treatment, the laboratory assessment showed a statistically significant improvement in total sperm count (41.5×10^6/ejaculate ± 22.1 vs. 48.2×10^6/ejaculate ± 22.4, $p = 0.002$),

sperm concentration (22.6 × 10^6/mL ± 9.5 vs. 25.7$10^6$/mL ± 8.1, $p = 0.0001$), total motility (48.3 ± % ± 13.8 vs. 59.0 ± % ± 12.8, $p = 0.0001$), and progressive motility (20% vs. 90%, $p = 0.0001$) (Table 2 and Figure 1).

The improvement of all the parameters was recorded previously at 1 and 3 months, with a progressive increase over time (Table 2). Sperm morphology, volume, and PH were not changed after treatment. The hormonal determinations were rather stable during follow-up (Table 3). The scrotal and transrectal prostate ultrasounds continued to be in the normal range.

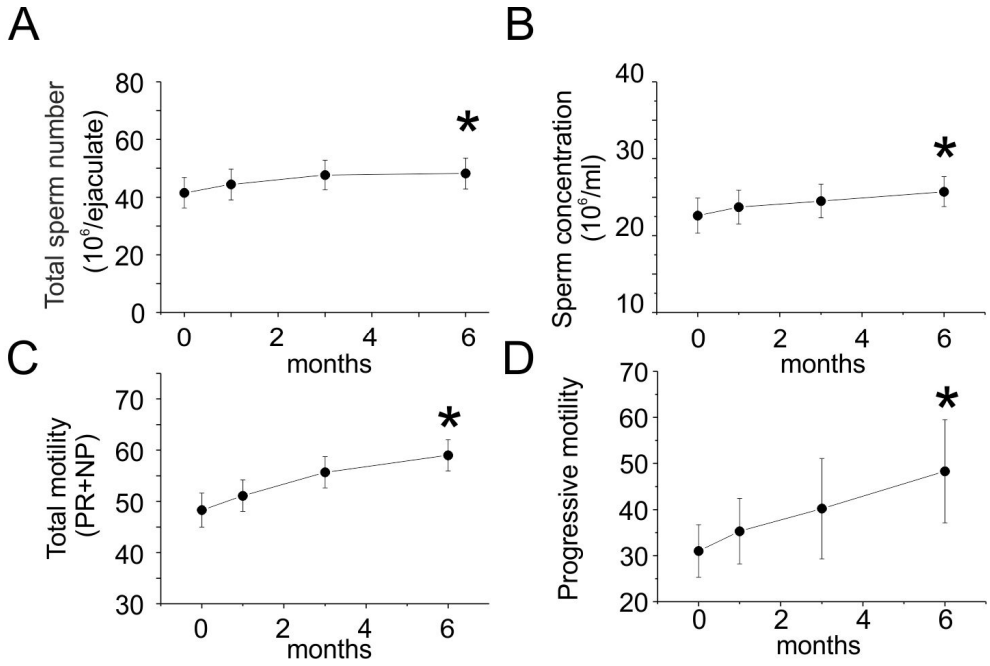


Figure 1. The laboratory assessment after six months of treatment. Data presented as ± SD. Significant differences between control and treated cells are denoted as * ($p \leq 0.05$). (**A**): change in the total number of spermatozoa in six months. (**B**): change in the sperm concentration in six months. (**C**): change in the total motility (PR+NP) in six months. (**D**): Change in progressive motility in six months.

Table 2. Semen parameters at baseline 1, 3, and 6 months after recruitment.

Parameters	Baseline	1 Month	3 Months	6 Months	*p*-Value
Normal Viscosity *n* (%)	20 (100)	20 (100)	20(100)	20 (100)	nd
Complete Fludification *n* (%)	11(100)	11(100)	11(100)	11(100)	nd
PH (mean ± SD)	8.1 ± 0.1	8.0 ± 0.3	8.0 ± 0.2	8.0 ± 0.3	0.219
Semen volume (mL, mean ± SD)	3.6 ± 0.7	3.61 ± 0.6	3.61 ± 0.5	3.7 ± 0.5	0.525
Total sperm number (10^6/ejaculate, mean ± SD)	41.5 ± 22.1	44.4 ± 22.4	47.7 ± 21.4	48.2 ± 22.4	0.002 *
Sperm concentration (10^6/mL, mean ± SD)	22.6 ± 9.5	23.7 ±9.2	24.5 ± 9.1	25.7 ± 8.1	0.0001 *
Total motility (PR + NP, % mean ± SD)	48.3 ± 13.8	51.1 ± 12.8	55.7 ± 12.7	59.0 ± 12.8	0.0001 *
Progressive motility (PR > 32% mean ± SD)	31 ± 5.7	35.3 ± 7.1	40.2 ± 10.9	48.3 ± 11.2	0.0001 *
Sperm morphology (normal forms%)	14 (66.7)	14 (66.7)	14 (66.7)	14 (66.7)	nd

PR: progressive motility. NP: non-progressive motility. nd: not determined * ($p \leq 0.05$).

Table 3. Hormonal evaluation at baseline 1, 3, and 6 months after recruitment.

Parameters	Baseline	1 Month	3 Months	6 Months	p Value
FSH mlU/mL (mean ± SD)	4.60 ± 1.3	4.62 ± 1.2	4.61 ± 1.5	4.61 ± 1.7	0.9
LH mlU/mL (mean ± SD)	3.87 ± 1.8	3.85 ± 1.3	3.87 ± 1.4	3.88 ± 1.4	0.87
Total Testosterone nmol/L (mean ± SD)	14.89 ± 0.3	14.84 ± 0.7	14.82 ± 0.2	14.87 ± 0.2	0.9
Estradiol pg/mL (mean ± SD)	25.2 ± 2.1	25.8 ± 2.4	25.5 ± 2.3	25.6 ± 2.0	0.86
Prolactin ng/mL (mean ± SD)	11.17 ± 1.9	11.13 ± 1.5	11.15 ± 1.3	11.19 ± 1.2	0.9
25-OH-Vitamin D3 ng/mL (mean ± SD)	55.8 ± 2.8	55.1 ± 2.2	55.7 ± 2.4	55.4 ± 2.1	0.9

FSH: follicle-stimulating hormone. LH: luteinizing hormone.

4. Discussion

It is known that a multitude of factors may lead to male factor infertility; however, in the majority of cases, the cause remains largely idiopathic, which reflects a poor understanding of the basic process of spermatogenesis and the mechanisms involved [19].

Sperm density may be influenced more by many nutraceuticals or micronutrients, such as vitamin D, B and folic acid, while limited nutrients influence sperm motility. Folic acid is known to increase sperm density significantly following three months of folic acid supplementation to patients with oligospermia or asthenospermia [35]. By contrast, no statistical correlations were found between seminal plasma vitamin B6 level and sperm motility, sperm count, or semen volume [36]. Folate and B12 are not correlated with any semen parameters [37] but are known to modulate homocysteine. Vitamin D demonstrates a direct and positive relationship between serum vitamin D level and overall semen quality, male reproductive potential, and testosterone levels [38] and may enhance sperm motility by promoting the synthesis of ATP through the cAMP/PKA pathway [39]. However, in the literature, controversial data exist regarding vitamin D status and reproductive parameters [40], and thus, the role of vitamin D in male fertility is still debated.

Our study shows that a resveratrol-based multivitamin treatment increases the concentration of sperm cells and motility, which suggests an improvement in both the spermatogenesis process and fertilization potential. The process of spermatogenesis comprises the differentiation of the primordial germ cells into spermatogonia, followed by the production of primary and secondary spermatocytes, spermatids, and ultimately highly specialized mature spermatozoa [41]. Sertoli cells (SCs) play a key role in spermatogenesis by providing the essential physical support for developing germ cells and ensuring that they have the appropriate nutrients, energy sources, hormones, and growth factors.

In fact, spermatogenesis is highly dependent on energy metabolism [42] and glycolytic metabolism, as the lactate produced by the Sertoli cells is the major substrate of germ cells [43]. The mitochondria of the isolated germ cells produce ATP potentially, at close to a maximal rate. Spermatogenesis, therefore, may be extremely sensitive to compounds which interfere with mitochondrial energy metabolism and respiratory control. Any alteration in the regulation of these cells' metabolic behavior may compromise the normal development of spermatogenesis and, consequently, male fertility [42,43]. It has been proposed that mitochondria also play a role in this degenerative process of the sperm, thereby assuring that good quality meiotic products enter the process of spermatogenesis to yield quality mature sperm [19]. Interestingly, resveratrol was demonstrated to increase the mitochondrial number (mitogenesis) and activity (ATP concentration) in several cell types, such as muscle cells [20] and granulosa cells [44]. Resveratrol improves mitochondrial function by activating sirtuin 1 (SIRT1) [20]. SIRT 1 is related to multiple age-associated diseases due to its capacity to deacetylate histones and non-histone proteins, such as tumor protein p53 (p53), kB-gene binding nuclear factor (NF-κB), heat shock factor 1 (HSF1), forkhead box transcription factor, class O (FOXOs), and peroxisome proliferator-activated receptor γ (PPARγ)

coactivator-1 (PGC-1). Thus, it can regulate the cell's biology, metabolism, and fate at various levels [45]. Therefore, sirtuins play an important role in a broad spectrum of biological processes. Their regulation of glycolytic metabolism and mitochondrial energy metabolism–respiratory control not only increases their physiological relevance to the testicular environment [46]; however, it also suggests that these metabolisms control sperm functionality and thus male reproductive health. Further studies are required to conclusively demonstrate if this effect on males occurs in male gamete cells during resveratrol treatment and if it is dependent on the SIRT-1 pathway.

Mitochondrial activity is also critical for mature sperm cells as it is correlated with sperm motility, an important factor for the penetration of the cumulus cells and zona pellucida of the oocyte [23]. In mature sperm, mitochondria cover the axosome and the associated dense fibers of the midpiece, via oxidative phosphorylation (OXPHOS), which increases the production of adenosine triphosphate (ATP) [18]. The inner mitochondrial membrane includes several complexes (electron transfer chain, ETC), which transport electrons derived from the oxidation of dihydroflavine–adenine dinucleotide (FADH2) and the nicotinamide adenine dinucleotide (NADH). In this process, an osmotic proton gradient is generated across the inner mitochondrial membrane and is subsequently used by the ATP synthase to phosphorylate adenosine diphosphate (ADP) to ATP. OXPHOS-derived ATP seems to be important for sperm motility [18]. The expression of several sperm mitochondrial proteins, including ETC complexes [47], may be altered in asthenozoospermic patients [23,48]. Many different ETC inhibitors have been shown to negatively affect sperm motility [49], both in humans [49,50] and in engineered mice [51]. Interestingly, there is a strong correlation with inner mitochondrial membrane potential (DP) and spermatic motility. In this context, the increase of motility observed in our study due to resveratrol treatment may be associated with this incremented mitochondrial membrane potential and metabolic activity. Mitochondria, in the mid-piece of mature mammalian spermatozoon, are fundamental for the creation of energy which is useful for sperm movement [19], and mtDNA genetic defects may compromise sperm physiology, and, in particular, motility [19]. Multiple mtDNA rearrangements are associated with decreased sperm motility [52]. In addition, the reduction of energy production may induce meiotic arrest during spermatogenesis [19].

