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No Benefit of Reduced Oxygen Level for the Culture of Human Embryos *In Vitro*

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Abstract

Introduction: Effects of low oxygen versus atmospheric oxygen have been the subject of many studies. Oxygen concentration is suggested to be correlated with reactive oxygen species production hence effect early embryo development.

Aim: The aim of our prospective randomized study was to investigate the effects of different oxygen concentrations (5% versus atmospheric oxygen) on the ICSI outcome parameters including fertilization, early embryo development, embryo quality, and pregnancy and implantation outcomes.

Materials and Methods: This is a prospective randomized trial that included 2682 couples who attend for ICSI treatment in IVF center of Medicana Camlica Hospital. Embryos were incubated either in a low O_2 concentration (5%) or ambient atmosphere concentration (~21%). The outcome parameters were analyzed and compared between two groups.

Results: None of the ICSI outcome parameters were affected significantly from the oxygen concentration although all parameters especially the top quality embryo rate was increased in 5% oxygen concentration.

Conclusion: It may be concluded that it is the metabolic state of an embryo that decide to use the oxygen, not the oxygen concentration it is exposed to.

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Introduction

The oxygen concentration in the female reproductive tract of numerous species is much lower than the atmospheric level. Fertilized oocytes of hamsters, rabbits and rhesus monkeys are exposed to approximately 8% oxygen concentration *in vivo* [1,2]. However, during *In-Vitro* Fertilization (IVF) treatments, the embryos were exposed to ambient oxygen concentration which is approximately 20%.

Although oxygen is an essential molecule for the survival and development of the embryo, reactive oxygen species such as Hydrogen Peroxide (H_2O_2), Hydroxil Radical (OH) and superoxide anion radical (O_2-) are generated during the oxygen metabolism and ATP synthesis [3]. Those molecules in physiological amounts are used for the normal physiological functions of the oocyte and embryo while the remaining is scavenged by the antioxidative defence systems of the embryo and surroundings [3-5]. However, ROS in excessive amounts may damage the DNA, lipid and protein components of the cell, and the mitochondria [6]. Those effects are manifested by low fertilization rates, early embryonic developmental block and retardation, high fragmentation rates and development of morphologically abnormal blastocysts [7,3]. Since ART culture media may be an exogenous source of excessive ROS generation because of the unphysiological level of oxygen around the embryo *in vitro*, oxygen controlled incubators had started to be widely used in order to annihilate the detrimental effects of high oxygen concentration on the embryos [8].

The results of several animal and human studies comparing 5% versus atmospheric oxygen concentration, document the beneficial effects of low oxygen tension on early embryo development [6,9-13] . Those effects include improved embryo development, increased proportion of blastocyst formation and increased number of total cells, ICM cells and trophectoderm cells within the blastocyst [14]. However some others reported no beneficial effect of decreased oxygen rates. On a systematic review and meta-analysis, Nastri *et al.* [15] reported similar results with a small

improvement (~5%) in live birth/ongoing pregnancy and clinical Pregnancy Rates (PRs), whose evidence is of very low quality and they concluded to be very uncertain about differences in the comparison [15]. Moreover a new debate has emerged regarding whether a further reduction after day 3 of development represents the most physiologic system which is based on the premise that oxygen tension is lower in the uterus than in the oviduct and that the embryo crosses the uterotubal junction sometime on day 3. While data are currently limited, recent experience with ultra-low oxygen (2%) after day 3 of development suggests that the optimal oxygen tension in embryo culture may depend on the stage of development [16].

The aim of our prospective randomized study was to investigate the effects of different oxygen concentrations (5% versus atmospheric oxygen) on the ICSI outcome parameters including fertilization, early embryo development, and embryo quality and pregnancy outcomes.

Materials and Methods

This is a prospective randomized trial that included 2682 couples who attend the IVF center of Medicana Hospital for ICSI treatment between May 2016 and December 2018. Written informed consents were obtained from all patients and the study was approved by the ethics committee of the hospital. The patients were allocated randomly to ~20% (atmospheric) and 5% O_2 groups. Patients with a maternal age of >37, cycles with testicular sperm extraction and preimplantation genetic screening, patients with genetic problems were discarded. ICSI treatment was used routinely for all patients.

Experimental design

Culture dishes of patients were incubated either in a low O_2 concentration (5%) or ambient atmosphere concentration (~21%). Patients were chosen randomly according to the number of patients in an incubator.

Ovarian stimulation

The stimulation of ovulation was performed using Gonadotropin Releasing Hormone (GnRH) analogues (Suprecur; Hoechst AG, Frankfurt, Germany) and human Menopausal Gonadotropins (HMG) (Pergonal; Serono, Aubonne, Switzerland, Humegon; Organon, Oss, Holland) or using a protocol including the usage of Follicle Stimulating Hormones (FSH) in combination. When the dominant follicle size was measured 18 mm or bigger and the blood oestrogen reached the required level, Human Chorionic Gonadotropin (HCG) injection was administrated. (5000 or 10000 IU: Profasi; Serono, Pregnyl; Organon). The ovarian response to the treatment was monitored by ultrasound examinations and serum oestradiol measurements.

Oocyte retrieval

Oocyte retrieval was performed at 36 hr post-HCG injection under general anesthesia. Transvaginal ultrasound guided aspiration was applied through single or double luminal needles based on the preference of the gynecologist.

