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Annals of Reproductive Medicine and Treatment

Case Report

Oxigen-Ozonetherapy Treatment Combined with Gonadotropin-Releasing Hormone Agonist in Endometriosis and IVF: Successful Pregnancy in a Case Series

Giorgia Scuderi, Giulia Giglione, Aikaterini Selntigia*, Mauro Cozzolino and Daniela Galliano

IVIRMA Global Research Alliance, IVIRMA Roma, Italy

***Corresponding author**

Aikaterini Selntigia, IVI RMA Rome, 00169 Rome, Italy, Tel: +393667465199

Submitted: 20 August 2024

Accepted: 08 September 2024

Published: 10 September 2024

ISSN: 2573-1092

OPEN ACCESS

Keywords

- Oxigen-Ozonetherapy
- Endometriosis
- GnRHa
- IVF
- Pregnancy

Abstract

Nowadays, oxygen-ozonetherapy is used in many chronic inflammatory diseases, especially in orthopedics, neurology, and infectious diseases. Recent studies have confirmed that oxygen-ozonetherapy could be used in urology and gynecology to treat inflammatory conditions and infections. We intend to understand if oxigen-ozone systemic therapy applied to In Vitro Fertilization (IVF) could improve pregnancy outcomes. We present three cases of long-term primary infertility with previous implantation failures suffering from endometriosis or adenomyosis. Before the embryo-transfer, the patients were treated with a 2/3-month course of Gonadotropin-Releasing Hormone Agonist (GnRHa) combined with 3-4 sessions of oxygen-ozone systemic therapy. We combined a mixture of oxygen and ozone, with ozone concentrations ranging from 30µg to 45µg, increasing the dosage at each session. The oxygen-ozonetherapy session started during the hormonal endometrial preparation and stopped 1-7 days before the embryo-transfer. All the patients were also treated with intravenous vitamins. In all our patients, we obtained a positive blood β- Human Chorionic Gonadotropin (hCG) test and an ongoing pregnancy.

INTRODUCTION

The properties of ozone in the medical field have been widely known, although they are still limited to only a few areas. The oxygen-ozone mixture can be administered locally or systemically depending on the specific case. The systemic pathway (oxigen-ozone systemic therapy) makes it possible to act on all tissues of the body, stimulating a beneficial response. To date, oxygen-ozone therapy is mainly used in orthopedics for the treatment of spinal pathologies [1], musculoskeletal disorders [2] and arthrosis [3]. It's use also in the treatment of COVID-19 [4], skin diseases [5], sepsis [6] and dental disorders [7,8]. Recent studies are controversial regarding the gynecological field. Many studies demonstrate a correlation between the use of local ozone and the resolution of genital tract infections [9,10], yielding better results than conventional therapies and reducing relapses. Ozone possesses many beneficial characteristics. Bactericidal, fungicidal, and virustatic effects [9]: ozone, with its high oxidation potential, can oxidize the essential cellular components of pathogens, deactivating them, as well as inhibiting the ability of virus to adhere to receptors. Analgesic and anti-inflammatory effects [11]: ozone inactivates halogen substances and their receptors, increases the expression of antinociceptive genes [12] and stimulates the antioxidant response. Ozone plays also an immune-stimulating role, regulating the immune response, reducing the production and activation of cytokines and interleukins [13]. Moreover, ozone has an impact on the microcirculation system, reducing capillary congestion, interstitial edema, and increasing arterial flow [14,15]. On the other hand, endometriosis and adenomyosis are chronic hormone-dependent inflammatory diseases with a negative impact on Assisted Reproduction Technique (ART) outcomes [16]. In the literature, there are study evaluating the effect of ozone sauna therapy on the IVF outcome, demonstrating that its known characteristics as vasodilatory, anti-inflammatory, and antioxidant could enhance endometrial receptivity and increase the number of formed embryos without increasing the number of oocytes retrieved, suggesting an improvement in oocyte quality [17,18].

We highlight some cases of women suffering from deep infiltrating endometriosis, adenomyosis and infertility with implantation failure, on treatment with oxigen-ozone systemic therapy in combination with Gonadotropin-Releasing Hormone Agonist (GnRHa) and its impact on implantation rate and ognoing pregnancy.

