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The Efficacy of Test Tube Warming Devices Used During Oocyte Retrieval for IVF

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***Purpose*:** To investigate whether commonly used test tube warming devices maintain a constant temperature in follicular fluid aspirates.
***Methods*:** By using a digital thermocouple, temperature was measured and comparisons were made between an analog dry block heater, a digital dry block heater, and a thermostatic test tube heater.
***Results*:** For small fluid volumes, temperature in the block heaters increased above 37°C after being in the block for over 2 min. The thermostatic heater maintained a constant temperature, but this was below the factory setting of 36.9°C. Temperature maintenance was influenced by fluid volume in each tube.
***Conclusions*:** One of the key factors in the handling of gametes and embryos is the maintenance of constant temperature. Test tube warming devices require verification of their ability to maintain fluid at the desired temperature. Temperature may vary with fluid volume and the type of test tube warming device used.

KEY WORDS: Fluid volume; heater; temperature; test tube; warming device.

INTRODUCTION

Providing optimal physiological conditions in vitro is of utmost importance to cell and tissue culture. Because variation in culture temperature alone can significantly compromise cell growth, many warming devices, such as incubators, warming stages, and dry block heaters have been designed to maintain constant temperature in laboratories. However, for routine assisted reproductive technologies (ART), temperature fluctuations can easily occur whenever gametes or embryos are manipulated. On the basis of the evidence from bovine studies, heat shock can alter the ultrastructural morphology in early embryos (1), affecting cleavage and subsequent blastocyst formation (2,3). With polarized light microscopy, it has been shown that human meiotic spindles are extremely sensitive to cooling and temperature fluctuations (4). Change in spindle integrity may result in abnormal chromosomal distribution which may in turn lead to abnormal fertilization and suboptimal embryo development.

Although sophisticated culture media have been developed to meet the metabolic requirements of gametes and/or embryos, there is limited information about the precision of equipment commonly used in human ART. A recent study (5) assessed the maintenance of temperature with different heating surfaces, types of incubator and culture vessels, and volume of media used. It was shown that when culture vessels are placed outside an incubator, there is rapid cooling but only slow rewarming of the culture medium (6).

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Since temperature fluctuation can negatively influence developmental potential, it is essential to sustain a constant environment once an oocyte is aspirated out of its in vivo environment. The aim of this study was to assess the ability of commonly used test tube warming devices to maintain constant temperature in various volumes of culture medium used for IVF. This would mimic the clinical situation where follicular aspirates of varying volumes are recovered during oocyte retrieval.

MATERIALS AND METHODS

Temperature was measured utilizing a digital thermometer (Model HH23, OMEGA Engineering, Inc., Stamford, CT) and a thermocouple (Type K, OMEGA Engineering, Inc., Stamford, CT). Calibration was performed by the manufacturer, with an accuracy of ±0.2°C. Three different test tube warming devices were assessed: 1) an analog dry block heater (Model 110004-2, Boekel Scientific, Feasterville, PA); 2) a digital dry block heater (Model MD-02-220, Major Science, Jershuenn Enterprise Co. Ltd., Taiwan); and 3) a thermostatic test tube heater (COOK, Queensland, Australia). While the test tube heater from COOK was specifically designed for IVF use and embraces the whole length of a test tube (Falcon 2057, New Jersey), the blocks inside the two types of dry block heaters cover only the bottom half of a test tube, equivalent to a volume below 6 mL, or a length of 43 mm measured from the bottom of the tube.

The dry block heaters were set according to manufacturers' instructions so that a Falcon 2057 tube with 11 mL of distilled water would measure 37.0 ± 0.5°C with a thermocouple after 1 h of equilibration. The test tube heater from COOK was preset at 36.9 ± 0.5°C and could only be reset by the manufacturer.

