Low-oxygen compared with high-oxygen atmosphere in blastocyst culture, a prospective randomized study

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Objective: To investigate birth rates with two oxygen (O_2) concentrations in blastocyst culture.
Design: Randomized trial.
Setting: Private in vitro fertilization (IVF) clinic.
Patient(s): Six hundred women undergoing IVF.
Intervention(s): Blastocyst culture in atmospheres with either 6% carbon dioxide (CO_2) in air, the equivalent to 19% O_2, a two-gas system; or 5% O_2, 6% CO_2, and 90% nitrogen (N_2), a three-gas system.
Main Outcome Measure(s): Birth rate.
Result(s): The inclusion criterion for blastocyst culture (at least five fertilized oocytes) was fulfilled in 396 women, randomized to 197 cultures with the three-gas system and 199 cultures with the two-gas system. The outcome with the three-gas system compared with the two-gas system showed a statistically significantly increased blastocyst rate (47.8% vs. 42.1%), mean number of blastocysts (3.8 vs. 3.3), and number of cryopreserved blastocysts (1.7 vs. 1.1). The mean number of transferred blastocysts was 1.2 versus 1.3. Culture with the three-gas system increased the relative birth rate by 10% compared with the two-gas system (42% vs. 32%, respectively), a statistically significant difference. The overall twin rate was 4.8%.
Conclusion(s): Blastocyst culture with low-oxygen (5%) versus high-oxygen (19%) concentration yielded a better blastocyst outcome and a marked improvement in birth rate. Generation of cytotoxic reactive oxygen species with prolonged embryo culture might deteriorate blastocyst viability. (Fertil Steril® 2009;91:2461–5. ©2009 by American Society for Reproductive Medicine.)

Key Words: In vitro fertilization, blastocysts, gas atmosphere, oxygen

Before sequential culture media was introduced in blastocyst culture during the late 1990s, the overall results for blastocyst development were poor, although co-culture with cells of other origins improved the outcome (1). It has been claimed that blastocysts enable easier evaluation of quality and selection for transfer, providing a higher implantation and birth rate. This could also make choosing single over multiple transfers easier, in particular when cryopreservation of spare high-quality blastocysts is possible (2). In our own observations, the birth rates increased when 5-day culture replaced 2- or 3-day culture (3); and in recent randomized studies, in vitro fertilization (IVF) outcomes have been favorable with blastocyst culture (4).

Blastocyst culture requires experience and optimal culture conditions, including appropriate media, as embryo development is markedly affected under different conditions. In addition, blastocyst culture requires a skilled assessment of the blastocysts and the timing of the transfer (5). Advocates of day-2 transfer argue that the losses of embryos from fertilization to blastocyst stage are too large. Fertilization rate in most programs is above 70%, whereas only 35% to 60% of 2-day embryos develop into blastocysts depending on whether the 2-day embryos have been selected or not (6–10). The prolonged culture must not only fulfill the nutritional needs of the growing embryo, but the surrounding atmosphere must be appropriate as well.

During culture, the atmosphere of traditional 5% carbon dioxide (CO_2) in air (with approximately 20% oxygen [O_2]) has a higher O_2 concentration than is physiologically normal in the oviduct and uterus. It has been claimed that this promotes the generation of cytotoxic highly reactive oxygen radicals detrimental to the embryo (11). Studies on animal models (mainly) have estimated the physiologic O_2 concentrations in the oviduct and uterus of different species at 5% to 10%, and have compared the results of different atmospheres in blastocyst culture. In general CO_2 in air has been compared with 5% CO_2, 5% O_2, and 90% nitrogen (N_2). In general the results have been promising for the latter atmosphere, but there also have been discrepancies (12).

Our study compared blastocyst culture in 6% CO_2 in air (two gases) with 6% CO_2, 5% O_2, and 89% N_2 (three gases), when all other conditions were equal and the patients were randomized into the two procedures.

MATERIALS AND METHODS

We randomized 600 patients to culture under 6% CO_2 in air, the equivalent to 19% O_2 (two-gas system), or 6% CO_2, 5%

Received June 14, 2007; revised and accepted March 18, 2008; published online June 12, 2008.
U.W. has nothing to disclose. A.E. has nothing to disclose. D.H. has nothing to disclose. S.N. has nothing to disclose.
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O_2, and 89% N_2 (three-gas system) under 37.0° to 37.5°C temperature. A 5% oxygen concentration was achieved by adding 6% CO_2 and 70% N_2 to air. Small variations in the CO_2 concentration could occur due to calibration of pH to 7.28 to 7.32. The same type of incubators (Labrum Klimat, Stockholm, Sweden) were used for the two-gas and three-gas systems.