CASE-REPORT 1

A 40-year-old patient with focal adenomyosis and severe Poor

Ovarian Reserve (POR) underwent IVF using donor oocytes. In her clinical story was described a positivity of Lupus Anticoagulant, an hysteroscopic removal of a myoma, a non-smoker with normal Body Mass Index (BMI), and having regular menstrual cycles. She presented with her partner on February 2022 to our clinic, after a 6-year of IVF course with previous homologous treatments and 4 embryos-transfers with negative results. The male partner was healthy, with normal semen analysis parameters. From the donation cycle 5 blastocysts were obtained. During the embryo-transfer preparation process, the patient underwent endometrial evaluation tests, which provided results of endometrial receptivity and pathogenic dysbiosis, respectively, treated with antibiotics. After 3 cycles of GnRHa (Triptoreline 3,75 mcg intramuscular every 30 days), the first embryo-transfer was performed under the Hormone Replacement Therapy (HRT) cycle for endometrial preparation. The first HRT cycle consisted of the administration of estrogens (Valerate estradiol 8 mg every day), and subsequent performance after seven days of ultrasound to evaluate the endometrial thickness (8 mm). According to the endometrial receptivity, progesterone was administrated 5 days (subcutaneous progesterone 25 mg every 12h). Furthermore, the patient was on treatment with enoxaparin 4000 mg/UI and acetylsalicylic acid 100 mg, metformine, prednisone 7.5 mg, folic acid but yielded unsuccessful results. The second attempt started after 3 months of GnRHa. The HRT cycle consisted on the administration of estrogens (Valerate estradiol 8 mg every day), and subsequent performance after eleven days of ultrasound to evaluate the endometrial thickness (8.5 mm). According to the endometrial receptivity, progesterone was administrated 5 days before the embryo-transfer (subcutaneous progesterone 25 mg every 12h). Furthermore, the patient was on treatment with enoxaparine 4000 mg/UI, acetylsalicylic acid 100 mg, prednisone 7.5 mg, metformine, folic acid associated with oxigen-ozone systemic therapy, simultaneously. The oxygen-ozone therapy consisted on 3 sessions (1 session every 7-10 days) with ozone concentrations ranging from 40µg to 45µg, associated with intravenous vitamins (vit c, folic acid, glutatione, L-carnitine, cianocobalamine, n-acetilcisteina, MgSo4, nicotinamide, ascorbic acid, dexpantenol, trivalent iron), stopped one day before the embryo-transfer. This attempt with the combined therapy (GnRHa and oxigen-ozone systemic therapy) resulted successful with an ongoing pregnancy without complications. The patient underwent a cesarean section for induction failure.

CASE-REPORT 2

A 39-year-old patient with ovarian endometriosis, adenomyosis and POR presented with her partner to our clinic on February 2022 with the desire to conceive using donor oocytes. They had a 4-year primary history of infertility and underwent four homologous Intra Cytoplasmic Sperm Injection (ICSI) treatments and 6 unsuccessful embryo-transfers in other clinics. She was on treatment with Levothyroxine 50 mg, smoking 1-2 cigarettes per day, normal BMI and regular menstrual cycles. The male partner was healthy, with normal semen analysis parameters.

