

Two Spontaneous Pregnancies after Treatment with Ovocyplus™ among an Infertile Patient with Two Failed IVF: A Case Report

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Abstract

The role of oxidative stress in female reproduction is becoming increasingly important, as recent evidence suggests that it has been implicated in the pathology of infertility of both known and idiopathic origin. Although its efficacy has yet to be well established, supplementation with antioxidants is a new tool being developed in the therapeutic armamentarium for female-factor infertility. We present a case of a spontaneous viable pregnancy in a 37-year-old patient with a history of two consequent *In Vitro* Fertilization (IVF) failures, occurring after a 5-month antioxidant treatment. This report suggests that oral antioxidants supplementation in female patients may provide an alternative or adjunct to conventional fertility therapies and improve their chances of becoming pregnant.

Keywords

Oxidative Stress, Antioxidant, Female Infertility, Pregnancy, IVF

1. Introduction

Infertility is a disease defined as “the inability to conceive following 12 or more months of unprotected and reg-

ularly timed sexual intercourse". Although the frequency and origin of different forms of infertility varies, 40% - 50% of cases of infertility studied are due to female causes including ovulatory disorders, poor egg quality, fallopian tube damage, endometriosis and unexplained infertility [1]. Recent studies have linked the prevalence of female infertility to an increase in oxidative stress (OS) levels, in the various critical micro- or macro-environments in the body [2]-[4]. The OS, a deleterious process that damages cell structures as well as lipids, proteins and DNA, is, by definition, an imbalance between the production of reactive oxygen species (ROS) and antioxidant defense systems [5]. The presence of ROS within the ovary, especially during the ovulation process and the endometrial has significant physiological and pathological implications in the female reproductive tract. It seems that physiological levels of ROS are required as modulators of a large spectrum of female reproductive pathways such as oocyte maturation, ovarian steroidogenesis, corpus luteum functions, ovulation and are involved in the processes of fertilization, embryo development and pregnancy [5] [6]. Conversely, abnormal increased ROS activity is negatively correlated with oocyte development, embryonic development and pregnancy outcome [5] [7]. Natural enzymatic antioxidants such as catalase, glutathione peroxidase and superoxide dismutase as well as nonenzymatic antioxidants (vitamin A, vitamin E, zinc and selenium) are essential in maintaining adequate levels of ROS in the cell by preventing and removing excess of free radicals [8] [9].

In an effort to enhance fertility, oral antioxidant supplementation may serve to prevent and alleviate ROS [10]. Thus, there is emerging enthusiasm in the use of natural or synthetic organic nutrients including vitamins, minerals and polyunsaturated fatty acids (PUFAs) that mimic antioxidant molecules. Some of the predominant antioxidants used in female subfertility are *N*-acetyl-cysteine; melatonin; vitamins A, C and E; folic acid; myo-inositol; zinc and selenium [11]. They may be administered as a single antioxidant or as combined therapy. Generally, antioxidant supplements are used in preparation for assisted reproductive techniques (ART) and/or simultaneously with the treatment, or alone with no ART in an attempt to improve natural fertility.

In this paper, we report a case of a spontaneous pregnancy in a woman occurred after a course of oral antioxidant supplementation (Ovocyplus™) with a review of the current literature highlighting the usefulness of antioxidant therapy to prevent and/or treat infertility and poor pregnancy outcomes.

2. Case Presentation

A 35-year-old woman has consulted our Reproduction Medicine Unit with a chief complaint of a 2-year primary infertility. Her surgical history included a fibro adenoma of the left breast at the age of 30. She was no smoker and had a body mass index of 26.3 BMI kg/m². Her menses were found to be irregular with a 28 - 32 day cycle. Her basal hormone parameters revealed that her follicle-stimulating hormone (FSH) levels was 4.9 mIU/mL, her luteinizing hormone (LH) level was 9.6 mIU/mL, her estradiol was 145 pg/mL and antimüllerian hormone (AMH) was 1.11 ng/mL. The patient had a normal prolactin and thyroid-stimulating hormone (TSH) levels. An ultrasound examination showed that she had normal ovaries and uterine cavity. A bilateral proximal tubal occlusion was diagnosed on an initial hysterosalpingography (HSG). An additional exploratory laparoscopy confirmed tubal obstruction and revealed the presence of mild peritoneal endometriosis.