It is unlikely that this is an increase in the expected number of mitochondria as the number of mitochondria is highly dependent on the neck volume of sperm cells. The beneficial effect of resveratrol agrees with the inverse correlation of mtDNA; in fact, mtDNA would be advantageous to developing spermatozoa [19], but not in mature sperm cells. Oligozoospermic and asthenozoospermic men have sperm containing significantly elevated levels of mtDNA [53], prompting the hypothesis of an optimal threshold for spermatozoa. Since the energy metabolism is important in both spermatogenesis and oxidative phosphorylation, it has been suggested as a determinant of sperm motility and functionality.

We are aware that this study may have some limitations. One is related to its small sample size and study design as a prospective clinical study. The second is the lack of evaluation of the impact of redox status in the effects observed since mitochondrial ETC promotes the production of ROS [18]. Balanced ROS levels are required for sperm motility, capacitation, the acrosome reaction, hyperactivation, and fertilization ability [27], so we can assume that ROS levels are inside of physiological range; however, further studies will be able to address the specific role of ROS in resveratrol's effects in promoting a better spermatic performance. The strengths of our study include the use of a supplement with a highly bioavailable form of resveratrol (REVIFAST™) with an increased pharmacokinetic profile [34]. It can bridge the gap between the interesting in vitro effects that are otherwise not possible in vivo.

5. Conclusions

In conclusion, GENANTE® improves the concentration and motility of sperm in idiopathic male infertility. This study also confirms that, taken together, the possibility of targeting the metabolic and energetic mechanisms involved in spermatogenesis and sperm motility with a promising molecule such as resveratrol could provide clinical benefits. However, a deeper understanding of the specific mechanisms involved is essential and further studies are needed to confirm our hypothesis.

Abbreviations

ADP	phosphorylate adenosine diphosphate
ATP	production of adenosine triphosphate
DP	mitochondrial membrane potential
ETC	electron transfer chain
FADH2	dihydroflavine-adenine dinucleotide
FOXOs	forkhead box transcription factor, class O
FSH	follicle-stimulating hormone
HSF1	heat shock factor 1
LH	luteinizing hormone
MTHFR	polymorphisms of the tetrahydrofolate methylene
NADH	nicotinamide adenine dinucleotide
NF-kB	kB gene binding nuclear factor
OXPHOS	oxidative phosphorylation
PPARγ	peroxisome proliferator-activated receptor γ
SIRT1	sirtuin 1

Author Contributions: Writing—original draft and validation, E.I.; data curation and methodology, F.T.; visualization, A.Z.; formal analysis and validation, R.G.I.; formal analysis and supervision, B.F.; conceptualization and supervision, E.C. All authors have read and agreed to the published version of the manuscript.

Funding: No funding was requested for this study.

Conflicts of Interest: The authors declare that they have no conflict of interests.

References

1. World Health Organization. *WHO Manual for the Standardized Investigation and Diagnosis of the Infertile Couple*; Cambridge University Press: New York, NY, USA, 2000.
2. National Institute for Health and Care Excellence. *Fertility Problems: Assessment and Treatment*; NICE: London, UK, 2013. Available online: www.nice.org.uk/guidance/cg156 (accessed on 12 July 2017).
3. Winters, B.R.; Walsh, T.J. The epidemiology of male infertility. *Urol. Clin.* **2014**, *41*, 195–204.
4. Nieschlag, E.; Behre, H.M.; Nieschlag, S. (Eds.) *Andrology: Male Reproductive Health and Dysfunction*; Springer: Berlin, Germany, 2010.
5. Jungwirth, A.; Giwercman, A.; Tournaye, H.; Diemer, T.; Kopa, Z.; Dohle, G.; Krausz, C.; EAU Working Group on Male Infertility. European Association of Urology Guidelines on Male Infertility: The 2012 Update. *Eur. Urol*. **2012**, *62*, 324–332.
6. Fisch, H.; Goluboff, E.T.; Olson, J.H.; Feldshuh, J.; Broder, S.J.; Barad, D.H. Semen analyses in 1283 men from the United States over a 25-year period: No decline in quality. *Fertil. Steril*. **1996**, *65*, 1009–1014.
7. Fisch, H.; Braun, S.R. Trends in global semen parameter values. *Asian J. Androl*. **2013**, *15*, 169–173.
8. Carlsen, E.; Giwercman, A.; Keiding, N.; Skakkebaek, N.E. Evidence for decreasing quality of semen during past 50 years. *BMJ* **1992**, *305*, 609–613.
9. Mohanty, G.; Samanta, L. Redox regulation & sperm function: A proteomic insight. *Indian J. Med. Res.* **2018**, *148*, S84–S91.
10. Palmero, S.; Bottazzi, C.; Costa, M.; Leone, M.; Fugassa, E. Metabolic Effects of L-Carnitine on Prepubertal Rat Sertoli Cells. *Horm. Metab. Res.* **2007**, *32*, 87–90.
11. Oeda, T.; Henkel, R.; Ohmori, H.; Schill, W.-B. Scavenging effect of Nacetyl-L-cysteine against reactive oxygen species in human semen:a possible therapeutic modality for male factor infertility? *Andrologia* **1997**, *29*, 125–131.
12. Ebisch, I.M.W.; Thomas, C.M.G.; Peters, W.H.M.; Braat, D.D.M.; Steegers-Theunissen, R.P.M. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. *Hum. Reprod. Update* **2007**, *13*, 163–174.
13. Iacono, F.; Prezioso, D.; Ruffo, A.; Di Lauro, G.; Romis, L.; Illiano, E. Analyzing the efficacy of a new natural compound made of the alga Ecklonia bicyclis, Tribulus terrestris and BIOVIS® in order to improve male sexual function. *J. Men's Health* **2011**, *8*, 282–287.

14. Ghanim, H.; Sia, C.L.; Korzeniewski, K.; Lohano, T.; Abuaysheh, S.; Marumganti, A.; Chaudhuri, A.; Dandona, P. A resveratrol and polyphenol preparation suppresses oxidative and inflammatory stress response to a high-fat, high-carbohydrate meal. *J. Clin. Endocrinol. Metab.* **2011**, *96*, 1409–1414.
15. Agarwal, A.; Sekhon, L.H. The role of antioxidant therapy in the treatment of male infertility. *Hum. Fertil.* **2010**, *13*, 217–225.
16. Barati, E.; Nikzad, H.; Karimian, M. Oxidative stress and male infertility: Current knowledge of pathophysiology and role of antioxidant therapy in disease management. *Cell. Mol. Life Sci.* **2019**, *77*, 93–113.
17. Lanzafame, F.M.; La Vignera, S.; Vicari, E.; Calogero, A.E. Oxidative stress and medical antioxidant treatment in male infertility. *Reprod. Biomed. Online* **2009**, *19*, 638–659.
18. Amaral, A.; Lourenço, B.; Marques, M.; Ramalho-Santos, J. Mitochondria functionality and sperm quality. *Reproduction* **2013**, *146*, R163–R174.
19. Rajender, S.; Rahul, P.; Mahdi, A.A. Mitochondria, spermatogenesis and male infertility. *Mitochondrion* **2010**, *10*, 419–428.
20. Price, N.L.; Gomes, A.P.; Ling, A.J.Y.; Duarte, F.V.; Martin-Montalvo, A.; North, B.J.; Agarwal, B.; Ye, L.; Ramadori, G.; Teodoro, J.S.; et al. SIRT1 Is Required for AMPK Activation and the Beneficial Effects of Resveratrol on Mitochondrial Function. *Cell Metab.* **2012**, *15*, 675–690.
21. Juan, E.M.; González-Pons, E.; Munuera, T.; Ballester, J.; Rodríguez-Gil, J.E.; Planas, J.M. Trans-Resveratrol, a natural antioxidant from grapes, increases sperm output in healthy rats. *J. Nutr.* **2005**, *135*, 757–760.
22. Shin, S.; Hee Jeon, J.; Park, D.; Jang, M.-J.; Choi, J.H.; Choi, B.-H.; Joo, S.S.; Nahm, S.-S.; Kim, J.-C.; Kim, Y.-B. Trans-Resveratrol relaxes the corpus cavernosum ex vivo and enhances testosterone levels and sperm quality in vivo. *Arch. Pharm. Res.* **2008**, *31*, 83–87.
23. Kasai, T.; Ogawa, K.; Mizuno, K.; Nagai, S.; Uchida, Y.; Ohta, S.; Fujie, M.; Suzuki, K.; Hirata, S.; Hoshi, K. Relationship between sperm mitochondrial membrane potential, sperm motility, and fertility potential. *Asian J. Androl.* **2002**, *4*, 97–103.
24. Zhao, C.; Huo, R.; Wang, F.; Lin, M.; Zhou, Z.; Sha, J. Identification of several proteins involved in regulation of sperm motility by proteomic analysis. *Fertil. Steril.* **2007**, *87*, 436–438.
25. Bahmanzadeh, M.; Goodarzi, M.T.; Farimani, A.R.; Fathi, N.; Alizadeh, Z. Resveratrol supplementation improves DNA integrity and sperm parameters in streptozotocin–nicotinamide-induced type 2 diabetic rats. *Andrologia* **2019**, *51*, e13313.
26. Alamo, A.; Condorelli, R.A.; Mongioì, L.M.; Cannarella, R.; Giacone, F.; Calabrese, V.; La Vignera, S.; Calogero, A.E. Environment and Male Fertility: Effects of Benzo-α-Pyrene and Resveratrol on Human Sperm Function In Vitro. *J. Clin. Med.* **2019**, *8*, 561.
27. Takeo, S.; Sato, D.; Kimura, K.; Monji, Y.; Kuwayama, T.; Kawahara-Miki, R.; Iwata, H. Resveratrol Improves the Mitochondrial Function and Fertilization Outcome of Bovine Oocytes. *J. Reprod. Dev.* **2014**, *60*, 92–99.
28. Barratt, C.L.; Björndahl, L.; De Jonge, C.J.; Lamb, D.J.; Martini, F.O.; McLachlan, R.; Oates, R.D.; Van Der Poel, S.; John, B.S.; Sigman, M.; et al. The diagnosis of male infertility: An analysis of the evidence to support the development of global WHO guidance—Challenges and future research opportunities. *Hum. Reprod. Update* **2017**, *23*, 660–680.
29. Deshpande, P.S., Gupta, A.S. Causes and Prevalence of Factors Causing Infertility in a Public Health Facility. *J. Hum. Reprod. Sci.* **2019**, *12*, 287–293.
30. Durairajanayagam, D. Lifestyle causes of male infertility. *Arab. J. Urol.* **2018**, *16*, 10–20.
31. Banyra, O.; Nikitin, O.; Ventskivska, I. Acute epididymo-orchitis: relevance of local classification and partner's follow-up. *Cent. Eur. J. Urol.* **2019**, *72*, 324–329.
32. La Vignera, S.; Vicari, E.; Condorelli, R.A.; D'Agata, R.; Calogero, A.E. Male accessory gland infection and sperm parameters (review). *Int. J. Androl.* **2011**, *34*, e330–e347.
33. Everaert, K.; Mahmoud, A.; Depuydt, C.; Maeyaert, M.; Comhaire, F. Chronic prostatitis and male accessory gland infection—Is there an impact on male infertility (diagnosis and therapy)? *Andrologia* **2003**, *35*, 325–330.
34. Spogli, R.; Bastianini, M.; Ragonese, F.; Ianninitti, R.G.; Monarca, L.; Bastioli, F.; Nakashidze, I.; Brecchia, G.; Menchetti, L.; Codini, M; et al. Solid Dispersion of Resveratrol Supported on Magnesium DiHydroxide (Resv@MDH) Microparticles Improves Oral Bioavailability. *Nutrients* **2018**, *10*, 1925.
35. Xie, C.; Ping, P.; Ma, Y.; Wu, Z.; Chen, X. Correlation between methylenetetrahydrofolate reductase gene polymorphism and oligoasthenospermia and the effects of folic acid supplementation on semen quality. *Transl. Androl. Urol.* **2019**, *8*, 678–685.

