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ASSISTED REPRODUCTION TECHNOLOGIES

Temperature variations within and between incubators—a prospective, observational study

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Abstract
Purpose To determine if there is a temperature variation within and between incubators.
Methods This prospective, experimental trial with external controls was performed at an Assisted Reproductive Technology laboratory in a tertiary-care, university hospital. Temperature values were taken at various locations within and between incubators.
Results Even though they were both set to 37.0 °C, the same make and model incubators had significantly different internal temperatures. Temperatures differed significantly among top, middle and bottom shelves and between fronts and backs of shelves.
Conclusion(s) We found temperatures differed within and between our front-loading incubators. Thus, laboratory personnel should evaluate their incubators for temperature variations within and between incubators and, if temperatures differ significantly, develop a plan to deal with discrepancies.

Keywords Temperature · Incubator · Quality control · Assisted reproductive technology · ART

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Capsule Temperatures differ at different locations within the same incubator and between incubators.

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Introduction

To have a successful Assisted Reproductive Technology (ART) program, there are many variables that laboratory personnel must take into consideration. One such variable is temperature. Scientists have conducted numerous studies to investigate the affect temperature has on embryo development and pregnancy rates [1–4]. Because temperature has a direct impact on embryo homeostasis [1], it is important to monitor and control temperatures in the ART laboratory [1–7].

Previous reports confirm that variations among temperature can alter embryo development. Eng and associates reported in vivo maturation of pig oocytes occurs better at 39 °C than at 37 °C [2]. Abramczuk and Lopata found the highest human in vitro embryo cleavage progression rate and pregnancy rate were obtained at 36.9°C [3]. In rat models, Kimmel and associates demonstrated that embryos were adversely influenced by elevated temperature and that an increase in culture temperature could be lethal [4]. Rat embryos cultured 2 °C above normal body temperature (normal body temperature of rats is 38.0 °C) demonstrated altered embryonic development and organogenesis [4]. Embryo culture below 37 °C is not as detrimental; however, embryo development is slowed [3].

Generally, among ART laboratories, temperature is recorded once daily at one position inside an incubator. However, within an incubator, internal temperature differentials between and among shelves may exist. Higdon and associates reported that human embryos cultured on middle shelves of front-loading incubators had better odds of generating a pregnancy compared with embryos placed on the top or bottom shelves [5]. The reason for improved embryo development on the middle shelf is unknown, but may be caused by temperature variations.

The aim of this study is to investigate temperature differentials inside front-loading incubators typically used in ART

laboratories. With the use of wireless temperature probes, we evaluated temperatures between the front and the back of incubators, between and among incubator shelves, and between incubators.

Materials and methods

Background

This was a prospective, observational study that was conducted in an Assisted Reproductive Technology laboratory located in a tertiary-care, university hospital in Greenville, South Carolina. This study was conducted between May 31, 2012 and June 25, 2013. Because no patient data were involved, this study did not require IRB approval.

Mechanism of action for the CIMScan

We used CIMScan (CIMTechniques, Inc., Beaufort SC) technology to monitor temperature changes within incubators. CIMScan technology used wireless temperature probes that were located inside incubators at predesignated locations. The probes sent temperature measurements to a monitoring station, which then routed data to a server where it was stored.

Validation of CIMScan

To ensure accuracy, CIMScan was validated with the use of two manufacturer-calibrated National Institute of Standards and Technology Greisinger GMH 3230 digital thermometers (NIST; Greisinger Electronics, Germany). Before the study began, temperature was recorded for randomly selected equipment within the laboratory to ensure both thermometers agreed within +/−0.1 °C.

We took 30 measurements with each of the NIST thermometers and compared the data with data from CIMScan. The CIMScan probes were recalibrated to match the average value obtained from the NIST thermometers. To insure values obtained from the CIMScan were reliable, this process was repeated a second time.

Protocol

Two, three-shelf Forma incubators (Series II/3110 Thermo Electron Corporation, Marietta, OH) were used in this study. Both incubators were set to deliver a temperature of 37.0 °C. We placed a CIMScan temperature probe in the front of the top shelf. Before temperatures were recorded, the door of the incubator was closed and the internal environment of the incubator was allowed to equilibrate. Temperatures were recorded every 5 min for 4 h. The probe was moved to the back of the shelf and the process was repeated. This procedure was repeated for every shelf in both incubators. Thirty temperatures were recorded for each position. The thirty temperatures, at each incubator position, were chosen during a time at which room temperature was most stable. Because room temperature affects equipment [6, 7], room temperature also was recorded.