Four different volumes of medium (Modified HTF Medium with HEPES, Irvine Scientific, Santa Ana, CA) were used in this study: 2, 5, 7, and 9 mL. These volumes were chosen as being representative of what would commonly be seen at the time of oocyte retrieval for in vitro fertilization. For the first two volumes, the medium was completely surrounded by the dry heating block; for the latter volumes, only medium below the 6-mL marking was surrounded. The thermocouple was inserted into the bottom of the test tube and secured with tape on the outside. To simulate the oocyte retrieval procedure, all test tubes were capped during the experiment. Each of the test tubes was brought to 37.0°C initially (to mimic the in vivo environment) and then held inside the palm of a gloved hand for 20 s (to mimic the transfer of a follicular aspirate from the clinical to the laboratory staff) before the tube was placed into the test tube warming device. Temperature measurements were taken every 10 s for a total of 5 min. The experiment was performed and repeated 10 times inside a Microflow Laminar Flow Work Station (Model 50548, MDH Limited, Hampshire, UK), with the laminar flow turned off and a constant room temperature of 23.5°C.

RESULTS

Temperature maintenance for the three test tube warming devices using increasing volumes of fluid is shown in Figs. 1–3. For all fluid volumes examined, the temperature decreased during the 20 s when the test tube was held in the hand and also during the first 10 s that the fluid in the tube was inside the warming device. For 2 mL volumes (Fig. 1), the three warming devices warmed the fluid, although none could maintain the initial and desired temperature of 37°C. In both the analog and the digital dry block heaters, the temperature rose to 37°C in 1 min after rewarming; however, the temperature then continued to rise over the next 4 min to above 38°C. At each 10-s interval, there was also a temperature range of at least ±0.8°C with the analog heater. The average temperature range with the digital heater was ±0.2°C. In the thermostatic test tube heater, the factory setting of 36.9°C was not reached and the temperature was kept constantly at around 36.4°C with a range of ±0.2°C.

Figure 2 shows the results for 5 mL volumes. After the 20 s of initial cooling, there was a further 20-s interval of temperature reduction inside all three warming devices. In both types of dry block heaters, the temperature rose to 37°C in 2 min after rewarming. Then the temperature continued to rise to above 37.5°C over the next 3 min, with an average temperature range of ±0.6°C in the analog heater and ±0.3°C in the digital heater. Similar to the results obtained with a 2 mL volume, the thermostatic test tube heater maintained a relatively constant temperature of around 36.4 ± 0.2°C.

Temperature measurements using a 7 mL volume are shown in Fig. 3. The fluid was again successfully rewarmed to 37°C in 2 min inside both types of dry block heaters. Temperature was then maintained constant at 37.2°C by the analog heater and at 37.3°C by the digital heater, with ranges of ±0.6°C and ±0.2°C respectively. Although the thermostatic