36. Banihani, S.A.; Aljabali, S.M. Seminal plasma vitamin B 6 levels in men with asthenozoospermia and men with normal sperm motility, a measurement using liquid chromatography with tandem mass spectrometry. *Andrologia* **2020**, *52*, e13556.
37. Murphy, L.E.; Mills, J.L.; Molloy, A.M.; Qian, C.; Carter, T.C.; Strevens, H.; Wide-Swensson, D.; Giwercman, A.; Levine, R.J. Folate and vitamin B12 in idiopathic male infertility. *Asian J. Androl.* **2011**, *13*, 856–861.
38. Ciccone, I.M.; Costa, E.M.; Pariz, J.R.; Teixeira, T.A.; Drevet, J.R.; Gharagozloo, P.; Aitken, R.J.; Hallak, J. Serum vitamin D content is associated with semen parameters and serum testosterone levels in men. *Asian J. Androl.* **2020**, *28*, doi:10.4103/aja.aja_9_20.
39. Jueraitetibaike, K.; Ding, Z.; Wang, D.-D.; Peng, L.-P.; Jing, J.; Chen, L.; Ge, X.; Qiu, X.-H.; Yao, B. The effect of vitamin D on sperm motility and the underlying mechanism. *Asian J. Androl*. **2019**, *21*, 400–407.
40. Rudnicka, A.; Adoamnei, E.; Noguera-Velasco, J.A.; Vioque, J.; Cañizares-Hernández, F.; Mendiola, J.; Jørgensen, N.; Chavarro, J.E.; Swan, S.H.; Torres-Cantero, A.M. Vitamin D status is not associated with reproductive parameters in young Spanish men. *Andrologia* **2020**, *8*, 323–331.
41. Neto, F.T.L.; Bach, P.V.; Najari, B.B.; Lira, N.F.T.; Goldstein, M. Spermatogenesis in humans and its affecting factors. *Semin. Cell Dev. Biol.* **2016**, *59*, 10–26.
42. Grootegoed, J.A.; Jansen, R.; Van Der Molen, H.J. The role of glucose, pyruvate and lactate in ATP production by rat spermatocytes and spermatids. *Biochim. Biophys. Acta Bioenerg.* **1984**, *767*, 248–256.
43. Han, Y.; Luo, H.; Wang, H.; Cai, J.; Zhang, Y. SIRT1 induces resistance to apoptosis in human granulosa cells by activating the ERK pathway and inhibiting NF-κB signaling with anti-inflammatory functions. *Apoptosis* **2017**, *22*, 1260–1272.
44. Michan, S.; Sinclair, D. Sirtuins in mammals: Insights into their biological function. *Biochem. J.* **2007**, *404*, 1–13.
45. Rato, L.; Alves, G.M.; Silva, M.B.; Sousa, M.; Oliveira, P.F. Sirtuins: Novel Players in Male Reproductive Health. *Curr. Med. Chem.* **2016**, *23*, 1084–1099.
46. Ruiz-Pesini, E.; Diez, C.; Lapeña, A.C.; Pérez-Martos, A.; Montoya, J.; Alvarez, E.; Arenas, J.; López-Pérez, M.J. Correlation of sperm motility with mitochondrial enzymatic activities. *Clin. Chem.* **1998**, *44*, 1616–1620.
47. Martínez-Heredia, J.; de Mateo, S.; Vidal-Taboada, J.M.; Ballescà, J.L.; Oliva, R. Identification of proteomic differences in asthenozoospermic sperm samples. *Hum. Reprod.* **2008**, *23*, 783–791.
48. Ruiz-Pesini, E.; Lapeña, A.C.; Díez, C.; Álvarez, E.; Enríquez, J.A.; López-Pérez, M.J. Seminal quality correlates with mitochondrial functionality. *Clin. Chim. Acta* **2000**, *300*, 97–105.
49. Mundy, A.J.; Ryder, T.A.; Edmonds, D.K. Asthenozoospermia and the human sperm mid-piece. *Hum. Reprod.* **1995**, *10*, 10116–10119.
50. Pelliccione, F.; Micillo, A.; Cordeschi, G.; D'Angeli, A.; Necozione, S.; Gandini, L.; Lenzi, A.; Francavilla, F.; Francavilla, F. Altered ultrastructure of mitochondrial membranes is strongly associated with unexplained asthenozoospermia. *Fertil. Steril.* **2011**, *95*, 641–646.
51. Nakada, K.; Sato, A.; Yoshida, K.; Morita, T.; Tanaka, H.; Inoue, S.-I.; Yonekawa, H.; Hayashi, J.-I. Mitochondria-related male infertility. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 15148–15153.
52. Kao, S.H.; Chao, H.T.; Wei, Y.H. Multiple deletions of mitochondrial DNA are associated with the decline of motility and fertility of human spermatozoa. *Mol. Hum. Reprod.* **1998**, *4*, 657–666.
53. Tremellen, K. Oxidative stress and male infertility—A clinical perspective. *Hum. Reprod. Update* **2008**, *14*, 243–258.

GENANTE™

Integratore alimentare

S&R Farmaceutici S.p.A.
Via dei Pioppi, 2
06083 Bastia Umbra
(Perugia) - Italia
www.srfarmaceutici.it

S&R FARMACEUTICI

GENANTE™

Studio clinico 2 GINECOLOGIA

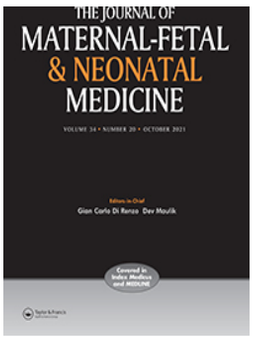
Biological and clinical effects of a Resveratrol-Based Multivitamin Supplement on intracytoplasmic sperm injection cycles: a single centre, randomized controlled trial.

Sandro Gerli[a,b]*, Chiara Della Morte [a,b,], Margherita Ceccobelli[a,b] , Monica Mariani[b], Alessandro Favilli[c] , Lucio Leonardi[d] , Alessandro Lanti[d] , Rossana G. Iannitti[d] and Bernard Fioretti[b,e]

a Department of Medicine and Surgery, Centre of Perinatal and Reproductive Medicine, University of Perugia, Perugia, Italy;
b Centre of Assisted Reproductive Technologies, S. Maria della Misericordia Hospital, Perugia, Italy;
c Department of Obstetrics and Gynecology, AOUI Verona, Verona, Italy;
d Department of R&D, S&R Farmaceutici S.p.A Bastia Umbra, Perugia, Italy;
e Department of Chemistry, Biology and Biotechnologies, University of Perugia, Perugia, Italy

**Corresponding author*

S&R FARMACEUTICI



The Journal of Maternal-Fetal & Neonatal Medicine

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ijmf20

Biological and clinical effects of a resveratrol-based multivitamin supplement on intracytoplasmic sperm injection cycles: a single-center, randomized controlled trial

Sandro Gerli, Chiara Della Morte, Margherita Ceccobelli, Monica Mariani, Alessandro Favilli, Lucio Leonardi, Alessandro Lanti, Rossana G. Iannitti & Bernard Fioretti

To cite this article: Sandro Gerli, Chiara Della Morte, Margherita Ceccobelli, Monica Mariani, Alessandro Favilli, Lucio Leonardi, Alessandro Lanti, Rossana G. Iannitti & Bernard Fioretti (2021): Biological and clinical effects of a resveratrol-based multivitamin supplement on intracytoplasmic sperm injection cycles: a single-center, randomized controlled trial, The Journal of Maternal-Fetal & Neonatal Medicine, DOI: 10.1080/14767058.2021.1958313

To link to this article: https://doi.org/10.1080/14767058.2021.1958313

Published online: 01 Aug 2021.

THE JOURNAL OF MATERNAL-FETAL & NEONATAL MEDICINE
https://doi.org/10.1080/14767058.2021.1958313

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Biological and clinical effects of a resveratrol-based multivitamin supplement on intracytoplasmic sperm injection cycles: a single-center, randomized controlled trial

Sandro Gerli[a,b], Chiara Della Morte[a,b], Margherita Ceccobelli[a,b], Monica Mariani[b], Alessandro Favilli[c], Lucio Leonardi[d], Alessandro Lanti[d], Rossana G. Iannitti[d] and Bernard Fioretti[b,e]

[a]Department of Medicine and Surgery, Centre of Perinatal and Reproductive Medicine, University of Perugia, Perugia, Italy; [b]Centre of Assisted Reproductive Technologies, S. Maria della Misericordia Hospital, Perugia, Italy; [c]Department of Obstetrics and Gynecology, AOUI Verona, Verona, Italy; [d]Department of R&D, S&R Farmaceutici S.p.A Bastia Umbra, Perugia, Italy; [e]Department of Chemistry, Biology and Biotechnologies, University of Perugia, Perugia, Italy

ABSTRACT

Background: Resveratrol display's positive effects on follicle growth and development in pre-clinical studies while there is scantly information from clinical trials. The aim of this study was to evaluate the biological and clinical impact of a resveratrol-based multivitamin supplement on intracytoplasmatic sperm injection (ICSI) cycles.