Her 39-year-old male partner had had an operation for bilateral varicocele. His hormone parameters and semen analysis were normal. Both partners had a normal karyotype and were seronegative for hepatitis B and C as well as human immunodeficiency virus. No infection with *Chlamydia trachomatis* or syphilis was detected.

Two consecutive IVF and embryo transfer with 7-month interval were carried out under controlled ovarian hyper stimulation according to the long-GnRH agonist protocol. During the first IVF cycle, four oocytes were picked-up and inseminated with partner spermatozoa. Three oocytes were normally fertilized and progressed to good quality embryos which were transferred into the uterine cavity on day 2. In the second attempt, a total of three oocytes were retrieved, of which two good quality embryos were available on day 2 for transfer, without difficulty under abdominal ultrasound guidance. Unfortunately, none pregnancy was achieved.

Before being subjected to a subsequent IVF cycle, the patient was offered to be supplemented with an oral antioxidant therapy in the form of Ovocyplus™ (DCMG™ Laboratory, France) which has a balanced combination of micronutrients and developed specifically for sub fertile women (composition of double capsules/day: Ascorbic acid [vitamin C] 160 mg; vitamin E 24 mg; Zinc 15 mg; Copper 2 mg; Selenium 50 µg; Folates 200 µg, Vitamin B 62 mg; Vitamin B 12 2.5 µg; Iron 14 mg; Magnesium 400 mg; Manganese 3.5 mg; Vitamin D 2 µg).

Two months after the beginning of antioxidant intake, the patient returned to her obstetrician with a spontaneous pregnancy that subsequently ended in early miscarriage. The patient was advised to extend Ovocyplus™

medication. Three months later, she became pregnant with a single viable intrauterine pregnancy on obstetrical ultrasound examination appropriate for a gestational age at 7 weeks. Fortunately, she is currently at 29 weeks of an ongoing singleton gestation. Amniotic fluid volume, umbilical artery Doppler flow velocimetry, foetal growth, morphology scan findings and the maternal blood pressure were all within normal limits. No maternal or fetal complications were reported during the regular follow-up of the pregnancy.

3. Discussion

In this study, we have reported a case of two spontaneous pregnancies in a woman with mild endometriosis after Ovocyplus antioxidant supplementation: the first pregnancy ended in miscarriage and the current one is healthy maintained. Endometriosis has been associated with adverse influence on the processes of fertilization, embryogenesis and/or implantation [12]. Although ART has encompassed the management of almost all types of infertility, patients with endometriosis continue to pose difficulties in achieving pregnancy [13]. Recently, the role played by free radicals has attained central stage in the pathophysiology of endometriosis [14]. Accordingly, endometriosis subjects may have increased OS due to increased oxidant free radical production, compromise of the antioxidant defenses systems, or both [15] [16]. Although the OS status was not determined in this case, it is possible to speculate that a decrease in peritoneal and systemic OS markers is to be expected with increased vitamin/ mineral intake, enhancing thereby patient's fertility mainly implantation. Özkaya *et al.* [17] investigated the effects of multivitamin/mineral supplementation on the concentrations of various minerals and vitamins in serum and follicular fluid of women undergoing IVF. They observed that copper, zinc and selenium levels in serum and follicular fluid were lower in IVF patients than in controls but normalized by the multivitamin/mineral supplementation. Similarly, Mier-Cabrera *et al.* [18] conducted a randomized control trial (RCT) of the effect of a high antioxidant diet which revealed a significant increase in the concentrations of serum retinol, alpha tocopherol, leukocytes and plasma ascorbate after 2 months of treatment. Antioxidant supplementation was also observed to increase the activity of antioxidant enzymes (Superoxide dismutase and Glutathione peroxidase), while decreasing markers of OS such as malondialdehyde and lipid peroxides. The same results were assessed in a controlled, randomized, double-blind trial of a 6-month supplementation of vitamins C and E in women with stage I or II endometriosis suggesting a role for antioxidant supplementation in decreasing the levels of OS afflicting patients with endometriosis-associated infertility [19].