The egg donation treatment in our clinic produced 4 blastocysts. After 2 cycles of GnRHa (Triptoreline 3,75mcg intramuscular every 30 days), the first embryo-transfer was performed on an HRT cycle for endometrial preparation with estrogens (Valerate estradiol 8 mg every day), subcutaneous progesterone 5 days before the embryo-transfer (25 mg every 12h) subsequent performance after ten days of ultrasound to evaluate the endometrial thickness (10 mm), folic acid and Acetylsalicylic acid 160 mg from the transfer, but yielded unsuccessful results. After that, the patient underwent Microbiome Expanded Typing Analysis (ES-META) which uses the qPCR technique to analyze the hypervariable regions of the gene that encodes the 16S and 18S subunit of ribosomal RNA, as well as specific regions of non-bacterial microorganisms, to analyze the endometrial microbioma resulted in non-pathogenic dysbiosis and treated with probiotics. Moreover, the couple underwent immunological exams and immunological mismatching of the HLA-KIR complex emerged. She started an HRT cycle for endometrial preparation (similar at the previous one), combining immunological therapy and oxigen-ozone systemic therapy. The second HRT cycle consists on the administration of estrogens (Valerate estradiol 8mg every day), subsequent performance after fifteen days of ultrasound to evaluate the endometrial thickness (9mm). Progesterone (subcutaneous progesterone 25 mg every 12h) was administrated 5 days before the embryo-transfer; the other therapy was desametasone 1 mg/day for the first 5 days of HRT and than prednisone 7.5 mg every day, filgrastim 13 mUI every 72h, Acetylsalicylic acid 160 mg, Metformin 500mg/day, folic acid and lactobacilli. The oxygen-ozone therapy consisted of 4 sessions with ozone concentrations ranging from 30 µg to 45 µg, associated with intravenous vitamins (vit c, folic acid , glutatione, L-carnitine, cianocobalamine, n-acetilcisteina, MgSo4, nicotinamide, ascorbic acid, dexpantenol, trivalent iron), and stopped seven days before the embryo-transfer. This attempt was successful, confirmed by a positive β-Human Chorionic Gonadotropin (β-hCG) with an ongoing pregnancy without complications. The patient underwent an inducted vaginal delivery.

CASE-REPORT 3

A 47-year-old patient with ovarian endometriosis and POR presented with her partner to our clinic on June 2022, intending to undergo IVF with their own gametes. The male partner had oligoasthenospermic semen. In her clinical story was described Hashimoto's thyroiditis in therapy with levotiroxine, an hysteroscopic removal of a myoma, non-smoker, normal BMI, and regular menstrual cycles. They had a 4-year primary history of infertility with the following treatments in other clinics: 3 homologous ICSI, a platelet-rich plasma ovarian treatment, and another homologous ICSI treatment; however, no transfers were performed as no embryos were formed. In our clinic, she underwent attempted another ovarian stimulation obtaining 3 mature oocytes but no blastocysts. After this failure, a treatment with donor oocytes was performed. The patient underwent hysteroscopy to remove a submucous fibroid and a second-look hysteroscopic control with hyaluronic acid treatment. The

first attempt in failed despite using 2 previous doses of GnRHa (Triptoreline 3,75 mcg intramuscular every 30 days). An HRT endometrial preparation was performed through administration of estrogens (hemihydrates estradiol patches 200 mg/48h), subsequent performance after sexteen days of ultrasound to evaluate the endometrial thickness (9 mm) and progesterone (subcutaneous injection 25 mg every 12h) was administrated per 5 days before the embryo-transfer. The patient during the preparation was on empirical therapy with Prednisone 10 mg/day, folic acid, Acetylsalicylic acid 160 mg from the embryo-trasfer. The second attempt started with 2 doses of GnRHa (Triptoreline 3,75 mcg intramuscular every 30 days) and then another equal HRT cycle; after the ultrasound to evaluate the endometrial thickness (8 mm) the patient starts progesterone (subcutaneous injection 25 mg every 12h) 5 days before the embryo-transfer. The patient during the preparation was on empirical therapy with Prednisone 10 mg/day, folic acid, and Acetylsalicylic acid 160 mg from the embryo-trasfer and oxigen-ozone systemic therapy. The oxygen-ozone therapy consisted in 4 sessions with ozone concentrations ranging from 35 µg to 45 µg, associated with intravenous vitamins (vit c, folic acid , glutatione, L-carnitine, cianocobalamine, n-acetilcisteina, MgSo4, nicotinamide, ascorbic acid, dexpantenol, trivalent iron), and stopped seven days before embryo-transfer. This attempt was successful, confirmed by a positive β-hCG blood test. Pregnancy is ongoing without complication. The patient underwent a cesarean section for induction failure.

DISCUSSION

The prevalence of adenomyosis in association with endometriosis in infertile women is up to 27–79%, contributing to its increased presence in ART centers [16]. In particular, recent studies have shown a correlation between the presence of adenomyosis and miscarriage [19]. There are several hypothetical mechanisms associated with adenomyosis-related infertility, including dysregulation of myometrial architecture and function, chronic inflammation, the presence of local oxygen, and altered endometrial function, all of which can contribute to implantation failure [20]. Despite numerous studies attempting to elucidate the relationship between adenomyosis and infertility, definitive conclusions have yet to be reached [21].