Methods: A randomized, single-center controlled trial conducted at the University Center of Assisted Reproductive Technologies involving 101 women infertile women undergoing ICSI cycles was conducted. A pretreatment with a daily resveratrol based nutraceutical was administered to the Study Group; Control Group received folic acid. The primary outcomes were the number of developed mature follicles (>16 mm), total oocytes and MII oocytes recovered, the fertilization rate and the number of cleavage embryos/blastocysts obtained. Secondary endpoints were the duration and dosage of gonadotropins, the number of embryos for transfer, implantation, biochemical, clinical pregnancy rates, live birth and miscarriage rates.

Results: A significantly higher number of oocytes and MII oocytes were retrieved in the Study Group than in Control Group ($p = .03$ and $p = .04$, respectively). A higher fertilization rate ($p = .004$), more cleavage embryos/patient ($p = .01$), blastocytes/patients ($p = .01$) and cryopreserved embryos ($p = .03$) were obtained in the Study Group. No significant differences in biochemical or clinical pregnancy, live birth, and miscarriage rates were revealed, but a trend to a higher live birth rate was revealed in the Study Group.

Conclusions: A 3 months period of dietary supplementation with a resveratrol-based multivitamin nutraceutical leads to better biological effects on ICSI cycles.

Trial registration number: ClinicalTrials.gov registration identifier: NCT04386499

ARTICLE HISTORY
Received 15 July 2021
Accepted 18 July 2021

KEYWORDS
Resveratrol; intracytoplasmic sperm injection; assisted reproductive technologies; embryo; oocyte

Introduction

In recent years, the demand for assisted reproductive technologies (ART) is constantly increasing, with more women encountering challenges with fertility. The reproductive capacity of the couple declines with age [1] and female aging is known to be associated with an impairment of the ART procedure's outcome, with a worsening of number and quality of oocytes, a reduced embryo quality and an increased incidence of miscarriages and embryo aneuploidy [2]. Chromosomal abnormalities depend on spindle instability, telomere shortening, chromosome misalignment, and mitochondrial dysfunction [3,4]. The mechanism of oocyte aneuploidy is not proven, but an energy-dependent mechanism is supposed to play a major role in oocyte development [5]. The production of energy for the metabolic requirements of the oocyte is provided solely by mitochondria and the oocyte has the largest number of mitochondria and mitochondrial DNA (mtDNA) copies of any cell [6]. During its maturation, the number and functionality of mitochondria increases rapidly, to prepare for the energy expenditure associated with the early stages of embryonic development [7]. Aged and unfertilized oocytes are frequently associated with loss of mitochondrial function, mainly due to mtDNA mutations and deletions [8].

The energy metabolism and production of adenosine triphosphate (ATP) occurs through the

CONTACT Sandro Gerli sandro.gerli@unipg.it Department of Medicine and Surgery, Centre of Perinatal and Reproductive Medicine, University of Perugia, Perugia, Italy; Rossana G. Iannitti r.iannitti@srfarmaceutici.com, Department of R&D, S&R Farmaceutici S.p.A. Bastia Umbra, Perugia, Italy

mitochondrial respiratory chain and this is a requirement at various stages of female gametogenesis [9]. ATP production in the follicle is impaired in older women and it has been demonstrated that embryo implantation potential is correlated with the ATP content of the embryo [5]. Granulosa cells (GC) have a critical role in regulating ovarian development and function by cross-talk signaling and crucial energetic support for the oocyte [10].

In a recent research, we showed that resveratrol, a natural polyphenolic compound, detected in a variety of plants, foods, and drinks, such as grapes, nuts, cranberries, and red wine, stimulates mitochondrial number (mitochondrial biogenesis) in GC and increase intracellular ATP levels [11] probably through the expression of the Silent Information Regulator 1 (SIRT1), the mammalian homolog of Sir2 (Silent information regulator 2) in yeast, a NAD-dependent deacetylase sirtuin [12]. All these mechanisms are reflected in better resistance to apoptosis and increased energy availability [13]. Resveratrol also modifies the electrophysiological properties by promoting resting membrane depolarization associated to the ultra-rapidly activating, slowly inactivating potassium current (IKur) currents reduction in h-GCs [11]. A vital interplay between oocytes and their associated GC occurs during follicular growth from the arrested primordial follicle stage to the ovulatory stage [14,15]. It is well established that oocyte development is highly dependent on molecular interactions with somatic cells [16], which provide the oocyte with nutrients that sustain basal metabolic activity and signals that regulate its differentiation [17]. Thus, resveratrol may also improve ovarian functioning and oocyte development *via* energy metabolism crosstalk between GCs and the oocyte, opening the possibility for treatment of infertile women undergoing ART cycles [18,19]. A protection against the reduction of fertility with aging in mice has been already indicated: long-term-oral administration of resveratrol improves healthy follicle number, telomere length, and telomerase activity, as well as oocyte quantity and quality [4,20]. We also reported that a resveratrol-based multivitamin supplement enhances spermatic parameters in men affected by idiopathic infertility [21,22]. Effects of resveratrol on human oocytes maturation, fertilization, and embryo development can be studied only during ART procedures; however, no specific trial has been yet conducted reporting this information. We, therefore, designed a randomized trial, with the aim to evaluate the biological and clinical effects of a resveratrol-based multivitamin supplement in women undergoing intracytoplasmatic sperm injection (ICSI) cycles.

Materials and methods

Study design

This randomized, single-blind, controlled, single-center, experimental study was performed at the Center of Assisted Reproductive Technologies, University of Perugia, Perugia, Italy, from January 2019 to March 2020. The study design was in accordance with the Helsinki Declaration, conforms to the Committee on Publication Ethics (COPE) guidelines. The study protocol was approved by the local Ethical Committee and that of the Aziende Sanitarie della Regione Umbria (n.15188/18/AV) on 20 December 2018 and retrospectively registered in the ClinicalTrials.gov Protocol Registration System (identifier: NCT04386499). The study was conducted according to the CONSORT guidelines [23]. All participating patients signed an approved informed consent.

We enrolled patients diagnosed with infertility with the following inclusion criteria: aged 18–42 years, body mass index (BMI) 18–30 kg/m^2, normal thyroid function and normal blood parameters, regular uterine cavity evaluated by hysterosalpingography or hysteroscopy.

Women were excluded if they had one of the following conditions: couples with severe male factor, women with primary or secondary ovarian failure or who adhered to the Bologna criteria [24], which meant to satisfy at least two of the following features: advanced maternal age ($>$ 40 years), a previous poor ovarian response ($\leq$3 oocytes with a conventional stimulation protocol), an abnormal ovarian reserve test (antral follicle count $<$7 or anti-Müllerian hormone $<$1.1 ng/mL); patients with inaccessible ovaries, ovarian cyst $>$20 mm, sactosalpinx, heterologous fertilization, significant systemic disease or other situations unsuitable for ovarian stimulation.

Randomization

All patients were randomized for no treatment (Control Group) and for treatment with resveratrol (Study Group) with a 1:1 ratio, *via* a sealed envelope with random numbers generated by a computer. The principal investigator generated the random allocation, enrolled patients and assigned patients to intervention. Participants were not blinded to the group assignment. The physicians and embryologists involved in the oocyte retrieval and embryo transfer

were blinded to the group assignments of the participants in the trial.

Intervention

Folic acid 400 μg/day was orally delivered to the Control Group patients while the Study Group received a nutraceutical (2 daily capsules) containing a formulation of sustained resveratrol (Resv@MDH, trademark Revifast® [25,26] and pure resveratrol for a total resveratrol amount of 150 mg), folic acid (400 mcg), vitamin D (25 mcg), vitamin B12 (2.5 mcg), and vitamin B6 (1.4 mg) (GENANTE™, S&R Farmaceutici, Bastia Umbra PG, Italy). Both treatments were started 3 months before the initiation of ovarian stimulation for ICSI procedures and were maintained until the oocyte pick-up.

Ovarian stimulation was carried out using a GnRH antagonist protocol. Gonadotropins, human highly purified FSH (Fostimon®, IBSA, Lugano, Switzerland), human FSH and LH (Meropur®, Ferring Italia, Milano, Italy) from menstrual cycle day 2 or 3, with a daily dose ranging from 150 to 375 UI, were administered. Type and dosage of gonadotropins were decided according to endocrinological patient's features, ovarian reserve, and results of previous attempts. From day 5, determinations of estradiol (E2) and progesterone (P) levels with follicular ultrasound monitoring were conducted to evaluate the ovarian response and follicular development. According to this, gonadotropins dose was daily adjusted.

Oocyte maturation was induced with 10,000 UI of human chorionic gonadotropin (hCG) (Gonasi HP®, IBSA, Lugano, Switzerland) when at least two dominant follicles of 18 mm were found. Patients with more than eight follicles with a diameter $\geq$16 mm were administered a subcutaneous (SC) injection with needle and syringe of 0.2 mg of GnRH agonist (Decapeptyl, Ferring Italy, Milano, Italy) to reduce the risk of ovarian hyperstimulation syndrome (OHSS). In these cases, a segmental approach with a "freeze all" strategy and a delayed transfer was planned. Transvaginal ultrasound-guided follicle aspiration was performed 34–36 h after trigger.

The maturation status of the oocytes was recorded according to ESHRE 2012 guidelines [27]. A high-quality oocyte, metaphase II (MII), was defined as a round, normal-sized oocyte with one regular polar body in the perivitelline space, homogeneous ooplasm without irregularities, and appropriate thickness of the zona pellucida [27]. The standard ICSI procedure was performed using ejaculated sperm from male partner.

All embryos were evaluated starting on day 2 and at the following days according to published criteria [28]: Veeck grades A, B, C, or D. Luteal phase was supported with 50 mg/day of natural, intramuscular progesterone (Prontogest®, IBSA, Lugano, Switzerland) or 800 mg/day micronized progesterone (Progeffik®, Effik Italia, Milano, Italy) and maintained until the day of hCG measurement (14 days after embryo transfer) or the evidence of a clinical pregnancy, in case of positive result. Embryo-transfer was performed between days 3 and 5 following the oocyte pick-up depending on the patient and embryo characteristics. In older women with one or two developing embryos and in women with difficulty to develop a blastocyst demonstrated in a previous attempt, a day 3 transfer was preferred. In all other cases, a day 5 transfer was performed. Only embryo-transfer from fresh cycles were considered. Beta hCG was done for the detection of pregnancy. Clinical pregnancy was established by the presence of a gestational sac, an embryo pole and heart activity by transvaginal ultrasound examination.