Our patient had been subjected to two unsuccessful IVF attempts despite adequate fertilization, cleavage and good embryo quality rates. Based on the understanding of the different effects of ROS on various stages of pre-implantatory and implantatory embryo growth, we reason that herein they may not affect fertilization and embryo development but rather implantation. The Ovocyplus supplementation provides a balanced combination of various well established antioxidant substances. Potentially these compounds could get to diverse district of reproductive tract and locally exert a valuable benefit in different phenotypes of infertile patients [20]. Therefore OvocyplusTM supplementation seems to be effective in quenching excessive generation of free radicals in the endometrium and plays a contributory role in pregnancy maintenance. Indeed, a growing body of evidence suggests that the redox state has a critical role in modulating implantation, implicating blastocyst/placental OS in the pathogenesis of spontaneous abortions, idiopathic recurrent pregnancy loss and fetal embryopathies [21]-[23]. Velthut *et al.* [24] demonstrated that achievement of IVF/ICSI clinical pregnancy was favored by elevated systemic total antioxidant status suggesting that a link between systemic antioxidant defense and endometrial receptivity. The main actors as first line of enzymatic defenses include Copper-Zinc superoxide dismutase (Cu-Zn-SOD), manganese-superoxide dismutase (Mn-SOD) [25] and selenium-dependent glutathione peroxidase (GSH-Px) [26]-[28]. Secondary antioxidants such as vitamins A, C and E may play a role in compensating for the oxidative burst during early pregnancy since their deficiency was be involved in the pathogenesis of recurrent pregnancy loss [19] [28] [29]. Moreover, vitamin B and folates are important for oocyte quality, implantation, placentation and fetal growth [30]. The aforesaid antioxidant vitamins and minerals are contained in the OvocyplusTM medication used in the current study. Hence, this supplement may have a beneficial effect on the endometrial environment and pregnancy outcome through its constituents. OvocyplusTM also has essential trace minerals such as iron and magnesium well-known to be important in cell division and neural-tube development.

In spite of the perceived hypotheses regarding the benefits of antioxidant supplementation on pregnancy outcomes, clinical trials in this area reported largely conflicting results, with most agreeing that these therapies result in only partial success.

Our observation is in agreement with several groups that have assessed the ability of antioxidants to improve considerably the female fertility. A prospective RCT assessing the impact of Vitamin E administration on infertile women undergoing controlled ovulation induction and intrauterine insemination (IUI) cycles found that Vitamin E had beneficial effects on improving the endometrial thickness. Another important result of this study was the higher implantation and ongoing pregnancy rates observed in the Vitamin E-administered group, even though the differences were not significant. These improvements may be a result of the improving antioxidant and anticoagulant effects of Vitamin E on the endometrial receptivity [31]. Other studies reported higher pregnancy rates in patients undergoing IVF-embryo transfer supplemented with vitamin C [32] and in women with endometriosis supplemented with Vitamins C and E [19] though not reaching significance, possibly because the study had so few participants that it failed to statistically demonstrate the observed benefits of taking supplements. The authors postulated that this unchanged pregnancy rate may be explained by an insufficient intake of vitamins C and E or an insufficient presence of other antioxidants such as vitamin A, selenium, zinc, manganese and copper [19]. Considering that each antioxidant product makes a unique contribution to preventing harmful effects of OS, it is postulated that an ideal combination of antioxidants, such as ovocypus formulation, might ensure protection against ROS-induced damage and provide a synergistic impact to support human reproductive health. Westphal *et al.* [33] studied the impact of (Foetpro-F) a nutritional supplementation formula containing chasteberry and green tea extracts, chasteberry, L-arginine; vitamins E, B6, B12 and folate; iron; magnesium; zinc and selenium, on the reproductive health of women who had unsuccessfully attempted to become pregnant for 6 to 36 months. They found that after 5 months, 33% of the women in the supplementation group were pregnant and none in the placebo group. They concluded that nutritional supplementation may play an important role in fertility increasing mean midluteal phase progesterone levels and number of days with basal temperatures $>37^{\circ}\text{C}$.

