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
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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.

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 (H2O2), Hydroxil Radical (OH) and superoxide anion radical (O
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- ) 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 trophoderm 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
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% O₂ groups. Patients with a maternal age of >37, cycles with testicular sperm extraction and preimplantation genetic screening, patients with genetic problems were excluded. ICSI treatment was used routinely for all patients.

**Experimental design**

Culture dishes of patients were incubated either in a low O₂ 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 oварian 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% CO₂, ~20% O₂ or in 6% CO₂, 5% O₂ 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 HEPS 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 HEPS HTFw/Hepes (LifeGlobal, America) which is pre-equilibrated. They were incubated in Labotect C-200 incubator equipped with O₂, N₂ and CO₂ control. The CO₂ and O₂ concentrations of incubators were checked daily using Labotec 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, p<0.05. The values of parameters are given as mean % unless otherwise stated. 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% O₂ and 12872 were incubated in ~20% O₂ 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.

**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% O₂ and 12872 were incubated in ~20% O₂ 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.
intrinsically by the metabolic activity of the cells. To decide to use the oxygen, not the oxygen concentration it is exposed to in the light of the data that oxygen consumption is regulated metabolically.

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 embryo quality by triggering apoptosis and thus oxidative stress. It is suggested to be harmful effects of atmospheric oxygen concentrations via reactive oxygen species produced [3-5]. Oxidative stress is suggested to be trigged because of the end product of oxygen metabolism. We may conclude that it is the metabolic state of an embryo that determine 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.

### 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 trigged 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.

### References