Primary and secondary outcomes

Primary outcomes were the number of developed mature follicles (>16 mm), total oocytes, MII oocytes recovered, fertilization rate, number of cleavage embryos/blastocysts obtained, and the number of available embryos for cryopreservation. Secondary outcomes were duration and dosage of gonadotropins, number of embryos per transfer, implantation, biochemical, clinical pregnancy rates, live birth rate and miscarriage rate.

Statistical analysis

A minimum number of subjects calculation (https://clincalc.com/stats/SampleSize.aspx) was performed with a level of 0.05, our sample size yielded sufficient power to detect at least a 20% difference between groups in the primary outcome with over 80% power ($\beta = 0.2$) and an estimated variability of parameter with SD of <30%. Reproductive outcomes were compared between the Resveratrol treated group (Study Group) and Control group with a *post hoc* calculation analysis. To evaluate differences between nonparametric variables which are expressed as frequencies and percentages, we used Chi-square with Yates' correction factor. Reproductive outcomes were compared between the Study group and Control group. An intention-to-treat analysis (ITT) of clinical results on the whole allocated population was performed. The

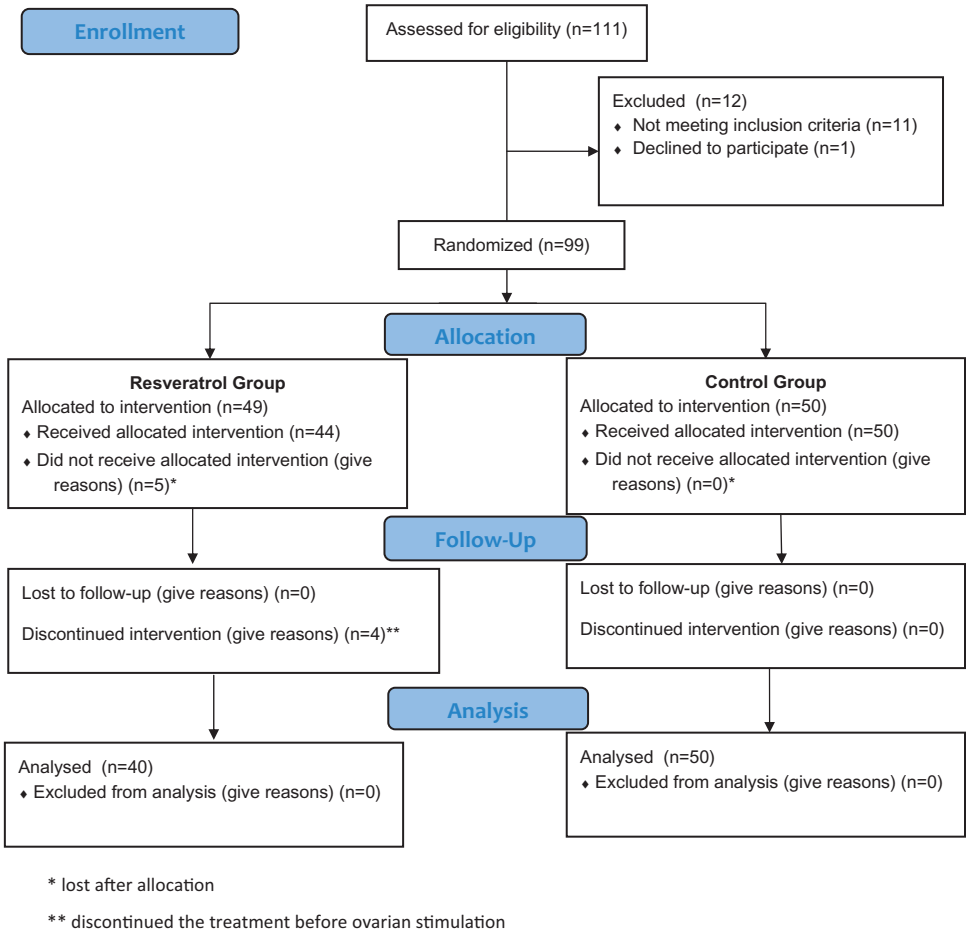


Figure 1. CONSORT flow diagram. Diagram depicts patient enrollment, allocation to different groups (Control and Study Group), follow-up, and analysis.

analyses included also all randomized and exposed women, except for the analyses of fertilization, cleavage embryos, blastocytes, and cryopreserved embryos which are based on patients with at least one oocyte retrieved following ICSI procedure. Analyses were performed using Origin 61version. Statistical significance was defined as a two-sided p value of $<.05$ and are presented as mean ± standard error (SE). Student's t-test was used for normally or near-normally distributed data.

Results

A total of 111 patients were initially considered eligible for the study. Twelve patients were excluded due to the presence of exclusion criteria (severe male factor or patients who adhered to Bologna criteria)and 99 were randomized. Forty-nine patients were allocated in the Study Group, while 50 in the Control Group. In total, five women were lost after allocation and four patients discontinued the pretreatment before starting the ovarian stimulation (all in the Study Group) (Figure 1). No statistical differences were found in the general characteristics of the patients, likewise the two groups were not significantly different even considering the different ovarian stimulation protocol, where preparations with FSH-only or human menopausal gonadotropins were used (Table 1).

Table 2 shows the outcomes of ovarian stimulation for both Groups. A not statistically significant longer stimulation was required for the Study Group ($p = .06$). There were no statistically significant differences in the total gonadotropins dosage used in the ovarian stimulation protocol. At the end of the hormonal treatment a trend to a higher number of leading follicles was shown in the Study Group ($p = .13$), with a significant increase of retrieved oocytes ($p = .03$) and MII oocytes ($p = .04$) in the Study group compared to the control group were found. A higher fertilization rate ($p = .004$), significantly more cleavage embryos, blastocysts ($p = .01$) were obtained and more embryos were cryopreserved ($p = .03$) for patient in the Study Group than in the Control Group. The two groups were similar regarding implantation, biochemical, clinical

Table 1. Patient demographic data, ovarian reserve and stimulation characteristics in the Control and Study groups.

	Control Group (± SE)	Study Group (±SE)	*p*
Patients (*n.*)	50	40	
Age (*n.*)	36.6 ± 0.6	36.1 ± 0.6	.62
BMI (kg/m^2)	22.0 ± 0.6	23.6 ± 0.7	.07
AMH (ng/mL)	2.8 ± 0.3	3.4 ± 0.4	.36
AFC (*n*)	10.8 ± 0.8	11.4 ± 0.8	.24
Basal FSH (mIU/mL)	7.6 ± 0.4	7.9 ± 0.4	.66
Type of gonadotropin			
h-HP-FSH / r-FSH	27 (54%)	23 (57%)	.74
FSH + LH (*n*) (%)	23 (46%)	17 (43%)	

Values are expressed as mean + SE or numbers. BMI: Body Mass Index; AMH: anti-Mullerian hormone; AFC: antral follicle count; FSH: follicle stimulating hormone; h-HP FSH: human highly purified follicle-stimulating hormone; r-FSH: recombinant follicle-stimulating hormone, LH: luteinizing hormone.

Table 2. Gonadotropins, follicles and oocytes in the Control and Study groups.

	Control Group (*n* = 50)	Study Group (*n* = 40)	*p*
Days of stimulation	10.5 ± 0.2	11.2 ± 0.3	.06
Gonadotropins (IU)	2693.4 ± 141.4	2480.6 ± 131.6	.27
Estradiol levels at the day of hCG (pg/mL)	2790.3 ± 567.3	2272.8 ± 378.6	.47
Number of follicles (>16 mm)	9.4 ± 0.6	10.9 ± 0.8	.13
Oocytes retrieved	7.1 ± 0.4	8.7 ± 0.7	.03
Ratio follicles/ oocytes retrieved	0.77 ± 0.02	0.82 ± 0.03	.24
MII oocytes	4.9 ± 0.4	6.4 ± 0.6	.04

Values are expressed as mean ± SE.

pregnancy rate, live birth, and miscarriage rate. A trend to a higher live birth rate was revealed in the Study Group. No adverse secondary effects for the pretreatment protocol or severe OHSS were observed in any of the two groups, but a trend in the reduction of delayed transfer (due to a "freeze all" strategy to prevent OHSS) (2.6% versus 10.6%) was observed in the Study Group (Table 3).

Discussion

This study demonstrated that a pretreatment with a resveratrol based multivitamin dietary supplement is able to provide better biological effects in ICSI procedures. The group of patients who received this supplementation developed a higher number of oocytes, more mature oocytes, with a higher fertilization rate. A higher number of cleavage embryos and blastocysts were also obtained leading to more cryopreserved embryos. Probably due to the small sample size, no significant differences in clinical data (biochemical, clinical pregnancy, live birth, and miscarriage rate) were revealed in the two study groups; however, we could expect a greater cumulative pregnancy rate in the Study Group. Results reported in our study have been achieved with the association of a 3 month pretreatment with resveratrol to the ovarian stimulation protocol. This pretreatment period of 3 months was based on the average duration of oogenesis in which oocytes go through the early stages of maturation in the ovary [29]. These results were similar to those obtained in an *in vitro* maturation (IVM) study, showing that resveratrol, at 1.0 μm in culture medium, significantly enhanced oocyte maturation and fertilization as well as the formation of blastocysts in mice [30]. There are many beneficial effects of resveratrol on humans, including antiaging, antioxidant, anti-inflammatory, insulin-sensitizing, cardioprotective, vasodilating, and anti-neoplastic properties [31]. Important emerging evidence in human reproduction indicate that resveratrol has potential positive effects in older women, PCOS, endometriosis, and uterine fibroids [32], but no reports have been published regarding the effects of resveratrol on oocytes and embryos, with clinical results, during ART procedures.

Only two very recent studies have been published until today evaluating effects of resveratrol on follicles, oocytes and embryos in humans, but they are not comparable with our analysis [19,33]. Ochiai *et al.* evaluated the impact of resveratrol supplementation on pregnancy outcomes in ET cycles, fresh or vitrified-warmed. Data on the effects of resveratrol on ovarian response after stimulation for oocyte retrieval were not collected, as only results regarding the effect on ET have been reported [19]. The outcome showed that resveratrol supplementation was strongly associated with a decrease in clinical pregnancy rate. In that study resveratrol was continued during the luteal phase of the treated cycle and the negative effect was probably due to adverse effects on decidualization of the endometrium, caused by the resveratrol-induced suppression of decidual senescence and deacetylation of significant genes for decidualization [19]. The same group also analyzed the effects of resveratrol in primary culture of human endometrial stromal cells demonstrating its interference with the reprogramming of the retinoic acid signaling pathway and remodeling of the endometrium [34]. It is well known that decidual change in the endometrium may cause implantation failure and recurrent miscarriage [35]. Considering that the half-life of resveratrol is 9–10 h [36], as affirmed by Ochiai et al. [19] it is sufficient to administer resveratrol before and during ovarian stimulation, and to discontinue the intake at the day of oocyte pick up to avoid adverse effects on endometrium. In our trial, resveratrol was given exactly during that period of time and this could explain the different clinical results obtained. However, further clinical studies are required

Table 3. Embryos and pregnancies in the Control and Study groups.

