Low versus atmospheric oxygen tension for embryo culture in assisted reproduction: a systematic review and meta-analysis

Carolina O. Nastri, Ph.D., Beatrice N. Nóbrega, M.D., Danielle M. Teixeira, M.D., Jowanka Amorim, M.Sc., Lívia M. M. Diniz, M.D., Marina W. P. Barbosa, M.D., Vanessa S. I. Giorgi, M.Sc., Vicky N. Pileggi, M.Sc., and Wellington P. Martins, Ph.D.

Department of Obstetrics and Gynecology, Ribeirao Preto Medical School, University of São Paulo, Ribeirao Preto, Brazil

Objective: To appraise the available evidence comparing low oxygen ($LowO_2$) and atmospheric oxygen tension ($AtmO_2$) for embryo culture.
Design: Systematic review and meta-analysis.
Setting: Not applicable.
Patient(s): Women undergoing assisted reproduction using embryo culture.
Intervention(s): Embryo culture using $LowO_2$ versus $AtmO_2$.
Main Outcome Measure(s): Reproductive, laboratory, and pregnancy outcomes.
Result(s): A total of 21 studies were included in this review. All used O_2 concentration between 5% and 6% in the $LowO_2$ group. Considering the studies that randomized women/couples, we observed very low quality evidence that $LowO_2$ is better for live birth/ongoing pregnancy (relative risk [RR] = 1.1, 95% confidence interval [CI] 1.0–1.3) and clinical pregnancy (RR = 1.1, 95% CI 1.0–1.2). Considering the studies that randomized oocytes/embryos, we observed low quality evidence of no difference of fertilization (RR = 1.0, 95% CI 1.0–1.0) and cleavage rate (RR = 1.0, 95% CI 1.0–1.1), and low quality evidence that $LowO_2$ is better for high/top morphology at the cleavage stage (RR = 1.2, 95% CI 1.1–1.3). No studies comparing pregnancy outcomes were identified. Several studies used different incubators in the groups—a new model for the $LowO_2$ group and an old model for the $AtmO_2$ group. The risk of detection bias for the laboratory outcomes was high as embryologists were not blinded.
Conclusion(s): Although we observed a small improvement (~5%) in live birth/ongoing pregnancy and clinical pregnancy rates (PRs), the evidence is of very low quality and the best interpretation is that we are still very uncertain about differences in this comparison. The clinical equipoise remains and more large well-conducted randomized controlled trials are needed. They should use the same incubators in both groups and the embryologists should be blinded at least when evaluating laboratory outcomes. (Fertil Steril® 2016;106:95–104. ©2016 by American Society for Reproductive Medicine.)
Key Words: Embryo culture, IVF, ICSI, oxygen, assisted reproductive techniques, meta-analysis

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/nastric-low-oxygen-embryo-culture/

Almost 40 years have passed since the birth of the first test tube baby (1) and, in spite of the extensive research on many new interventions and refinements (2–5), the success rates have only slightly changed. Some research aiming to improve these rates has focused on oxygen (O_2) concentration. Embryo culture is traditionally carried on in atmospheric O_2 concentrations of about 20%, whereas the physiological intrauterine O_2 concentration is lower, corresponding to conditions provided by using 2%–6% of O_2 in the air (6). Reducing the O_2 tension requires, however, especial incubating systems that allow the use of nitrogen gas to purge oxygen out of the incubator. These incubators have sensors for carbon dioxide and O_2 that guide the interior atmosphere rebalance each time the incubator is handled. All of these add costs and maintenance procedures make the fertility treatment even more expensive and less accessible for couples worldwide.

Received January 6, 2016; revised February 18, 2016; accepted February 22, 2016; published online March 21, 2016.
C.O.N. has nothing to disclose. B.N.N. has nothing to disclose. D.M.T. has nothing to disclose. J.A. has nothing to disclose. L.M.M.D. has nothing to disclose. M.W.P.B. has nothing to disclose. V.S.I.G. has nothing to disclose. V.N.P. has nothing to disclose. W.P.M. has nothing to disclose.
Reprint requests: Wellington P. Martins, Ph.D., Avenue. Bandeirantes, 3900 – 8 andar - HCRP - Campus Universitário, Ribeirao Preto, Sao Paulo, Brazil 14048-900 (E-mail: wpmartins@gmail.com).

Fertility and Sterility® Vol. 106, No. 1, July 2016 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.02.037

Low O_2 human embryo culture has been performed since the early 1970s (7). Small studies evaluating the effect of O_2 concentration, combined with other interventions on animal embryo culture, have been published with conflicting results (8–12). Further studies linking high O_2 concentration with increased oxidative stress and developmental blockage of embryos cultured in vitro (13–15) impelled further studies. Theoretically, higher O_2 tensions could compromise embryo quality and viability, which raises concern regarding the possibility of congenital abnormalities. During the past 2 decades, many studies have been published and low O_2 culture has been empirically adopted in many centers.

