Comparison of 5% and ambient oxygen during days 3–5 of in vitro culture of human embryos

Laszlo Nanassy, Ph.D.,[a] *C. Anthony Peterson, B.S.,*[a] *Aaron L. Wilcox, B.S.,*[a] *C. Matthew Peterson, M.D.,*[b] *Ahmad Hammoud, M.D.,*[b] *and Douglas T. Carrell, Ph.D.*[a,b,c]

[a] Department of Surgery, [b] Department of Obstetrics and Gynecology, and [c] Department of Physiology, Andrology and IVF Laboratories, University of Utah School of Medicine, Salt Lake City, Utah

Objective: To compare the effect of two oxygen concentrations used during days 3–5 of human embryo culture on embryo quality and pregnancy outcome.
Design: Retrospective analysis of the use of two culture conditions.
Setting: University-based infertility clinic.
Patient(s): Three hundred eighty-two patients undergoing IVF.
Intervention(s): Embryos were cultured in 5% CO_2 balanced (~20% O_2) gas phase until day 3 then assigned to ~20% or reduced (5%) oxygen concentration groups and cultured until ET.
Main Outcome Measure(s): Embryo quality, pregnancy rates, and implantation rates.
Result(s): There were no differences in demographic features (age, type of infertility) between the two groups. The embryo scores at day 3 and day 5, blastulation rate, and transfer score did not differ between groups. No differences were observed between the 5% and 20% oxygen concentrations in the chemical pregnancy rate (71.27% vs. 78.72%), clinical pregnancy rate (58.56% vs. 64.36%), or implantation rate (44.06% vs. 44.16%).
Conclusion(s): Reduced oxygen concentration in the gas mixture from day 3 until ET did not support better embryo development or result in higher pregnancy or implantation rates. These data do not support the hypothesis that beneficial effects of reduced oxygen concentration can be gained by employing the strategy during the latter stages of embryo culture (days 3–5) only and highlight the need for further studies through all stages of in vitro culture. (Fertil Steril® 2010;93:579–85. ©2010 by American Society for Reproductive Medicine.)

Key Words: In vitro fertilization, oxygen concentration, blastocysts, implantation rate, embryo culture

Reduced oxygen tension in the gas mixture during embryo culture has been shown to be beneficial in a number of mammalian species including mice (1–3), cattle (4–6), and pigs (7, 8). The oxygen tension in the female reproductive tract is $\leq$40% of atmospheric O_2 (9). During human IVF, embryo culture is usually carried out in conventional incubators that use atmospheric oxygen concentration, and it has been proposed that excessive oxygen is detrimental to early embryos through the generation of oxygen-centered free radicals (10, 11).

Studies investigating the effect of oxygen concentration in humans have provided inconsistent results. Some studies have failed to reveal any positive effects of reduced oxygen concentration on embryo quality and/or implantation or pregnancy rates (12–16). However, improved clinical pregnancy and implantation rates (17) and higher birth rates (18) have also been reported after using a lower O_2 concentration during preimplantation embryo culture.

A lower oxygen concentration has been observed in the uterus than in the oviduct in many mammalian species. The oxygen concentration in the oviduct of the rhesus monkey has been found to range from 5% to 8.7%, while in the uterus it was only ~2% (9). Thus, the embryos are exposed to reduced oxygen tension by the time they arrive in the uterus, which corresponds to blastocyst formation and implantation. A recent preliminary study has reported that reducing the oxygen in the gas atmosphere during days 3–5 of in vitro culture of human embryos improves cleavage and pregnancy rates similar to reduced oxygen culture throughout days 1–5, indicating that the benefits of lower oxygen may be critical during the 8-cell to blastocyst stages and coinciding with the studies showing lower oxygen in the uterus than in the oviduct (9, 19).

The objective of this study was to evaluate the effect of high (~20%) and reduced (5%) oxygen concentration in the gas mixture on human embryos during the culture period from day 3 to ET. Embryo development, embryo quality, and pregnancy and implantation rates were compared in patients undergoing identical culture protocols from day 1 to day 3 and then using the two different oxygen concentrations on days 3–5 of culture.

MATERIALS AND METHODS

Data were analyzed from infertility patients who underwent IVF treatment between May 2006 and November 2007 using a protocol of alternating two different gas mixtures during days 3–5 of embryo culture. Data were initially analyzed as

Received August 13, 2008; revised January 12, 2009; accepted February 17, 2009; published online April 1, 2009.
L.N. has nothing to disclose. C.A.P. has nothing to disclose. A.L.W. has nothing to disclose. C.M.P. has nothing to disclose. A.H. has nothing to disclose. D.T.C. has nothing to disclose.
Reprint requests: Douglas T. Carrell, Ph.D., Andrology and IVF Laboratories, 675 South Arapeen Drive, Suite 205, Salt Lake City, Utah 84108 (FAX: 801-581-6127; E-mail: douglas.carrell@hsc.utah.edu).

a retrospective chart review as part of a quality assurance program. The study was approved by the Institutional Review Board of the University of Utah.