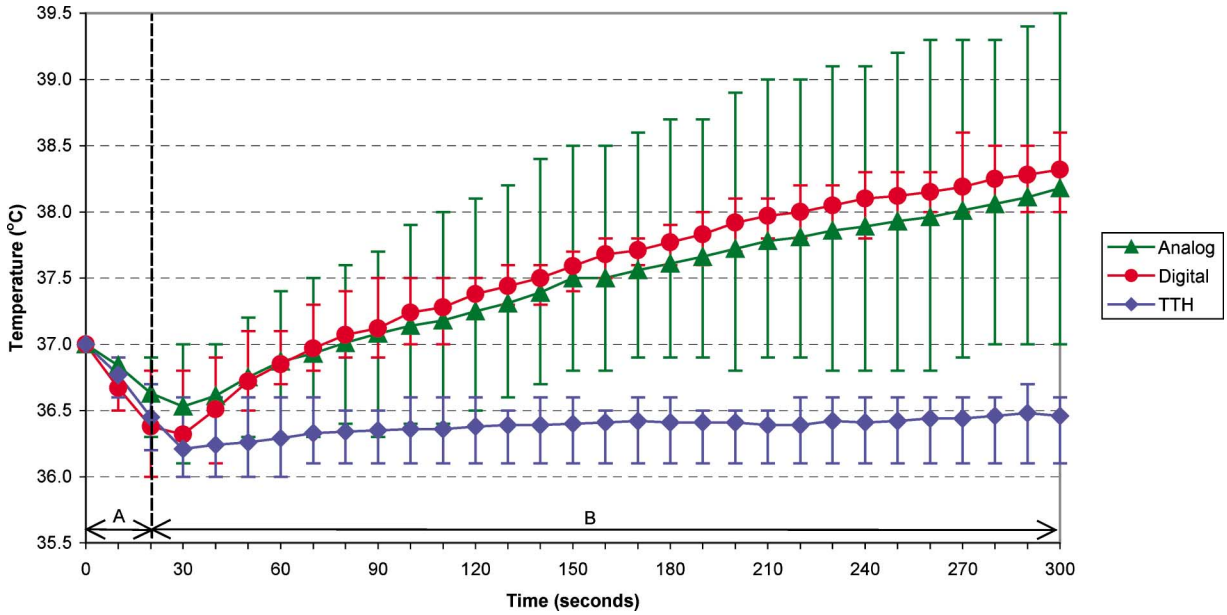

Fig. 1. Temperature maintenance in a 2-mL volume by three types of test tube warming devices. The error bar at every 10-s interval represents the range spanned by the maximum and minimum temperatures obtained for that interval. Period A denotes the time when the tube is held in hand while period B denotes the time when it is held in the warming device. Analog: analog dry block heater; Digital: digital dry block heater; TTH: Cook's test tube heater.

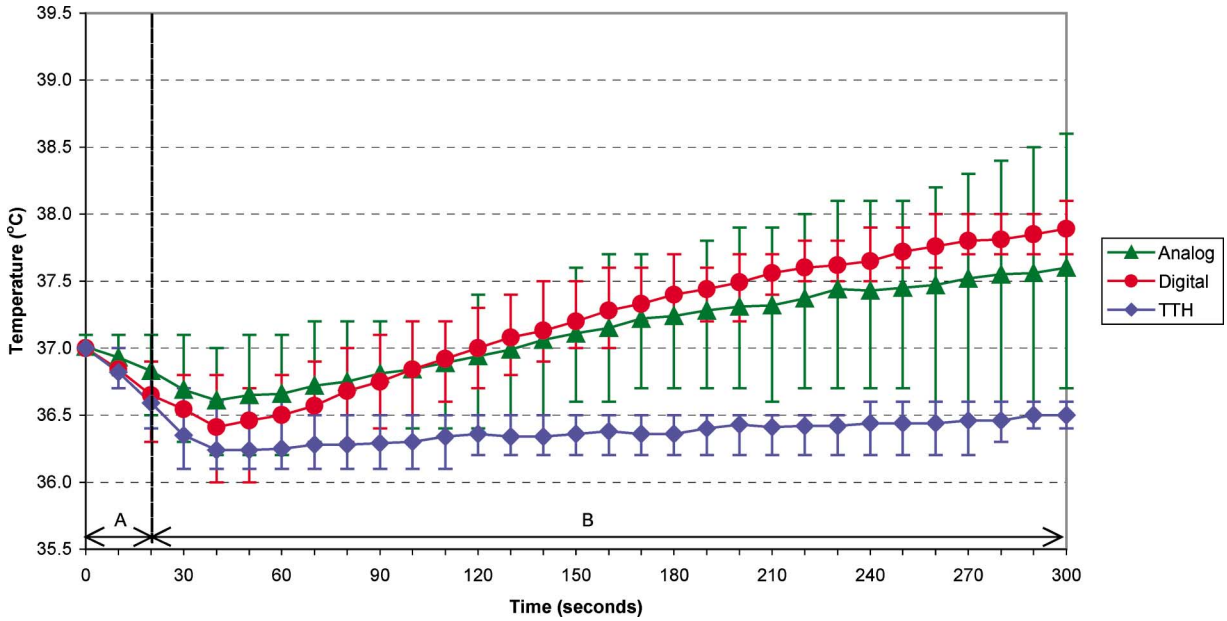

Fig. 2. Temperature maintenance in a 5-mL volume by three types of test tube warming devices. The error bar at every 10-s interval represents the range spanned by the maximum and minimum temperatures obtained for that interval. Period A denotes the time when the tube is held in hand while period B denotes the time when it is held in the warming device. Analog: analog dry block heater; Digital: digital dry block heater; TTH: Cook's test tube heater.