	Control Group ($n = 50$)	Study Group ($n = 40$)	p
Fertilization rate	64.6% (210/325)	75.4% (230/305)	.004
Fertilized oocytes/patient	4.4 + 0.4	5.7 + 0.4	.01
Cleavage rate	94.1% (208/210)	96.5% (222/230)	.08
Cleavage embryos/patient	4.2 ± 0.4	5.7 ± 0.4	.01
Blastocysts/patient	2.3 ± 0.3 (89/38)	3.5 ± 0.3 (104/30)	.01
Transferred embryos/patient	1.8 ± 0.1	1.9 ± 0.1	.3
Embryos transferred day 3	39% (31/79)	27% (20/74)	.11
Embryos transferred day 5	61% (48/79)	73% (54/74)	.11
Cryopreserved embryos/patient	1.1 ± 0.21	1.5 ± 0.3	.03
Implantation rate	25.3% (20/79)	24.3% (18/74)	.96
Biochemical pregnancy rate (BPR)	42.8% (18/42)	43.2% (16/37)	.84
BPR (transfer day 3)	40.0% (6/15)	44.4% (4/9)	.83
BPR (transfer day 5)	44.4% (12/27)	42.9% (12/28)	.91
Clinical pregnancy rate (CPR)	40.5% (17/42)	37.8% (14/37)	.99
Clinical pregnancy rate (CPR)[a]	34.0% (17/50)	28.6% (14/49)	.56
CPR (transfer day 3)	33.3% (5/15)	33.3% (3/9)	.99
CPR (transfer day 5)	44.4% (12/27)	39.3% (11/28)	.70
Live birth rate (LPR)	30.9 % (13/42)	35.1 % (13/37)	.87
Live birth rate (LBR)[a]	26.0 % (13/50)	26.5 % (13/49)	.95
LBR (transfer day 3)	13.3% (2/15)	22.2% (2/9)	.57
LBR (transfer day 5)	40.7% (11/27)	39.3% (11/28)	.91
Delayed transfer rate	10.6% (5/47)	2.6% (1/38)	.31
Delayed transfer rate[a]	10.0% (5/50)	2.0% (1/49)	.1
Miscarriage rate	23.5% (4/17)	21.4% (3/14)	.77
Miscarriage rate[a]	8.0% (4/50)	6.1% (3/49)	.71

Values are expressed as mean ± SE or percentages (numbers in parenthesis).
[a]The statistical analysis was made on the intent-to-treat (ITT) population (Ctrl = 50 and Study Group = 49, for details see Figure 1).

to establish optimal doses and periods of resveratrol intake while preventing adverse effects on implantation, and pregnancy as also suggested in a recent review [37].

The second clinical trial conducted by Bahramrezaie et al. determined the effect of resveratrol on the angiogenesis pathway specifically on the expression of VEGF and hypoxia-inducible factor (HIF) 1 genes in GC of PCOS patients who underwent an ART treatment [33]. According to the results, significant higher rates of high-quality oocytes (81.93 ± 10.81 versus 69.13 ± 18.71; p .002) and high-quality embryos (89.80 ± 11.53 versus 78.83 ± 23.04; p .024) were obtained in the resveratrol group [33].

Several hypotheses coming from basic research could explain the positive effects of resveratrol on oocytes. In regard of the oocyte aging, indeed, recent studies demonstrated that resveratrol effects GCs and, moreover, the ovarian physiology. Specifically, resveratrol induces GCs proliferation through activation of the PI3K pathway and promotes primordial follicle activation [38]. Compared to untreated tissues, a higher proportion of growing follicles has been reported after human ovarian tissue are cultured in the presence of resveratrol [38,39]. The activation of SIRT1 would be a key therapeutic action needed to ameliorate oocyte competence in oxidative stress-mediated ovarian aging [40,41]. Resveratrol is a natural activator of sirtuins able to upregulate the production of SIRT1 in response to oxidative stress [42] and, therefore, its administration may compensate for the physiologic decrease of SIRT1 expression in aged oocytes. *In vitro* studies showed that resveratrol decreases DNA damage, indeed is clearly evident that its addition in culture may have beneficial effects on the ovarian tissue because DNA damage was only 15.2% in those follicles cultured in 2 μM resveratrol versus 55.2% in not enriched culture medium [43]. Regarding animals, in aged mice resveratrol also improved the number of follicles [4]. Resveratrol also effects vascular endothelial growth factor (VEGF), a potent inducer of angiogenesis and vascular permeability that plays a crucial role in ovarian folliculogenesis [44–47].

There are some limitations in our study. First the small sample size of population considered for the analysis. Second, we evaluated only patients who completed the treatment with a fresh transfer. Frozen-thawed ET cycles were not considered and therefore we did not calculate cumulative pregnancy rates per patient. Furthermore, two different protocols of ovarian stimulation were used, with LH or without LH, according to the endocrinological patient's features and to the results of previous attempts, and although a bivariate analysis demonstrated that the two groups were well balanced, this may represent a possible

bias. Regarding gonadotropins, we decided to administer a tailored-dosage to optimize the outcome of the ovarian stimulation: this customized choice may also represent a potential bias of the study.

Conclusions

In conclusion, the treatment with a resveratrol-based multivitamin supplement in infertile patients, before and during ovarian stimulation in ICSI cycles, seems promising to ameliorate the biological patterns related to ovarian aging by enhancing the quality of oocytes and embryos. This study showed no significant differences in the clinical and chemical pregnancy rates by the addition of a resveratrol-base multivitamin supplementation. However, the number of cryopreserved embryos was significantly higher in the Study Group and this may predicted a greater cumulative pregnancy rate from frozen embryos. This is the first randomized, clinical trial and further studies could contribute to reinforce these conclusions.

Acknowledgments

The authors thank S&R Farmaceutici S.p.A [Italy], for kindly providing the resveratrol based multivitamin nutraceutical for our studies. All authors would like to thank Sara Iannitti Pereira for finalizing the English editing and proofreading of our manuscript.

Institutional review board statement

The study design was in accordance with the Helsinki Declaration, conforms to the Committee on Publication Ethics [COPE] guidelines. The study was approved by the Ethical Committee of Aziende Sanitarie della Regione Umbria [n.15188/18/AV] on 20 December 2018 and retrospectively registered in the ClinicalTrials.gov Protocol Registration System (identifier: NCT04386499) on 13 May 2020.

Informed consent statement

Informed consent was obtained from all individual participants included in the study.

Disclosure statement

Sandro Gerli, Chiara Della Morte, Margherita Ceccobelli, Monica Mariani, Alessandro Favilli, and Bernard Fioretti declare they have no financial interests. Alessandro Lanti and Rossana G. Iannitti receive a salary from S&R Farmaceutici. Lucio Leonardi is Executive Director of S&R Farmaceutici S.p.A.

Author contributions

Sandro Gerli, Bernard Fioretti, and Rossana G. Iannitti made substantial contributions to the conception or design of the work; Sandro Gerli, Bernard Fioretti, Rossana G. Iannitti, Chiara Della Morte, Margherita Ceccobelli, and Monica Mariani made substantial contributions to the acquisition, analysis, or interpretation of data; Sandro Gerli, Bernard Fioretti, Rossana G. Iannitti, Alessandro Favilli, Lucio Leonardi, and Alessandro Lanti revised it critically for important intellectual content; All authors approved the version to be published

ORCID

Sandro Gerli http://orcid.org/0000-0003-1082-3062
Bernard Fioretti http://orcid.org/0000-0002-9570-4195

Data availability statement

All data and materials as well as software application comply with field standards.

References

[1] Crawford NM, Steiner AZ. Age-related infertility. Obstet Gynecol Clin North Am. 2015;42(1):15–25.
[2] Bentov Y, Esfandiari N, Burstein E, et al. The use of mitochondrial nutrients to improve the outcome of infertility treatment in older patients. Fertil Steril. 2010;93(1):272–275.
[3] Woods DC, Tilly JL. Autologous Germline Mitochondrial Energy Transfer (AUGMENT) in human assisted reproduction. Semin Reprod Med. 2015;33(6):410–421.
[4] Lee Y, Drake AC, Thomas NO, et al. Dietary resveratrol increases mid-life fecundity of female Nothobranchius guentheri. Comp Biochem Physiol C Toxicol Pharmacol. 2018;208:71–76.
[5] Seifer DB, DeJesus V, Hubbard K. Mitochondrial deletions in luteinized granulosa cells as a function of age in women undergoing in vitro fertilization. Fertil Steril. 2002;78(5):1046–1048.
[6] May-Panloup P, Chretien MF, Malthiery Y, et al. Mitochondrial DNA in the oocyte and the developing embryo. Curr Top Dev Biol. 2007;77:51–83.
[7] Jansen RP, Bowman MC, de Boer KA, et al. What next for preimplantation genetic screening (PGS)? Experience with blastocyst biopsy and testing for aneuploidy. Hum Reprod. 2008;23(7):1476–1478.
[8] Keefe DL, Niven-Fairchild T, Powell S, et al. Mitochondrial deoxyribonucleic acid deletions in oocytes and reproductive aging in women. Fertil Steril. 1995;64(3):577–583.
[9] Collado-Fernandez E, Picton HM, Dumollard R. Metabolism throughout follicle and oocyte development in mammals. Int J Dev Biol. 2012;56(10–12):799–808.
[10] Bertoldo MJ, Faure M, Dupont J, et al. AMPK: a master energy regulator for gonadal function. Front Neurosci. 2015;9:235.

