

H.A.R.O.T. Human assisted reproduction ozone therapy: the use of Oxygen-Ozone therapy as an adjunct in the therapy of couple sterility

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Abstract

The increase in couple sterility and the average increase in the age of women seeking pregnancy has placed the scientific community in front of the need to improve the quality of oocytes and the percentage of the embryonic implantation. The oxygen- ozone therapy seems to be able to help in this research thanks to its modes of action. A therapeutic protocol, identified with the acronym H.A.R.O.T (Human assisted reproduction ozone therapy) has been developed, the first results of which seem to be considerably encouraging in order to obtain a greater number and quality of oocytes and improve the percentage of embryonic implantation.

Introduction

Sterility is commonly defined as the absence of conception after 12 months of unprotected sexual intercourse.

With the term *infertility* we mean said absence in couples who already procreated.

However, this definition should not be intended absolutely, in fact in the definition of *sterile couple* to be initiated to the diag-

nostic-therapeutic process also intervene important factors, such as for example: *age, abnormal lifestyle, medical history* that may induce to anticipate or delay the therapeutic approach.

The oxygen-ozone therapy is a therapeutic procedure that uses a mixture of gases: oxygen and ozone.

Its first use dates back to 1915, then it has been studied and tested all over the world. In Italy its official use dates back to 1983.

Scientific studies show that this is the therapeutic method with less risks of side effects (Jacobs 1981, 0.0007% side effects). The only side effects are cough in case of accidental inhalation and hypotension in case of bad practice.

Precautions for safe operation: i) right administration route; ii) use of proper equipment; iii) use by experienced physicians, qualified by the *Società Scientifica di Ossigeno-Ozono Terapia* (SIOOT); iv) no inhalation.

The only contraindications are: i) hyperthyroidism; ii) favism; iii) pregnancy in progress;

Administration routes are multiple and depend on the indication, we distinguish between: i) big blood auto-infusion intravenously; ii) anal insufflation; iii) vaginal-uterotubal insufflation; iv) small blood infusion intramuscularly.

Couple sterility is a continuously increasing disease; today more and more couples address to *in vitro* fertilization (IVF) techniques to solve their problem.

ISTAT (Italian National Statistics Institute) data show that about 35% of couples are affected by this disease; the numbers are constantly increasing so as to define this pathology as a real social disease.

The causes of this increase are to be found in the increasingly advanced age of women in search of their first child, in the environmental pollution factors that affect the quality of human gametes, and the presence of some individuals who in the past did not reach the age to procreate due to diseases that today can instead be solved thanks to the progress of medicine.

Since July 25, 1978, when Louise Brown was born thanks to the first IVF performed by Robert Edwards, so much progress has been made in the execution of these methods.

The two key points of this method that are capable of positively influencing the outcome are the quality of the oocytes, and the understanding and improvement of embryonic implantation, as arises from the most recent congresses and debates between operators.¹⁻⁵³

Object

The study object is to demonstrate how the oxygen-ozone therapy applied to IVF can help the treatment by positively influ-

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encing the quality of oocytes and the mechanisms of embryonic implantation.

Rationale

The study rationale is based on the effects of oxygen-ozone therapy, validated by the consensus of the scientific community (Viebahn, 1994; Richelmi *et al.*, 2001; Fabris, 2009), on some functions and processes of the organism, as follows: i) *anti-inflammatory activity*: reduction of TNF-alpha levels; ii) *anti-oxidant activity*: it activates anti-oxidant enzymes (catalase, superoxide dismutase, glutathione), particularly at mitochondrial level, free radicals' reduction; iii) *neoangiogenesis induction*: improvement and reactivation of blood circulation and microcirculation; iv) *rising of O₂ release in tissues by haemoglobin*, by rising 2,3DPG (diphosphoglycerate) production, rising of red blood cells deformability while improving their pass inside small capillaries, viscosity reduction; v) *dysbiosis normalizing activity*; vi) *rising of energy production (ATP)* by accelerating fat acids catabolism, glycolysis acceleration; vii) *immunomodulatory activity*, by acting on lymphocytes and monocytes, lowering of circulating immunocomplexes in autoimmune diseases; viii) *it improves the functionality of emune organs*, by promoting the elimination of metals and toxic substances.

But also disclosing the modes of action with which the oxygen therapy could improve the embryonic implantation and the quality of the oocytes, as follows: i) improvement of subendometrial microcirculation; ii) release of growth factors with triggering of tissue regeneration mechanisms; iii) improvement of tissue oxygenation; iv) reduction of TNF- α and increase of interleukin 10; v) endometrial inflammation reduction; vi) improvement of the vaginal uterine and intestinal microbiota; vii) improvement of perifollicular microcirculation; viii) release of growth factors with triggering of regeneration mechanisms; ix) reduction of free radicals' concentration in the follicular fluid; x) better O₂ concentration in the follicular fluid; xi) increased activity of oocyte mitochondria; xii) slowing down the reduction of telomere bases; xiii) reduction of heavy metals concentration in the follicular fluid; xiv) reduction of biogenic amines by promoting the elimination of dysbiosis.

