Evaluation of two incubation environments—ISO class 8 versus ISO class 5—on intracytoplasmic sperm injection cycle outcome

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Objective: To evaluate the impact of two different incubation environments—class 8 versus class 5—on embryo quality and pregnancy rate.
Design: Retrospective comparative study.
Setting: Private fertility and gynecology clinic.
Patient(s): 123 consecutive intracytoplasmic sperm injection (ICSI) cycles were analyzed from January 2002 to February 2005. Cycles were divided into two groups: in group I (n = 60), the embryo culture was performed in class 8 air quality; in group II (n = 63), the embryo culture was performed in class 5 air quality.
Intervention(s): None.
Main Outcome Measure(s): Number of embryos available for transfer, number of good quality embryos transferred, implantation rate, and clinical pregnancy.
Result(s): Age of women, duration of stimulation, total doses of gonadotropins, endometrial thickness on the day of human chorionic gonadotropin (hCG) administration, number of oocytes metaphase II retrieved, number of embryos available, number of good quality embryos transferred, fertilization and cleavage rates, implantation rate, and clinical pregnancy were not statistically different between the groups.
Conclusion(s): Our study demonstrated that incubation environment class 8 is as good as incubation environment class 5 when compared in relation to the parameters analyzed. We believe that there is still room for improvement in the overall outcome of ICSI embryos. (Fertil Steril® 2009;91:1780–4. ©2009 by American Society for Reproductive Medicine.)

Key Words: ICSI, embryo culture, ART outcome, oocyte, in vitro development, air quality, cleanroom

A gradual but important increase in the success rates in assisted reproduction procedures has been observed. Many of the environmental conditions for in vitro embryo production, such as media composition, temperature, pH, water quality, and atmospheric composition, have been relatively standardized with very little change over the past years in either human or animal in vitro production systems (1, 2). Laboratory air quality and in vitro fertilization (IVF) outcome have been receiving considerable attention because human embryo development in vitro is considered to be related, among other factors, to the conditions of the culture, especially to the air quality in the IVF laboratory and in the adjacent semen collection and preparation rooms. Many laboratories have installed specialized incubator filtration units with the intent of enhancing air quality for embryo culture. According to Battaglia (3), these filtration units are expensive; hence, it may be prudent for programs to determine whether the units are effective in their own laboratory environments as the efficacy of this approach is not yet conclusive.

In the International Congresses of Reproductive Medicine, works on this topic have been systematically presented correlating the presence of pollutants in the air of the IVF laboratory to the decline in the implantation rates (4, 5) and to a lower formation of in vitro blastocysts when exposed to volatile organic compounds (VOCs) (6). It has been shown that the use of activated carbon in the incubators reduces the concentration of VOCs (7). Another study compared the air quality in the IVF laboratory before and after moving the laboratory to another physical space (8). A comparative study between two laboratories—class 100 and class 1000—showed that in the class 100 facility there was an improvement in the embryo development, higher cleavage and gestational rates, and lower abortion rate (9). Previous studies have suggested that a class 100 facility is the one that most resembles the sterile environment of in vivo culture (10).

At least four studies discuss this topic in full detail (11–14). To date, however, the influence the quality of ambient air and gas environment in incubators can have on the embryo development is still little recognized.

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It is known that mammals are protected by their epithelial, immune, and digestive systems against environmental agents to which they are exposed on a large and small scale. Nevertheless, classic studies on toxicology (15) do not apply to oocytes and in vitro embryos because in this situation mechanisms of absorption of toxic substances can work more directly on the oocyte with effects on the embryo after fertilization.

In vitro fertilization clinics and laboratories are usually located in urban centers with a high rate of environmental pollution or close to industrialized areas. Considering that around 95% of the internal air in the incubator comes from the opening of the door and only 5% from the CO_2 cylinder used to supply the incubator, it is would be correct to say that the presence of pollutants from different originating sources present in the room could interfere in the in vitro embryo development.

The International Standard ISO 14644-1 (16) defines terms, identifies procedures for collecting and testing the air, and interprets the data concerning the cleanroom classification. The ISO 14644-1 establishes that ISO class 5 ambient air presents a maximum 3530 particles of ≥ 0.5 $\mu m/m^3$ and ISO class 8 presents 3,530,000 particles of ≥ 0.5 $\mu m/m^3$.