On the other hand, ozone therapy is a medical treatment that employs a mixture of oxygen and ozone as a therapeutic agent to address a diverse spectrum of diseases. The rationale for its application is grounded in the concept that low concentrations of ozone can exert a significant impact on cellular functions, and numerous demonstrated mechanisms of action validate this clinical evidence [22]. For medical applications, the Oxygen-Ozone mixture is generated in concentration using specialized equipment certified in compliance with established standards (EC directives). The fundamental principle of the generator is to convert a portion of the incoming oxygen into medical ozone in quantities that permit variable concentration dosing. The machine, mandatory to be equipped with a photometer, comprises generators connected to an electronically controlled high-voltage transformer for generating adequate voltage. The supplied energy facilitates the cleavage and recombination of the O_2 molecule, forming a gaseous mixture of O_2O_3 whose ratio varies based on the applied voltage and incoming O_2 flow. The incoming oxygen must be pure and delivered through medical cylinders or a centralized system. The administration and usage routes, as described below, have all been appropriately tested, with no recorded adverse effects using the reported dosages within the therapeutic range. This range aligns with guidelines provided by the Ozone Therapy International Library ISCO3, Levels of Evidence Working Group, OCEBM, Review on Evidence-Based Ozone Therapy, WFOT'S, and incorporates the insights of Dr. V. Bocci in the book "Ozone: A New Medical Drug."

Specifically, 150 ml of venous blood is collected in ozone-resistant plastic bags equipped with anticoagulants, following European Union regulations. Subsequently, it is mixed with an ozone concentration ranging, preferably, between 30 ug/mL and 45 ug/mL. This blend is promptly re-administered into the patient's vein without interrupting the circuit [23]. The limited availability of human studies investigating the impact of oxigen-ozone systemic therapy on gynecological and reproductive disorders is notable. An examination of existing research highlights a study on tubal recanalization utilizing catheter-mediated pressure injections of ozone, revealing significantly higher rates of postoperative tubal recanalization and pregnancy after 12 months in the ozone-treated group compared to controls [24]. Another preliminary study, involving 12 healthy ovulating women, demonstrated that intrauterine ozonated saline infusion led to a statistically significant increase in columnar epithelial cell height, an augmentation in the number of endometrial blood vessels, and an elevation in the number of stromal cells in the endometrium [25]. Moreover, a case series reported that 2 out of 3 infertile patients with an extremely thin endometrial lining and multiple failed in vitro fertilization cycles achieved pregnancy after IVF following treatment with ozone and Pulsed Electromagnetic Field (PEMF) therapy [26]. The treatment, facilitated by the Hyperthermic Ozone & Carbonic Acid Transdermal Therapy (HOCATT) machine, notably improved Endometrial Lining Thickness (EMT) [27]. Another study underlined the connection between local ozone therapy and IVF treatment outcomes [28].

These case reports aim to highlight not only the effects of oxygen-ozone therapy, validated by the consensus of the scientific community on various functions and processes of the organism, such as anti-inflammatory activity (reduction of TNF-alpha levels) and antioxidant activity (activation of antioxidant enzymes). In particular, our case series elucidates the modes of action through which oxygen therapy could improve the outcome of implantation. Three women with a clinical story of infertility, endometriosis, adenomyosis, and implantation failure treated with the combination of GnRH-a long protocol and oxigen-ozone systemic therapy had a positive outcome in terms of pregnancy after a single embryo-transfer. In conclusion, these cases are the first to assess the effect of oxigen-ozone systemic therapy in patients with endometriosis undergoing IVF. These results could help discover a supportive therapy for women with severe

endometriosis who had repeated failed IVF cycles. More research is needed in this field through well-designed large cohort studies and even randomized trials better to assess the efficacy of oxigen-ozone systemic therapy in Assisted Reproductive Technology.

ACKNOWLEDGMENT

GS, GG, AS and DG contributed to the conception and designed the manuscript. MC contributed to the diagnosis and indication for the treatment. GS and GG wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

Conflicts of Interest: The authors declare no conflict of interest.

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