Perhaps due to competition and aiming to improve results, we encounter a demand for new processes and technologies in reproductive medicine that are sometimes promoted without there being adequate evidence to support a change (2). To enable an informed decision we aimed to identify, appraise, and summarize the available evidence comparing the effectiveness and safety between low and atmospheric O_2 tension for embryo culture in women undergoing assisted reproduction.

MATERIALS AND METHODS

Registration and Eligibility Criteria

The review protocol was registered in PROSPERO (CRD42015025487). The studies compared embryo culture under low O_2 tension ($LowO_2$; 2%–8%) versus atmospheric O_2 tension ($AtmO_2$; ~20%) in women undergoing assisted reproduction. The primary outcomes of this review were live birth/ongoing pregnancy and congenital anomalies.

We divided this review in three parts to properly evaluate the effectiveness and safety of this comparison. Each part had a particular objective (16). For each objective, the eligibility criteria regarding study design was slightly modified. Therefore the best approach was used to identify the relevant evidence.

Clinical outcomes. Only randomized controlled trials (RCTs) that allocated women/couples were considered eligible. The clinical outcomes were live birth/ongoing pregnancy per randomized women, clinical pregnancy per randomized women, and miscarriage per clinical pregnancy. All terms used were as previously defined (17). In studies where live birth was not reported, ongoing pregnancy was used as a surrogate outcome because late pregnancy loss is not a common event. Not including data from these studies would represent a partial view of the available evidence (4, 16, 18). Nonrandomized studies were not considered eligible, as they are associated with a high risk of bias and there are several RCTs evaluating these outcomes.

Laboratory outcomes. The RCTs that randomized oocytes or embryos were considered eligible. The laboratory outcomes were fertilization rate; cleavage rate, embryos of high/top morphology at cleavage stage, blastocyst rate; embryos of high/top morphology at blastocyst stage. For these outcomes we considered the number of randomized oocytes/embryos as the denominator. For the definition of high/top morphology, any classification system was accepted. Nonrandomized studies were not considered eligible, as they are associated with a high risk of bias and there are several RCTs evaluating these outcomes.

Pregnancy outcomes. Because some of the important outcomes regarding safety in reproductive medicine are rare and therefore unlikely to be satisfactorily analyzed by RCTs, prospective and retrospective cohort studies were considered eligible (16). Case-control studies were not included. The outcomes that would be assessed were congenital anomalies per clinical pregnancy; preterm birth per clinical pregnancy; very preterm birth per clinical pregnancy; low weight at birth per clinical pregnancy; and very low weight at birth per clinical pregnancy. Although observational studies are at a higher risk of bias, it is extremely unlikely that such outcomes would be properly assessed by RCTs. Therefore at least 50,000 participants should be included to have sufficient power to detect a clinically relevant increase on birth defects. In addition, such outcomes are rarely related by RCTs in reproductive medicine (16, 18).

Searches for Studies

The electronic searches were run in the following electronic databases from their inception: Cochrane Central Register of Controlled Trials (CENTRAL), PubMed, Scopus, and Web of Science. We searched for study protocols and ongoing trials on the following databases: ClinicalTrials.gov (https://clinicaltrials.gov/) and ISRCTN registry (http://isrctn.com). In addition we hand-searched the reference list of included studies and related reviews. There was no limitation regarding language, publication date, or publication status. The full search strategy may be found in Supplemental Table 1, available online.

Study Selection and Data Collection Process

The study selection and data collection were performed independently by two review authors. Manual searches were performed by all authors. Disagreements were solved by consulting another author. The original studies' authors were contact by e-mail, as required.

Summary Measures and Synthesis of Results

Dichotomous variables were summarized as risk ratio (RR) and the precision of the estimates evaluated by the 95% confidence interval (CI). Where a significant difference was observed within the first part of the review—effect on clinical outcomes—either the number needed to treat for an additional beneficial outcome or an additional harmful outcome was calculated.

Where the studies were considered to have sufficiently similar data, these were combined using a random effects model in the following comparisons: [1] $LowO_2$ versus $AtmO_2$ during all embryo culture; [2] $LowO_2$ versus $AtmO_2$ before Day 3 followed by $LowO_2$ in both groups; and [3] $LowO_2$ versus $AtmO_2$ only after Day 2.

Results were combined for meta-analysis using Review Manager 5.3.5 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Cumulative meta-