IVF

Ovarian stimulation was performed using the long-stimulation protocol. GnRH agonist was combined with recombinant FSH (rFSH; Follistim, Organon, Ravensburg, Germany; and Gonal-f; Serono Inc., Rockland, MA) and urinary-derived gonadotropin stimulation (Repronex; Ferring Pharmaceuticals, Suffern, NY). Oocyte retrieval was performed approximately 36 hours after hCG administration using ultrasound-guided transvaginal aspiration. Before fertilization, oocytes were incubated for 3 hours in Quinn's Fertilization Medium (Quinn's Sage, Biopharma, Trumbull, CT) supplemented with 10% SPS (Quinn's Sage). Two models of incubators were used for culture. For culture at the atmospheric oxygen concentration, a Forma Scientific (Evanston, IL), model no. 3110 incubator was used. For concentration at 5% oxygen, a Thermo Electron Corporation (Marietta, OH) model no. 3130 incubator was used.

Standard density gradient centrifugation was used for sperm preparation for the IVF procedures (Isolate; Irvine Scientific, Santa Ana, CA). For washing and resuspension of sperm samples, Quinn's Fertilization Medium supplemented with 10% Quinn's SPS was used.

Standard IVF microdrop insemination was performed when patients had a normal sperm penetration assay (SPA) result (20). Standard IVF insemination was performed by adding 125,000–300,000 progressively motile spermatozoa to each 50 μL droplet containing the oocyte. Patients with oligozoospermia (<20 million sperm/mL) or a poor SPA value underwent intracytoplasmic sperm injection (ICSI). Fertilization was checked 18–20 hours postinsemination, and the zygotes were placed into culture media. Half of the embryos were cultured in 100 μL of a one-step culture medium (Global, LifeGlobal, Guelph, Ontario, Canada), and half were cultured in sequential medium (Quinn's Sage) droplets in 5% CO_2 balanced with ambient air (~20% O_2) gas phase until day 3.

On the morning of day 3 of culture, the embryos were placed in fresh droplets of culture medium (including the embryos cultured in the single-step medium). All embryos from each patient were either cultured in ~20% or reduced (5%) oxygen concentration until ET. Patients were selected for the treatment groups by their IVF identification number (odd numbered cycles underwent culture with 5% oxygen).

Only cases suitable for a possible day 5 ET were included in the study. The inclusion criterion was the presence of at least two 6-cell embryos with moderate or less fragmentation on day 3. Generally, the two embryos with the best morphological quality scores were transferred at the end of the culture period. Before transfer, all transferred embryos were placed in Quinn's Blastocyst Medium (Quinn's Sage) supplemented with 50% Quinn's SPS and kept for 1 hour.

The day 3 embryo score was calculated by subtracting the embryo grade from the number of blastomeres present. Embryo grade has been determined based on cellular fragmentation and blastomere morphology, with 0 being the best and 3 being the worst (21). An embryo score at day 5 was determined by the developmental stage the embryo reached (blastocyst, early blastocyst, cavitating morula, and morula) and also considering the level of fragmentation, the expansion of the trophectoderm, and the morphology of the inner cell mass (ICM) and trophectoderm.

Positive chemical pregnancy was determined by a serum hCG >5 mIU/mL; positive clinical pregnancy results were based on the presence of a gestational sac with fetal heart beat at 6 weeks after ET. Implantation rates were calculated by dividing the number of embryos with heartbeats by the number of embryos transferred and multiplying by 100.

Statistical Analysis

Statistical analysis was performed using the Stata 9.2 statistical software package (StataCorp, College Station, TX). Continuous variables were subjected to Student's *t*-test; percentage data were analyzed using χ^2 analysis. One-way analysis of variance with the Bonferroni correction was carried out where four different groups were compared. $P<.05$ was considered statistically significant. A post hoc power calculation was performed, which indicated that the study is powered at 80% to detect a 15% difference in pregnancy rate and powered at 50% to detect a 10% difference with the same sample size.

RESULTS

In total, 542 IVF procedures were performed during the study period. Twenty-six cases were excluded for miscellaneous reasons from the study, including 14 cases because of embryo manipulation for preimplantation genetic diagnosis and eight cases because of pronuclear stage embryo freezing for ovarian hyperstimulation. One hundred thirty-four patients underwent day 3 transfer and were therefore also excluded. After exclusion, 382 day 5 ET cases were analyzed in this study: 189 cases in the 5% O_2 group and 193 cases in the ~20% O_2 group (Fig. 1).

The demographics of the study groups are given in Table 1. No differences were observed in mean age of the patients, type of infertility, number of retrieved oocytes, number of prior IVF cycles, or other relevant factors (Table 1). Additionally, no differences were seen in the fertilization rates (91.00% vs. 91.68%) between the patients. Neither oxygen tension nor culture media had an effect on day 3 cleavage rates and embryos scores. No differences were found at this developmental stage (Table 2).

We did not find any difference in the embryo developmental rates between the groups at day 5 (51.45% and 56.93% in ~20% oxygen in Global and Quinn's Sage; 55.21% and 55.74% in 5% oxygen in Global and Quinn's Sage,