[11] Ragonese F, Monarca L, De Luca A, et al. Resveratrol depolarizes the membrane potential in human granulosa cells and promotes mitochondrial biogenesis. Fertil Steril. 2021;115(4):1063–1073.
[12] Tatone C, Di Emidio G, Barbonetti A, et al. Sirtuins in gamete biology and reproductive physiology: emerging roles and therapeutic potential in female and male infertility. Hum Reprod Update. 2018;24(3): 267–289.
[13] Han Y, Luo H, Wang H, et al. SIRT1 induces resistance to apoptosis in human granulosa cells by activating the ERK pathway and inhibiting NF-κB signaling with anti-inflammatory functions. Apoptosis. 2017;22(10): 1260–1272.
[14] Albertini DF, Barrett SL. Oocyte-somatic cell communication. Reprod Suppl. 2003;61:49–54.
[15] Wigglesworth K, Lee KB, Emori C, et al. Transcriptomic diversification of developing cumulus and mural granulosa cells in mouse ovarian follicles. Biol Reprod. 2015;92(1):23.
[16] Biase FH, Kimble KM. Functional signaling and gene regulatory networks between the oocyte and the surrounding cumulus cells. BMC Genomics. 2018;19(1): 351.
[17] Clarke H. Control of mammalian oocyte development by interactions with the maternal follicular environment. Results Probl Cell Differ. 2017;63:17–41.
[18] Schube U, Nowicki M, Jogschies P, et al. Resveratrol and desferoxamine protect human OxLDL-treated granulosa cell subtypes from degeneration. J Clin Endocrinol Metab. 2014;99(1):229–239.
[19] Ochiai A, Kuroda K, Ikemoto Y, et al. Influence of resveratrol supplementation on IVF-embryo transfer cycle outcomes. Reprod Biomed Online. 2019;39(2): 205–210.
[20] Liu M, Yin Y, Ye X, et al. Resveratrol protects against age-associated infertility in mice. Hum Reprod. 2013; 28(3):707–717.
[21] Illiano E, Trama F, Zucchi A, et al. Resveratrol-based multivitamin supplement increases sperm concentration and motility in idiopathic male infertility: a pilot clinical study. JCM. 2020;9(12):4017.
[22] Mongioì LM, Perelli S, Condorelli RA, et al. The role of resveratrol in human male fertility. Molecules. 2021; 26(9):2495.
[23] Schulz KF, Altman DG, Moher D, et al. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. BMJ. 2010;340:c332.
[24] Ferraretti AP, La Marca A, Fauser BCJM, et al. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod. 2011;26(7):1616–1624.
[25] Spogli R, Bastianini M, Ragonese F, et al. Solid dispersion of resveratrol supported on Magnesium DiHydroxide (Resv@MDH) microparticles improves oral bioavailability. Nutrients. 2018;10(12):1925.
[26] Iannitti RG, Floridi A, Lazzarini A, et al. Resveratrol supported on Magnesium DiHydroxide (Resv@MDH) represents an oral formulation of resveratrol with better gastric absorption and bioavailability respect to pure resveratrol. Front Nutr. 2020;7:570047.
[27] Rienzi L, Balaban B, Ebner T, et al. The oocyte. Hum Reprod. 2012;27(Suppl 1):i2–i21.
[28] Veeck LL. Atlas of the human oocyte and early conceptus, Vol. 2. Baltimore (MD): Williams and Wilkins; 1991. pp. 427–444.
[29] Gougeon A. Human ovarian follicular development: from activation of resting follicles to preovulatory maturation. Ann Endocrinol (Paris). 2010;71(3): 132–143.
[30] Liu MJ, Sun AG, Zhao SG, et al. Resveratrol improves in vitro maturation of oocytes in aged mice and humans. Fertil Steril. 2018;109(5):900–907.
[31] Neves AR, Lucio M, Lima JL, et al. Resveratrol in medicinal chemistry: a critical review of its pharmacokinetics, drug-delivery, and membrane interactions. Curr Med Chem. 2012;19(11):1663–1681.
[32] Ho Y, Sh Yang YC, Chin YT, et al. Resveratrol inhibits human leiomyoma cell proliferation via crosstalk between integrin αvβ3 and IGF-1R. Food Chem Toxicol. 2018;120:346–355.
[33] Bahramrezaie M, Amidi F, Aleyasin A, et al. Effects of resveratrol on VEGF & HIF1 genes expression in granulosa cells in the angiogenesis pathway and laboratory parameters of polycystic ovary syndrome: a triple-blind randomized clinical trial. J Assist Reprod Genet. 2019;36(8):1701–1712.
[34] Ochiai A, Kuroda K, Ozaki R, et al. Resveratrol inhibits decidualization by accelerating downregulation of the CRABP2-RAR pathway in differentiating human endometrial stromal cells. Cell Death Dis. 2019;10(4):276.
[35] Okada H, Tsuzuki T, Murata H. Decidualization of the human endometrium. Reprod Med Biol. 2018;17(3): 220–227.
[36] Gescher AJ, Steward WP. Relationship between mechanisms, bioavailability, and preclinical chemopreventive efficacy of resveratrol: a conundrum. Cancer Epidemiol Biomarkers Prev. 2003;12(10):953–957.
[37] Ochiai A, Kuroda K. Preconception resveratrol intake against infertility: friend or foe? Reprod Med Biol. 2020;19(2):107–113.
[38] Bezerra MÉS, Gouveia BB, Barberino RS, et al. Resveratrol promotes in vitro activation of ovine primordial follicles by reducing DNA damage and enhancing granulosa cell proliferation via phosphatidylinositol 3-kinase pathway. Reprod Domest Anim. 2018;53(6):1298–1305.
[39] Hao J, Tuck AR, Sjödin MOD, et al. Resveratrol supports and alpha-naphthoflavone disrupts growth of human ovarian follicles in an in vitro tissue culture model. Toxicol Appl Pharmacol. 2018;338:73–82.
[40] Passos JF, Saretzki G, von Zglinicki T. DNA damage in telomeres and mitochondria during cellular senescence: is there a connection? Nucleic Acids Res. 2007; 35(22):7505–7513.
[41] Li Q, Geng X, Zheng W, et al. Current understanding of ovarian aging. Sci China Life Sci. 2012;55(8): 659–669.
[42] Di Emidio G, Falone S, Vitti M, et al. SIRT1 signalling protects mouse oocytes against oxidative stress and is deregulated during aging. Hum Reprod. 2014;29(9): 2006–2017.

[43] Grabowska W, Sikora E, Bielak-Zmijewska A. Sirtuins, a promising target in slowing down the ageing process. Biogerontology. 2017;18(4):447–476.

[44] Ortega I, Duleba AJ. Ovarian actions of resveratrol. Ann N Y Acad Sci. 2015;1348(1):86–96.

[45] Artini PG, Monti M, Cristello F, et al. Vascular endothelial growth factor in females of reproductive age. Gynecol Endocrinol. 2003;17(6):477–492.

[46] Peitsidis P, Agrawal R. Role of vascular endothelial growth factor in women with PCO and PCOS: a systematic review. Reprod Biomed Online. 2010;20(4):444–452.

[47] Qiao J, Feng HL. Extra- and intra-ovarian factors in polycystic ovary syndrome: impact on oocyte maturation and embryo developmental competence. Hum Reprod Update. 2011;17(1):17–33.

GENANTE™

Integratore alimentare

S&R Farmaceutici S.p.A.
Via dei Pioppi, 2
06083 Bastia Umbra
(Perugia) - Italia
www.srfarmaceutici.it

S&R FARMACEUTICI

sciendo

The EuroBiotech Journal

CASE REPORT
Medicine and Biotechnology

Successful Mestrual Regularity and Spontaneous Pregnancies with a Resveratrol-Based Multivitamin Supplement in Women with Idiopathic Premature Ovarian Insufficiency

Prof. Michele Vignali

Abstract

Premature ovarian insufficiency (POI) is a clinical syndrome defined by loss of ovarian activity before the age of 40 years and is characterized by menstrual disturbance, follicle stimulating hormone (FSH) concentration above 40 IU/l and infertility. In some patients the best option is to conceive spontaneously since many treatment strategies remain unsuccessful or involve eggs donation. In this case report series, we describe the effects of a resveratrol-based multivitamin supplement containing trans-resveratrol, folic acid, vitamin B6, B12 and D, in six women with poor prognosis of pregnancy due to POI and evaluate the achievement of desired conception.

These women, aged less then 40 years, suffered from menstrual irregularities, anovulation and infertility. They all had normal karyotype, and no history of ovarian surgery, radiation exposure or chemotherapy. Blood test showed at least two values of FSH above 40 IU/l.

Four out of six patients with POI conceived after 3-6 months of a resveratrol-based multivitamin supplement, ultimately giving birth to a full-term baby. Regular menstrual cycle was restored in all patients after two to four months the start of treatment. In conclusion the treatment with a resveratrol-based supplement improved menstrual regularity and suggest a useful potential of this supplementation in some cases of POI.

Keywords: infertility, resveratrol, menstrual irregularity, POI

Introduction

Premature Ovarian Insufficiency, also known as primary ovarian insufficiency, is characterized by premature depletion or dysfunction of ovarian follicle (1), with irregular menses (intermittent or unpredictable) before the age of 40. There is no unanimous consensus on what the correct criteria are for identifying POI in adolescent, and delay in diagnosis is common (1). Despite the description of different genetic, immune and iatrogenic factors of POI, the etiology in most cases of this disease are unexplained. POI is characterized by the presence of oligo/amenorrhea in association with menopausal serum level of FSH above 40 IU/l and must be distinguished from natural premature menopause characterized by serum level of FSH very high (between 16 and 134 IU/L), estradiol very low (less than 20 pg/ml), permanent amenorrhea and absence of ovulatory cycles. POI occurs in approximately 1-2% of women aged under 40 years and in 0.1% of women aged under 30 years that present abnormal bleeding pattern (3). Clinical symptoms are similar to premature menopause, including hot flashes, night sweats, vaginal dryness, irritability, difficult on concentration (3). Five to ten percent of women with POI manage to conceive spontaneously while no women affected by premature menopause can conceive naturally.

The diagnosis of POI is psychologically devastating in reproductive-aged women because of deleterious impact on fertility. As long as ovulation is extremely rare and unpredictable in women with POI and none of the ovulation induction regimens have been shown to be effective, treatment strategies are lacking. Successful pregnancy with assisted reproductive technology (ART) rarely occurs in POI patients and the most successful

Dipartimento di Scienze Biomediche per la Salute – Università degli Studi di Milano. UOC di Ostetricia e Ginecologia, P.O. Macedonio Melloni – ASST Fatebenefratelli Sacco, Milano

DOI: 10.2478/ebtj-2022-0005

method remains eggs donation, with important ethical limitations (3,4).