Materials and Methods

In order to demonstrate the object of said study, we recruited: i) 500 couples with primary sterility; ii) 100 couples owning 2 vitrified embryos.

The 500 couples were divided into 2 homogeneous groups taking into account the age and the cause of sterility

Group A and Group B

It should be noted that in order to make the division more homogeneous in the two groups, 150 couples of group B are the same who performed the same procedure a few months earlier; these couples have been identified as subgroups B1 and A1.

The age of the recruited women was between 30 and 46 years. Only couples affected by the following causes were considered:

pathological semen (sperm count between 1,000,000 and 7,000,000 sperm cells), tubal pathology or bilateral imperviousness and 3rd degree endometriosis.

The 100 couples with at least 2 vitrified embryos were also divided into two groups - Group C and Group D. In dividing the couples, the age of the patients, the quality of the vitrified embryos and the stage of evolution (embryos in 3rd day and blastocysts) were taken into account.

Women belonging to the group of 500 couples have all been subjected to: i) diagnostic hysteroscopy to assess the uterine cavity; ii) stimulation protocols with follitropin alpha 300 IU per day for women under the age of 35 and Menotropin 300 IU for women over the age of 35 and for women with endometriosis; iii) post-transfer supplementation therapy with progesterone 100 mg, acetylsalicylic acid 100 mg and prednisone 5 mg and Nadroparin Calcium 2850 IU.

The women of group B underwent 3 LBI (large blood infusion): i) before ovarian stimulation (about one week); ii) in the middle of the stimulation (follicles between 12 and 14 mm); iii) at embryo transfer.

Women belonging to the group of 100 couples with vitrified embryos have all been subjected to: i) diagnostic hysteroscopy to assess the uterine cavity; ii) endometrium preparation with estradiol valerate 8 mg/day associated to progesterone 100 mg, when the proliferative endometrium exceeded 8 mm in thickness; iii) post-transfer supplementation therapy with progesterone 100 mg, acetylsalicylic acid 100 mg and prednisone 5 mg and Nadroparin Calcium 2850 IU.

The women of group D underwent 3 LBI (large blood infusion): i) a few days before the start of endometrial preparation; ii) associated with progesterone (24 h before or after); iii) at transfer.

The parameters used to highlight the differences between group A and B induced by oxygen-ozone therapy are the following: i) dosage of 17 β -estradiol on the day of induction; ii) number of mature follicles; iii) number of oocytes; iv) oocytes quality; v) fertilization index; vi) sO₂, pO₂; vii) number of embryos; viii) embryos quality; ix) embryonic implantation percentage.

The parameters used to highlight the differences between group C and D induced by oxygen-ozone therapy are the following: i) endometrial size at the time of association with progestin and transfer; ii) sO₂ and pO₂ values at transfer; iii) implantation percentage.

In order to carry out this study, we used the IVF Chianciano Salute laboratory and the TOMA SUD clinical analysis laboratory. We used the following instruments: i) MEDICAL99IR Portable Unit for Oxygen Ozone Therapy by Multiossigen; ii) blood POC analyzer by i-STAT Abbot; iii) Samsung A30 and Samsung mysono U6 ultrasound machine.

Results

The results of the IVF techniques of couples A and B and of the transfers of couples of group C and D are shown in Tables 1 and 2.

Table 1. The parameters used Group B.

Evaluated parameters	Group B
Dosage of 17 beta-estradiol on the day of induction (pg/mL)	+++
Number of mature follicles (>16 mm)	+++
Number of oocytes	++
Oocytes quality (1 st -2 nd -3 rd -4 th)	+
Fertilization index	++
sO ₂ (%), pO ₂ (mmHg)	+++
Number of embryos	++
Embryos quality (1 st -2 nd -3 rd -4 th class)	+
Embryonic implantation percentage	16% (12% B-B1)

With the + sign we meant to indicate a percentage increase between 1 and 10%, with the ++ sign an increase from 11 to 15%, with the +++ sign the increase of more than 16%. It should be noted that the maximum percentage increase was 24% of implantation with transfer of vitrified embryos. The analysis of the results shows an increase in all parameters, *i.e.* a better condition of the uterus at the implantation; in fact, we observed a better perfusion of the uterus and a greater growth of the endometrium. The response of the ovary also has been satisfactory; a greater production of hormones and follicles has been observed, but at the same time the number and quality of oocytes has not gone hand-in-hand, even if they provided higher values. The good percentage of embryos implantation is linked to the greater number and quality, but above all to the better conditions of the uterus.

Conclusions

Our observations show that the oxygen-ozone therapy is certainly an adjuvant therapy in IVF treatments. The feeling is that by selecting the best patients we can obtain even more satisfactory results; in fact, even if not reported in the tables for comparisons, the best results were obtained by comparing in the two groups particularly the various parameters in the most advanced age groups and in cases of sterility with underlying atrophic-inflammatory processes.

For this reason, further studies and observations are underway and procedures are being developed with topical application at the level of the uterus and ovary.

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Table 2. The parameters used Group C.

Evaluated parameters	Group C
Endometrial size at the time of association with progestin and transfer	++
sO ₂ and pO ₂ values at transfer	+++
Implantation percentage	24%

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