To better understand the effects of air quality used in the culture of human embryos, the present study compared the results obtained in the intracytoplasmic sperm injection (ICSI) cycles when using two incubators in two environments, ISO class 8 and ISO class 5.

MATERIALS AND METHODS

Patients

The study was performed at G&O Barra, a fertility and gynecology private clinic in Brazil. G&O Barra is one of the 28 clinics in the Centro Médico BarraShopping. For this study, an analysis of 123 consecutive ICSI cycles of 113 patients of the IVF program was performed in the period between January 2002 to February 2005. All women patients age ≤ 37 years were included in this study. In the first 60 cycles analyzed (group I), a Forma Scientific incubator, model 3165 (Forma Scientific Inc., Marietta, OH), without a high-efficiency particulate air (HEPA) filter airflow system, was used. The composition of the internal air, however, was similar to the ambient air classified as class 8 as there was no other air filtering system in the laboratory. In the following 63 cycles (group II), a Thermo Forma incubator, model 3110, serial II, class 100 (Forma Scientific, Inc.), was used. This incubator had a HEPA filter with 99.99% efficiency in removing particulate contaminants ≤ 0.5 μm and a continuous filtered airflow system of the entire chamber volume at every 60 seconds so that class 5 ambient air inside the incubator could be restored 5 minutes after closing the door.

The Institutional review board of Centro Médico BarraShopping approved the study protocol, and all patients gave an oral consent for data utilization as they had already signed a full written consent for assisted reproduction technology (ART) procedures.

Ovarian Stimulation

Ovarian stimulation was carried out using recombinant follicle-stimulating hormone (FSH; Gonal-F; Serono Laboratoires, São Paulo, Brazil) (17). It was initiated on cycle day 2, and the doses varied from 150 to 300 IU/day, depending on the patient's age, body mass index, ovarian pattern, menstrual cycles, basal hormones, and response to previous controlled ovarian stimulations. Follicle growth was assessed by vaginal ultrasound. A daily dose of 0.25 mg of GnRH antagonist (Cetrotide; Serono Laboratories) administration was initiated when the leading follicle reached 14 mm and continued until the day of human chorionic gonadotropin (hCG) admnistration. Oocyte maturation was trigged by subcutaneous administration of hCG (Ovidrel; Serono) when at least one follicle had reached 18 mm in diameter and two had reached 16 mm. Transvaginal oocyte retrieval was scheduled 34 to 36 hours later (18, 19). The supplementation of the luteal phase was started on the day of oocyte retrieval and consisted of 600 mg per day of natural micronized progesterone intravaginally (20) in three divided doses (Utrogestan; Farmoquimica, Rio de Janeiro, Brazil).

Semen Preparation

Samples were obtained by masturbation, after a minimum of 2 days and maximum of 5 days of abstinence. After liquefaction, the evaluation of semen density and motility was carried out according to the recommendations of the World Health Organization (21). The samples were prepared to remove seminal fluid, debris, viruses and bacteria, and concentrate on the spermatozoa with good motility and morphology (21) using two discontinuous gradients (22) (40% to 80%; Sperm Grad; Vitrolife, Kungsbacka, Sweden). The final density and motility were assessed, and, whenever necessary, dilutions were performed.

Oocyte Preparation and ICSI Procedure

During ovum pick-up, the cumulus–corona cell complexes were immediately separated from the follicular fluid and transferred into 1 mL of IVF medium (Vitrolife) in Falcon dishes. The cells of the cumulus and corona radiata were removed by incubation for 30 seconds in Gamete medium with 40 IU hyaluronidase/ml (Type IV; Sigma Aldrich, St Louis, MO) (23). The removal of the cumulus and corona cells was enhanced by aspiration of the complexes in and out of a 135-μm internal diameter pipette (Stripper Tips; Mid-Atlantic Diagnostics Inc, Medford, New Jersey). Afterward, the oocytes were carefully observed under the inverted microscope at $\times 200$ magnification for maturity assessment.

The ICSI procedures were carried out on an inverted microscope (Eclipse TE 300, Nikon Corporation, Tokyo, Japan) at $\times 200$ magnification using the Hoffman Modulation Contrast System.