The inclusion criterion for blastocyst culture was at least five fertilized oocytes. Randomization was made at start of ovarian hyperstimulation because three-gas or two-gas culture was to start immediately at insemination. Based on previous observation, we estimated that 400 of the 600 randomized patients would fulfill the inclusion criterion of at least five fertilized oocytes and that some additional patients could not participate in the study for other reasons. Randomization and subsequent culture were blinded for the physician.

Controlled ovarian stimulation and oocyte retrieval were performed according to a standard "long" gonadotropin-releasing hormone agonist protocol, or in a few cases a standard antagonist protocol. For ovarian stimulation, a recombinant follicle-stimulating hormone (FSH) was used, as has been described previously elsewhere (13). Sequential embryo cultures were in general performed with media from the original Blast Assist System (MediCult, Jyllinge, Denmark). After fertilization, embryos were transferred to Medium 1 on day 1 and to Medium 2 on day 3, which was changed for fresh Medium 2 on day 4. Embryos were cultured no more than five together in dishes without oil.

At embryo transfer, the physician in general recommended that couples transfer one blastocyst. Two blastocysts sometimes were transferred when exceptions were older age, several previous IVF failures, or only poor-quality blastocysts available (n = 96, 26%). Only fresh blastocysts were used for transfer in this study. Assisted partial hatching was performed with Tyrode's solution when there was a thick zona pellucida, according to the embryologist's experience. Spare good-quality blastocysts were frozen in freezing media from MediCult. Embryo transfer was performed in all cases where the best embryo was at least a morula.

Blastocyst quality was scored according to the criteria of Schoolcraft et al. (14). A high-quality blastocyst was fully expanded with the blastocele volume larger than that of the early embryo, a tightly packed inner cell mass, and an outer cell mass (trophectoderm) consisting of many cells forming a cohesive epithelium, with or without hatching.

The results were calculated on the JMP 3.1 statistical program (SAS Institute, Cary, NC). A chi-square test (likelihood ratio) was used for nominal variables and *t*-test for continuous variables. Multifactorial analyses, by logistic regression (log likelihood test), were used when adjustment for possible confounding factors was made.

Ethics approval was not obtained as both culture atmospheres were already simultaneously and randomly used in clinical routine when the study was initiated. There were no conflicts of interest.

RESULTS

We randomized 600 women to one of two culture atmospheres at the start of ovarian hyperstimulation. As expected, 173 women did not fulfill the study's entrance criteria because less than five oocytes were fertilized. An additional 31 patients could not be included in the study mainly because of their decision only to cryopreserve the embryos.

Of the remaining patients, 199 were assigned to culture in the two-gas system and 197 to the three-gas system. Background data for the 199 patients in the two-gas group and the 197 patients in the three-gas group are given in Table 1. Women in the two groups were of similar age, number of previous IVF cycles, number of retrieved oocytes, fertilization method, fertilization rate, and number of transferred embryos.

No blastocysts developed in 13 patients (6.5%) in the two-gas group and 14 patients (7.1%; not statistically significant) in the three-gas system. Culture in three-gas system had a statistically significant correlation with a higher mean number blastocysts and frequency of fertilized oocytes that developed to blastocysts (Table 2). When the three-gas system was used, relatively more patients had at least one high quality blastocyst but neither the overall quality nor the trophectoderm and inner cell mass quality differed significantly between the two study groups. There was a highly statistically significant (almost 50%) increase in number of blastocysts of good quality that could be cryopreserved when three-gas atmosphere was used compared with the two-gas atmosphere.

In vitro fertilization outcomes in terms of positive human chorionic gonadotropin (hCG) levels, viable pregnancy, and childbirth were all statistically significantly better in women for whom the three-gas atmosphere was used compared to the two-gas atmosphere (Table 3). The differences in birth rate per embryo transfer were 42.1% for the three-gas system compared with 32.2% for the two-gas system.

DISCUSSION

Our results showed a considerable and statistically significantly higher (plus 10%) birth rate when the three-gas atmosphere was used (5% O_2) compared with the two-gas system (equivalent to 19% O_2). The fertilization rate was similar in the two groups, but the three-gas atmosphere produced superior blastocyst rates and blastocyst quality (as judged by number of good quality blastocysts available to cryopreserve), and more chemical pregnancies, viable pregnancies, and childbirths.

One hypothesis is that highly reactive oxygen species (i.e., free radicals) continuously increase during 5-day culture. These increase with increased O_2 tension. Catalysts for production of reactive oxygen species, such as superoxide (O_2^-), hydrogen peroxidase (H_2O_2), and hydroxyl radical HO, could be xanthine oxidase, flavin mononucleotide, thyazines, acridines, and phenazines (15). Noda et al. (16) found that elimination of hypoxanthine, copper(II) sulphate