Recent studies have demonstrated the beneficial effects of resveratrol in humans. Resveratrol has been reported to decrease oxidative stress and attenuate inflammation, and these mechanisms may account for many of its health benefits. Important evidence is also emerging in the field of human reproduction indicating that resveratrol has potential positive effects in older women, PCOS, endometriosis, uterine fibroids and menopause (5). Concerning the oocyte aging, recent research have demonstrated that resveratrol is effective on granulosa cells and impacts positively on the ovarian physiology. Specifically, resveratrol induces granulosa cells proliferation through activation of the PI3K pathway and promotes primordial follicle activation (6). Compared to untreated tissues, a higher proportion of growing follicles in human ovarian tissue culture in the presence of resveratrol has been reported (6,7). However, recently conflicting findings on the actions of resveratrol on decidualization of human endometrial stromal cells (HESCs) have been published. Ochiai et al. demonstrated that resveratrol inhibits decidual transformation of primary cultured HESCs (8) while Mestre Citrinovitz et al. showed that resveratrol enhances decidualization of HESCs in culture (9). Nevertheless, because of the lack of robust data, more studies are required to verify these effects.

Furthermore in premature ovarian failure (POF) animal models, resveratrol effectively improved the ovarian function and the productive capacity of FGSCs via relieving oxidative stress and inflammation and a mechanism involving the hh signaling pathway, suggesting that resveratrol is a potential agent against POF (10).

Case Report Series

In this case report series, we describe the effects of a resveratrol-based multivitamin supplement containing trans-resveratrol (150mg /day in two administrations), folic acid, vitamin B6, B12 and D for at least 60 days, in 6 infertile women, with poor prognosis of pregnancy due to POI, with particular attention to the resumption of regular menstrual flow and/or the achievement of spontaneous conception, during an observation period of 12 months. None of the patients observed could access ART procedures because of an insufficient ovarian reserve whereas every partner has normal seminal test. When POI was diagnosed, all six patients had FSH level above 40 UI/L, irregular menses, normal karyotype, no history of ovarian surgery, radiation exposure or chemotherapy, and a BMI < 30 kg/m2.

Hereafter the description of the individual 6 cases.

Case 1

A 35-year-old female who was diagnosed with POI when she was 34, at the time of the first visit reported vasomotor symptoms, night sweats and being in amenorrhea for six months. The FSH serum level was 39,5UI/L.

After starting resveratrol supplementation, at the second visit two months later, she reported that her period has resumed about six weeks after starting treatment and that vasomotor symptoms and night sweats have disappeared. The FSH serum level in early follicle fase was 13UI/L.. She continued the treatment and returned for a check-up visit after 10 months in which she reported that she was back in amenorrhea. A transvaginal ultrasound was performed and a gestational sac with a viable embryo was recognized. She delivered a healthy full-term baby.

Case 2

A 29-year-old woman presented to our clinic with a 6-month history of oligo-amenorrhea and no vasomotor symptoms or night sweats. Laboratory blood tests performed while the patient was in amenorrhea revealed an elevated serum FSH level (38 IU/l) and AMH 0.01 ng/ml. She was given a diagnosis of POI. Since the patient wished to become pregnant it was suggested to her that egg donation or IVF were her best option to have a child. She chose to attempt conception with her own rather than using donor eggs. She started resveratrol-based multivitamin supplementation and her period was restored after 40 days of treatment. Three months later she conceived a baby naturally, without the need for assisted reproductive technology. This was confirmed by serial serum beta HCG measurements. The pregnancy proceeded uneventfully until the 35th week of gestation when she developed gestational hypertension. The baby was born at 39 weeks and 4 days of gestation, without any serious complications.

Case 3

A 38-years-old patient presented to our clinic reporting that she had been in amenorrhea for 8 months, with vasomotor symptoms and night sweats, and that she wished to become pregnant. She was diagnosed with POI and treated with various hormonal regimens coupled with close ultrasound follicle monitoring. She referred that she had very few ovulatory cycles over the last 2 years. Her serum FSH value at the moment of diagnosis was 55 IU/L. After about 4-months of treatment with resveratrol-based supplement her period resumed. The serum FSH level decreased to 18 IU/L. She continued to have menstrual bleeding every 60 days, but unfortunately, she was unable to get pregnant.

Case 4

A 39-years-old patient presented to our clinic reporting oligo-amenorrhea over the last 2 years and 4 years of infertility. Laboratory blood tests performed at the age of 37, revealed FSH serum level of 45 IU/L and AMH 0.02 ng/ml. The patient reported to have undergone several hormone replacement cycles and ovulation induction cycles and even an unsuccessful trial of intrauterine insemination (IUI) over the last 3 years. She said she was on the waiting list for eggs donation, thus we decided to start a resveratrol-based multivitamin supplementation. Four months after starting the treatment she began to menstruate regularly (every 40days), the serum FSH level decreased to 12,2 IU/L and she was able to conceive after eight

months of treatment with resveratrol-based multivitamin supplementation and delivered a healthy baby after 38 weeks of gestation.

Case 5

A 34-year-old patient came to our observation reporting that she had not menstruated for three months. Her female hormone panel showed serum FSH level of 49IU/mL and AMH of 0,01 ng/ml. Seven-weeks after starting the treatment with resveratrol-based supplement she reported having started menstruating again and a FSH serum level that was decreased to 16 IU/L. She was monitored for one year and her menstrual cycle appeared regularly every 32.4 +4 days, but unfortunately, she was unable to get pregnant.

Case 6

A 30-year-old patient with an 8-month history of amenorrhea and a previous diagnosis of POI at age of 28 presented to our clinic, manifesting her maternity desire. We confirmed diagnosis of POI (FSH value 40,8IU/L) and treated her with a resveratrol-based multivitamin supplement. After about 6 months of treatment her menstrual cycle appeared regularly every 30.4+3.8 days and the FSH value decreased to 11IU/L. This patient underwent to close ultrasound follicle monitoring; spontaneous ovulation occurred and the resulting pregnancy was confirmed by serum HCG and ultrasonography performed 28 days after ovulation. She delivered a healthy baby after 38 weeks of gestation.

Discussion

Several regimens have been employed in the setting of POI to restore menses and to achieve pregnancy however none have been proven to be effective (11,12). Oocyte donation is the most frequent suggested route of treatment in order to get pregnant, nevertheless this practice is not yet available in many countries. There is also no evidence that assisted reproductive technology (ART) without oocyte/embryo donation may improve pregnancy rate of POI patients without any other infertility factors within a spontaneous ovulatory cycle (11,12). Analysis of the six cases presented herein revealed restoration of menstrual cycle flow in all the patients after supplementation with resveratrol for at least 2 months Moreover four out of six patients with a diagnosis of POI successfully conceived spontaneously without the need of assisted reproductive technologies. Only one preclinical study, conducted on animal models, discussed the opportunity of resveratrol supplementation for women with premature ovarian insufficiency (10). The potential pitfall of this present case report is the lack of homogenous classifications of the POI population and the little number of cases. We are aware that case reports cannot decide a therapeutic management but certainly these findings can be useful to pave the way for new treatment hypotheses especially in this selected population (POI) whose treatment is not yet universally defined. Further studies are required to verify these possible beneficial effects of resveratrol observed in this limited case series.

References

1. Committee Opinion Reaffirmed 2020. Primary Ovarian insufficiency in adolescents and young women.
2. The ESHRE Guideline Group on POI, L. Webber, M. Davies, R. Anderson, J. Bartlett, D. Braat, B. Cartwright, R.

Table 1. Summary of POI women

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Age of POI diagnosis	34	29	38	37	34	28
Age at menarche	12	11	13	13	11	12
Age at initial visit to our clinic	35	29	38	39	34	30
FSH UI/L at diagnosis (medium value)	39,8	38	55	45	49	40,8
FSH UI/L early follicular fase	13	15	18	12,2	16	11
Menses /DAYS (medium value)	55	39	62	43	80	38
Treatments: Resveratrol-based multi supplement	+	+	+	+	+	+
Method for pregnancy	Spontaneous	Spontaneous	-	Spontaneous	-	Spontaneous
Pregnancy outcome	40 wks, female 3900g	39 wks, female 3455g	-	38 wks, female 3100g	-	38 wks, male 3840g

Cifkova, S. de Muinck Keizer-Schrama, E. Hogervorst, F. Janse, L. Liao, V. Vlaisavljevic, C. Zillikens, N. Vermeulen, ESHRE Guideline: management of women with premature ovarian insufficiency,Human Reproduction, Volume 31, Issue 5, May 2016, Pages 926–937

3. Nelson LM (2009) Clinical practice. Primary ovarian insufficiency.N Engl J Med 360: 606-614.
4. Blumenfeld Z. What Is the Best Regimen for Ovarian Stimulation of Poor Responders in ART/IVF? Front Endocrinol (Lausanne). 2020 Apr 17;11:192. doi: 10.3389/fendo.2020.00192.
5. Ho Y, Sh Yang YC, Chin YT, Chou SY, Chen YR, Shih YJ, Whang-Peng J, Changou CA, Liu HL, Lin SJ, Tang HY, Lin HY, Davis PJ. Resveratrol inhibits human leiomyoma cell proliferation via crosstalk between integrin αvβ3 and IGF-1R. Food Chem Toxicol. 2018;120:346-55.
6. Bezerra MÉS, Gouveia BB, Barberino RS, Menezes VG, Macedo TJS, Cavalcante AYP, Monte APO, Santos JMS, Matos MHT. Resveratrol promotes in vitro activation of ovine primordial follicles by reducing DNA damage and enhancing granulosa cell proliferation via phosphatidylinositol 3-kinase pathway. Reprod Domest Anim. 2018;53:1298-305.
7. Hao J, Tuck AR, Sjödin MOD, Lindberg J, Sand A, Niklasson B, Argyraki M, Hovatta O, Damdimopoulou P. Resveratrol supports and alpha-naphthoflavone disrupts growth of human ovarian follicles in an in vitro tissue culture model. Toxicol Appl Pharmacol. 2018;338:73-82.
8. Ochiai A, Kuroda K, Ozaki R, Ikemoto Y, Murakami K, Muter J, Matsumoto A, Itakura A, Brosens JJ, Takeda S. Resveratrol inhibits decidualization by accelerating downregulation of the CRABP2-RAR pathway in differentiating human endometrial stromal cells. Cell Death Dis 2019; 10:276.
9. Mestre Citrinovitz AC, Langer L, Strowitzki T, Germeyer A. Resveratrol enhances decidualization of human endometrial stromal cells. Reproduction. 2020 Apr;159(4):453-463.
10. Jiang Y, Zhang Z, Cha L, Li L, Zhu D, Fang Z, He Z, Huang J, Pan Z. Resveratrol Plays a Protective Role against Premature Ovarian Failure and Prompts Female Germline Stem Cell Survival. Int J Mol Sci. 2019 Jul 23;20(14):3605.
11. De Vos M, Devroey P, Fauser BC (2010) Primary ovarian insufficiency. Lancet 376: 911-921. 17.
12. Nelson LM, Covington SN, Rebar RW (2005) An update: spontaneous premature ovarian failure is not an early menopause. Fertil Steril 83: 1327-1332.