Table 1 Temperatures within two Forma (Series II, Model 3110) front-loading incubators. (Data are pooled for both incubators)

Shelf position	Front of shelf temperatures (°C; n =60/location)	Back of shelf temperatures (°C; n =60/location)	P value
Top	36.92±0.04	36.65±0.05	< .001
Middle	36.78±0.24	36.64±0.07	< .001
Bottom	36.85±0.05	36.75±0.05	< .001
P value	< .001	< .001	

Means ± standard deviation

Statistical methods

No changes to the methods were employed during this trial and no data were lost. Analysis of the data was performed with *SPSS* Version 16.0 (IBM Corporation, Somers, New York). Paired t test, chi-square test, and 1-way ANOVA were used to evaluate differences among temperature values. We reported

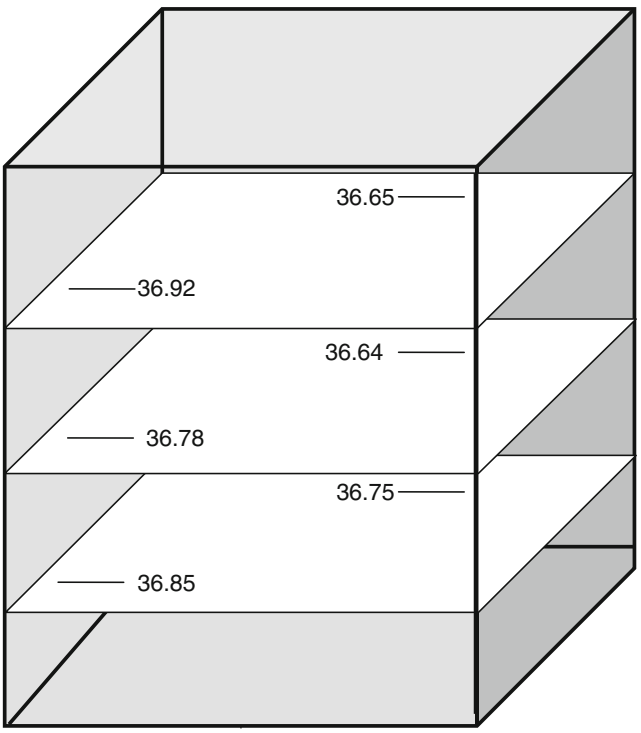

Fig. 1 Pooled temperatures (°C) for two Forma (Series II, Model 3110) front-loading incubators (60 values per location). Note that temperatures differ between fronts and backs of shelves and among shelves

temperature as mean ± standard deviation with significance set at $P<.05$.

Results

Temperature varied between incubators. We pooled the data from both incubators for all of the following analyses. The temperature difference between the two incubators was significant ($P=<.001$; 36.79 °C±0.14 °C versus 36.73 °C± 0.15 °C). Inside temperatures between the fronts and backs of incubators (all shelves included) were significantly different (36.85 °C±0.15 °C in the front of the incubators versus 36.68 °C±0.08 °C in the back; $P<.001$).

When the three shelves were evaluated as separate units, temperatures between the fronts and backs of shelves were significantly different (Table 1; Fig. 1). When we evaluated the front of the shelves of both incubators separately, we found them to be significantly different ($P<.001$; Table 1). Furthermore, the same was true for the backs of these shelves ($P<.001$; Table 1). When we combined the values for the fronts and backs of the three shelves for the two incubators, temperatures were still significantly different ($P<.001$; 36.80 °C±0.14 °C for the top shelves, 36.71 °C±0.19 °C for the middle shelves, and 36.78 °C±0.07 °C for the bottom shelves).

Discussion

The study objective was to investigate temperature variation within a front-loading incubator. With the use of wireless sensors, we were able to evaluate temperature in two similar front-loading incubators. Temperatures between incubators and among shelves were significantly different. In addition, temperatures between the front and back of each shelf and among the fronts and backs of all shelves were significantly different. These temperature differences may have an effect on gamete/embryo development.

In a preliminary study ($n=74$), we compared ART patient pregnancy rates (PR) for each shelf. All embryos were located in the back of the incubator so only shelf differences, and not location on shelves, could be compared. We found that PR did not differ among the three shelves, but there was a trend towards significance ($P=.083$). In the future, temperatures of various shelves that contain gametes/embryos should be monitored to determine if temperature differences do alter development and pregnancy rate.

Besides the advantage of being able to monitor a piece of equipment continuously, there are other advantages to using CIMScan as well. We have the ability to set acceptable temperature ranges for our incubators and if temperatures fall outside these ranges, an alarm notifies us so that adjustments can be made. Furthermore, real-time temperature data can be accessed from a remote site, which allows for more careful monitoring.

Most laboratory personnel record incubator temperatures once daily from one location. Our study is superior to other studies because of the use of wireless technology that gathers data from wireless sensors and allows for continuous monitoring of various sites within incubators.

Because we had few temperature probes, a limitation to this study was that not all temperatures were taken at the same time. Because of this limitation, temperatures may not be as comparable as when numerous probes are in the same incubator recording values at the same time. This limitation introduces the possibility of changes because of alterations in room temperature. In the future, it would be beneficial to use several probes to record temperatures simultaneously.

In conclusion, temperature variation does exist within the internal environment of front-loading incubators typically used in ART laboratories. Each ART laboratory is different; to ensure consistent culture conditions are maintained, technicians should evaluate and monitor temperature variations within their own incubators.

Conflicts of interest None of the authors has a conflict of interest.

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