Preparation of the semen sample

Culture: Following cumulus dissection and maturity evaluation, the oocytes were placed in separate drops of Human Tubal Fluid (HTF) (GMHT-100; LifeGlobal, America). They were incubated either in 6% CO_2, ~20% O_2 or in 6% CO_2, 5% O_2 incubators. The patient distribution was made randomly according to the capacity of the incubators in the laboratory. After 3 hrs of incubation, microinjection procedure was carried out. Same incubator brands were used in both groups (Labotect C-200) and the embryologists were blinded during evaluation of embryos.

ICSI procedure: In order to remove the remaining cumulus cells, the oocytes were incubated in a HEPES buffered medium containing 1:10 hyaluronidase (Hyase 10 X, Vitrolife) for 20 seconds, rinsed thoroughly and incubated in the culture medium until microinjection. ICSI was performed by using an inverted microscope equipped with Hoffman modulation. PVP (LPVP-001; LifeGlobal, America) was used to slow down the sperm motion. Morphologically normal and motile spermatozoa were selected for ICSI procedure. Oocyte and sperm morphology, and were recorded. The oocytes were then rinsed and transferred into the culture medium medium with HEPES HTFw/Hepes; LifeGlobal, America) which is pre-equilibrated. They were incubated in Labotect C-200 incubator equipped with O_2, N_2 and CO_2 control. The CO_2 and O_2 concentrations of incubators were checked daily using Labotect InControl analyzer which is calibrated every 3 months by the distributor.

Assessment of fertilization: After 12 hrs to 18 hrs of incubation the oocytes were checked for fertilization. Fertilization was defined as the presence of 2 pronuclei and 2 polar bodies. Fertilized oocytes were transferred into fresh Global medium (LGGG-050; LifeGlobal, America) and were cultured until embryo transfer.

Assessment of cleavage: The embryos were not checked before the third day. On the third day, the morphological appearance of the embryos, number of blastomeres they include, fragmentation ratio and cytoplasmic structures were evaluated to assess the cleavage stage embryos. Gardner's embryo scoring system was used to evaluate the embryos at cleavage stage or blastocyst.

Embryo transfer: When a patient had 2 or less embryos and/or poor quality embryos, 2nd day transfer, when she had 4 or less good quality embryos, 3rd day transfer and if the embryo number is higher than 4 blastocyst (5th day) transfer was performed. Embryos were placed in a separate drop in the morning of the transfer day. Embryo transfers were performed by the guidance of ultrasound and Wallace 1816N catheter.

Statistical analysis: SPSS for Windows 10.0 software package was utilized for the statistical analysis. Chi-square test and Mann Whitney U test was used to evaluate the rates and proportions and differences between the groups respectively. The results were evaluated in 95% confidence interval and the statistical significance was defined as, $p<0.05$. The values of parameters are given as mean % unless otherwise stated.

Results

No difference was found between the demographic data of patients for any of the parameters listed in (Table 1).

There was no difference between groups by means of ART indications, maternal age, number of oocytes collected, number of embryos transferred (Table 2). Oocytes of 4425 patients were retrieved, and 3986 of them had at least one embryo transfer. Surplus embryos of 1121 patients were frozen for future use. A total of 17239 embryos were incubated in 5% O_2 and 12872 were incubated in ~20% O_2 concentration. None of the ICSI outcome parameters were affected significantly from the oxygen concentration although all parameters especially the top quality embryo rate was increased in 5% oxygen concentration.

Table 1: Demographic data of patients in both groups.

Oxygen concentration	5% O_2	20% O_2	p value
Number of patients	2612	1813	NS
Maternal Age (years)	29.9 ± 8.4	30.7 ± 3.8	NS
Estrogen level on the day of oocyte pick-up	1359 ± 256	1115 ± 413	NS
Number of previous IVF attempts	1.36 ± 0.7	1.45 ± 0.7	NS
Number of oocytes collected	6.6 ± 2.14	7.1 ± 2.2	NS
Number of embryos transferred	1.2 ± 0.7	1.18 ± 0.4	NS
Day of transfer	3.03 ± 0.7	3.78 ± 0.6	NS

Table 2: ICSI outcome parameters of patients in both groups.

	5% O_2	20% O_2	p value
Fertilization rate (%)	73.8	72.4	NS
Embryo development rate (%)	90.6	89.3	NS
Top quality embryo rate (%)	36.1	23.3	NS
Clinical pregnancy rate (%)	40.9	36.6	NS
Implantation rate (%)	35.9	24.4	NS
Live birth rate (%)	22.3	18.6	NS

Conclusion

Limiting the oxygen concentration in embryo culture systems have been used widely because of the reported data about the harmful effects of atmospheric oxygen concentrations via reactive oxygen species produced [3-5]. Oxidative stress is suggested to be triggered because of the end product of oxygen metabolism. We compared the outcome of ICSI in both oxygen concentrations and found no significant difference between the embryos of low and atmospheric oxygen culture conditions. Embryo quality was the only parameter that a slight decrease was obtained in favor of low oxygen concentration but with no statistical significance. Our findings are in accordance with the data of a recent meta-analysis that summarize the data of 21 studies on the subject [15].

A possible explanation for the situation may be explained by the hypothesis that oxidative stress is an inducer of apoptosis in many cells and effects embryo quality by triggering apoptosis and thus fragmentation. The embryos are subjected to physiologic oxygen concentrations during their travel towards uterus. No study is present in the literature analyzing the effects of oxygen concentration on metabolic consumption.

We may conclude that it is the metabolic state of an embryo that decide to use the oxygen, not the oxygen concentration it is exposed to in the light of the data that oxygen consumption is regulated intrinsically by the metabolic activity of the cells.

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