In 2012, another prospective RCT [34] demonstrated that in women undergoing ovulation induction for anovulatory or unexplained subfertility using standard ovulation-induction regimens and who were on multiple micronutrient nutritional (MMN) supplementations as an adjuvant therapy had significantly higher ongoing pregnancy and cumulative clinical pregnancy rates compared with those who were on folic acid alone. Further, women on MMN supplementation achieved pregnancy in significantly fewer attempts compared with control group. Ruder *et al.* [35] corroborated that female antioxidant intake and OS may influence the timing and the maintenance of a viable pregnancy. They observed that higher intakes of antioxidants b-carotene, vitamin C and vitamin E were associated with a shorter time to conception among couples being treated for unexplained infertility, but this effect varies with BMI and age. Our report, as well of those of Westphal *et al.*, Mier-Cabrera *et al.*, Agrawal *et al.* and Ruder *et al.*, demonstrated that women who took antioxidant supplementation had a higher chance of pregnancy.

On the other hand, there are some trials that failed to report this beneficial effect of oral antioxidant supplementation in the treatment of female infertility. In a study by Youssef *et al.* [36], oral antioxidants in the form of a combination of multivitamins and minerals did not improve oocyte quality and pregnancy rates in women with unexplained infertility undergoing IVF/ICSI treatment. The lack of significant differences between the study and control groups could be explained by a lack of follicular fluid antioxidants during *in vitro* fertilization that may disturb the oxidant-antioxidant balance, rendering the culture media less protected against oxidation. Furthermore, ROS may develop in an IVF culture media as a consequence of oocyte metabolism, increased oocyte number per dish, long incubation time with spermatozoa used for insemination (150×10^3 - 200×10^3 per dish), visible light and metallic cations acting as exogenous sources of OS [37]. Another limitation of this study was short duration of antioxidant treatment (2 and half months) and follow-up.

In 2013, the Cochrane Collaboration reviewed antioxidant use in subfertile women [11]. This review included 28 randomized controlled trials that compared antioxidants with placebo or no treatment/standard treatment, or with another antioxidant in a total of 3548 women. Antioxidants were not found to be effective for increasing rates of live birth or clinical pregnancy. The quality of the evidence in this review for live birth, clinical pregnancy and adverse effects was rated “very low” to “low” limited because of the lack of large randomized controlled studies and the heterogeneity between them regarding indications for subfertility and types of administered antioxidants.

Concerning the occurrence of spontaneous intrauterine pregnancies in our patient regardless of bilateral tubal occlusion detected at laparoscopy, we support the opinion that this technique should not be considered the “gold” standard in the diagnosis of tubal infertility. The false appearance of proximal tube occlusion may be actually

related to technical problems: insufficient anesthesia, insufficient injection of a blue dye, or quick injection of the dye causing the cornual spasm [38]. Anyway, it's worth taking into account that a diagnostic test for tubal patency could in itself be responsible for fertility enhancement. Indeed, a Cochrane review meta-analysis found some evidence that tubal flushing can increase pregnancy rates which were significantly higher with oil-soluble media than water-soluble media [39]. Theoretical mechanisms for this finding include an effect inside the pelvic cavity near the ovary that influences the environment in which eggs grow, or an improvement of endometrial receptivity after exposure to contrast agents. Since tubal testing with either HSG or laparoscopy was performed early as part of the work-up for infertility in this patient, it seems unlikely that spontaneous pregnancy with a 26-month delay to be associated to the potential therapeutic effects of these procedures, at least at the level of the endometrium.

An important limitation of our report is that the OS has not been determined in the peripheral blood and/or the peritoneal fluid, before and after the treatment, to assess its capacity to reduce oxidative stress markers and improve the chances for natural conception. In addition, inflammatory markers were not measured either in serum or in reproductive tract since OS is known to induce a local inflammation resulting in elevated levels of cytokines and other factors that promote endometriosis.

4. Conclusions

Current evidence supports the use of oral supplementation with antioxidants to overcome OS and boost the exhausted antioxidant defense of the female reproductive microenvironment. This approach seems especially plausible in light of the fact that oral antioxidant treatment in males has been proven to be effective to treat male infertility and is widely employed in current clinical practice.

In this case of woman with endometriosis, the establishment of a successful pregnancy is consistent with the hypothesis that a 5-month antioxidant intake is positively associated with changes in endometrial receptivity. Accordingly, such therapy could be suggested as an alternative or complement to conventional fertility therapies with the aim to enhance both natural and assisted conception.

Nevertheless, future work should include prospective randomized, controlled studies within a larger population to confirm these preliminary data in order to ascertain the potential efficacy and safety of that medication in the improvement of female fertility.

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Conflict of Interests

The authors has declared that no conflict of interest exists

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