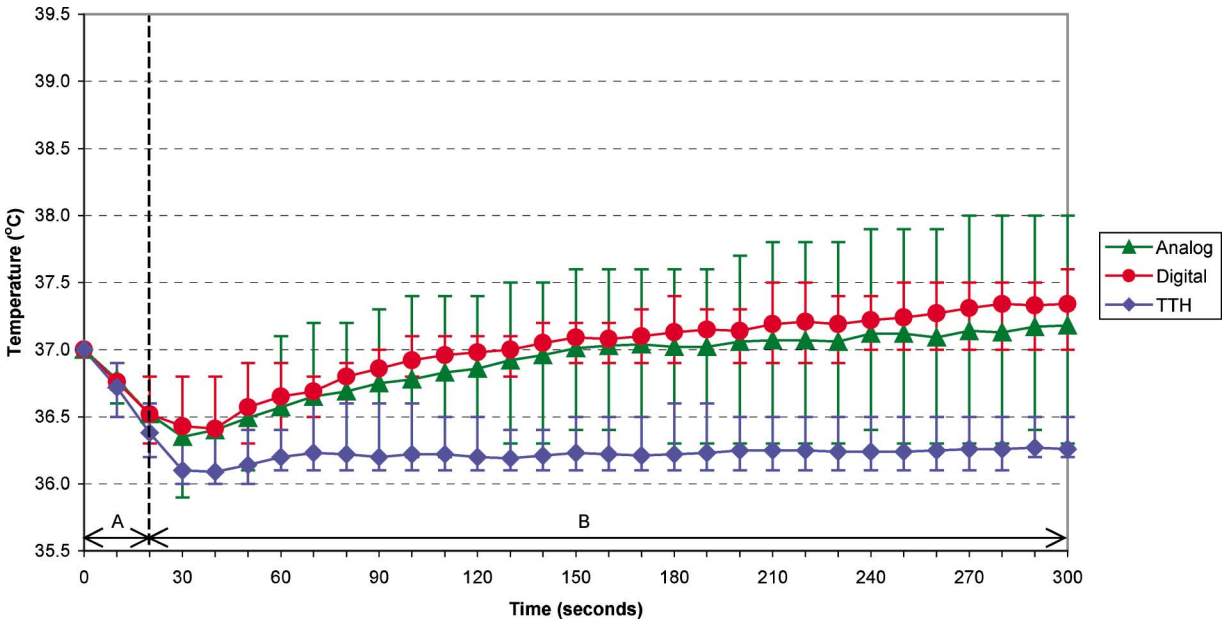

Fig. 3. Temperature maintenance in a 7-mL volume by three types of test tube warming devices. The error bar at every 10-s interval represents the range spanned by the maximum and minimum temperatures obtained for that interval. Period A denotes the time when the tube is held in hand while period B denotes the time when it is held in the warming device. Analog: analog dry block heater; Digital: digital dry block heater; TTH: Cook's test tube heater.

test tube heater also sustained a constant temperature, this was around 36.3°C within 5 min, with a range of ±0.2°C.

Results for 9 mL volumes were very similar to those obtained for 7 mL volumes (data not shown). The fluid was rewarmed to 37°C and maintained by the analog dry block heater and the digital dry block heater at 37.1 ± 0.6°C and 36.7 ± 0.3°C, respectively. Temperature was sustained at 36.3 ± 0.2°C by the thermostatic test tube heater.

DISCUSSION

Some fluctuation of temperature in the fluid containing gametes and/or embryos is inevitable whenever these are handled outside of the incubator. For over a decade, clinical ART laboratories have relied on test tube warming devices to keep aspirated oocytes at body temperature when transiting from follicular fluid to in vitro culture. In this study we have compared the efficacy of a device designed specifically for such a purpose with traditional dry block heaters commonly used for general warming.

In theory, the heat output from a dry block heater remains constant once the desired temperature is achieved, unless the thermostat becomes faulty or the setting is altered. The setting of an analog dry block heater involves adjustment using a knob while that of a digital heater utilizes a key-in control. However, the work to be done by either type of heater is to transfer heat energy from the heater to the dry block and then from the dry block into the test tubes. Because of this indirect mechanism, the device may require a setting above 37°C to achieve body temperature because of the influence of ambient temperature. Verification of the temperature of the medium inside a test tube is required to accurately set the dry block heater to the desired temperature. In our study, verification was performed using 11 mL of distilled water (the volume of an almost completely full Falcon tube). Although this method seemed logical, it was not appropriate when volumes lower than 11 mL were used in the dry block heaters. Thermodynamically, the heat capacity of a larger volume of fluid is greater than that of a smaller volume, i.e. a larger volume can buffer heat gain and heat loss more easily; therefore, the 7 and 9 mL volumes used in this study more closely resembled changes in the verification volume than did the 2 and 5 mL volumes. We found that temperature could be well controlled in the two larger volumes at around 37°C on average, while with the two smaller volumes, the fluid became overheated within 2 min. On the other hand, the thermostatic test tube

heater used in our study was preset at 36.9 ± 0.5°C and it warmed the tubes of various volumes by direct heat transfer from the back panel. Although the device did not overheat the medium and the temperature of all volumes was constantly maintained, a temperature of around 36.4°C was found which was lower than that desired and lower than the preset temperature.

A major factor contributing to the performance of the warming devices is the design of the heating block itself. The dry block of both traditional heaters surrounds the bottom half of Falcon test tube, covering a volume up to 6 mL. Therefore, heat is gained where the fluid is surrounded by the block while heat is lost where the tube is exposed at the top. The rate is dependent on the ambient temperature and the time spent in the block. Although this study only investigated two volumes of less than 6 mL and two volumes over 6 mL, the temperature differences between these two groups were obvious. While the larger volumes could almost be maintained at the desired 37°C, the smaller volumes became overheated if left too long. The test tube heater overcomes this problem by evenly warming the whole length of a test tube.

It is clear that variance in warming capacity and efficiency existed among the three test tube warming devices, especially when considering the overall temperature range spanned by the various volumes. However, in an oocyte retrieval procedure following controlled ovarian stimulation, ovarian follicles are not uniform in size and contain different volumes of follicular fluid. Depending upon the size of the follicles aspirated, the volume in each tube given to the laboratory personnel who is identifying whether an oocyte is present will be large, and will cover the volumes investigated in this study. It is impractical to restrict the clinician to provide follicular aspirates of one uniform volume. In addition, the time that aspirates are left in the warming device will vary depending on the speed at which the follicles are aspirated and also on the total number of aspirates from each patient. The longer the aspirates are left in the block awaiting inspection, the more dependent we are on the efficiency of the warming device. If a dry block heater is being used to warm test tubes during an oocyte retrieval procedure, the clinician may have to coordinate with the laboratory staff so that each test tube of follicular aspirate spends no more than two min warming in the device.

The thermostatic test tube heater used in this study provided the most consistent temperature at all volumes tested, but the temperature did not reach the setting claimed by the manufacturer. Although the clear front panel allows continuous observation of test tube contents and the unit gives more precise temperature control compared with traditional dry block heaters, there are a number of disadvantages associated with it. Firstly, it is impossible for the user to set or adjust the temperature. As shown in our study, the temperature achieved was around 36.4°C as opposed to the preset 36.9 ± 0.5°C. Secondly, this preset temperature may not be suitable for all ART laboratories because of possible differences in ambient conditions. Thirdly, the unit can only hold six test tubes while a dry block can hold 12 tubes. Finally, this equipment is more expensive than a traditional dry block heater, and the device may need to be sent back to the manufacturer for inspection which is both costly and time-consuming.

One unexpected finding in our study was that the temperature decreased both when the tube was in the gloved hand and also during the first 20 s inside the warming device. In an oocyte retrieval procedure, the aspirated follicular fluid is often cooled while it transverses the aspiration needle. An oocyte has to experience some cooling in the follicular fluid from the moment it is aspirated out of the body and is being transported to the laboratory even if this takes only seconds. Further time lapses occur before it can be identified and transferred to a more physiological environment with the desired body temperature. We simulated the above process by allowing the various fluid volumes to be in transition inside the warm and gloved palm of our technician for 20 s. At least 10 s of further temperature decrease ensued before the four different fluid volumes tested could be rewarmed.

On the basis of our study, units are advised to check their own equipment to ensure that the temperature in aspirated fluid is kept within whichever limits they are satisfied with. If a dry block heater is used, aspirates should not be kept in the block too long and small aspirates should be examined as soon as possible. This attention to detail should help to keep oocytes in good condition as they enter the culture process.

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