

Physical validation of a novel climate-controlled embryo-view enabled miniature incubation chamber for uninterrupted embryo culture

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Abstract

Introduction

Embryo culture chambers were utilized in the 1980's through late 1990's and thereafter were phased out because it was cumbersome and caused damage to the embryo especially if these plastic chambers were filled with un-warmed incubation gas. In these incubation chambers the dishes had to be taken out of the chamber for routine microscopic examination which interrupted and altered the climate-controlled conditions drastically contributing to poor embryo viability.

Materials and methods

A cheaper alternative that permitted uninterrupted embryo culture was investigated. Physical functionality validation tests were performed on a novel air-tight miniature incubation chamber (MIC) obtained from Androcryogenics Malaysia (Patent pending no. PI2023000385).

Results

The drop in temperature in the chamber of the MIC where the culture dishes are held is insignificant with no or insignificant loss of temperature, 0°C, -2°C, -4°C and -1.2°C at 1, 3, 5 and 10 minutes respectively. The MIC allowed the microscopic examination of the developing embryos cultured in the MIC without the need to take the culture dishes out of its chamber.

Discussion and Conclusion

The present study indicate excellent climate-control by the MIC. The drop in temperature in the chamber of the MIC where the culture dishes are held is insignificant with no or insignificant loss of temperature, 0°C, -2°C, -4°C and -1.2°C at 1, 3, 5 and 10 minutes respectively when the MIC was held outside of the incubator on a warm microscope stage. Furthermore the air-tight MIC held the humidity and incubation gas mixture within its chamber unaltered so that the oxygen and carbon dioxide tension, pH, osmolality and other culture conditions remained unaffected providing conducive uninterrupted culture conditions with the potential to prevent damaging environmental insults. The next course of investigation is to perform a clinical trial on the MIC.

Disclaimer: The first author (JA) is the inventor of the MIC, remaining authors have no conflicts of interest.

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Introduction

In the 1980s and early 1990s some workers utilized culture chambers in an attempt to maintain climate-controlled conditions which was supposedly thought to be conducive for embryo culture. Most of the commercial culture chambers were made of plastic material and, were large and cumbersome. An example of which was the Billups-Rothenberg Chamber. This method of "controlled-climate " was tried

but discontinued because it did not improve culture conditions nor the pregnancy rate, which hovered around a dismal 9 to 15% at that time. The major disadvantage of this mode of culture was it takes a lot of time for the incubation gas inside the plastic chamber to warm and stabilize to 37°C. The poor heat conductivity of the plastic material of the chamber made warming the incubation gas mixture inside the chamber

Fig. 1: The miniature incubation chamber (MIC)



Fig. 2: Component parts of the miniature incubation chamber (MIC)



difficult because of the poor heat conductivity and besides that, the chamber was large and bulky. Its large size made it was more difficult to warm up the incubation gas in its chamber. Some workers used gas warmed in a water bath held at 37°C but this did not improve the culture conditions. In retrospect, it is an extremely ill-advised method of embryo culture because by the time the incubation gas inside the incubation chamber took to stabilize at 37°C, the viability of the gametes and embryos may have been compromised. Furthermore the chamber had to be taken out of the incubator and then the dishes out of the chamber for routine microscopic examination of the culture dish, all which will interrupt (Alhelou et al., 2018) and alter the climate-controlled culture conditions drastically contributing to poor embryo viability.

Over the last two decades innovations in all aspects of laboratory IVF, and in particular climate-controlled incubator design, improved culture conditions. However with almost all incubators, with the exception of the time-lapse incubator (TLI), there is a break in the climate-control chain because the incubator has to be opened and the embryo culture dish taken out for routine microscopic examination.

At the present time the TLI alone appears to maintain the climate-control chain throughout the culture or incubation period but it is expensive and is beyond the means of many clinics worldwide. It is common knowledge those clinics that purchased the TLI passed on the cost to their clients, which raised the cost of treatment.

The question is how to maintain the controlled-climate chain and provide uninterrupted culture conditions during embryo incubation without investing large sums of funds? The challenge is to maintain embryo culture conditions identical to that in the incubator when the embryo culture dish is taken out for routine microscopic examination or handling. Here we describe the physical functional evaluation of an embryo-view enabled miniature incubation chamber provided by Androcryogenics Malaysia Sdn Bhd (MIC; Patent pending; no. PI2023000385; Ali, 2023) that could provide climate-control and uninterrupted culture conditions.

The MIC can be held outside of the incubator for 3 to 5 without significant perturbations and even up to 10minutes with minimal alteration in the chamber temperature outside of the incubator during routine microscopic embryo examination, specifically, without loss of climate-controlled culture conditions. The embryo can be microscopically observed without the need to take out the culture dishes out of the chamber because the MIC allows the embryos to be visualized through its glass base and top. Furthermore the air-tight MIC held the humidity and incubation gas mixture within its chamber unaltered so that the carbon dioxide and oxygen tension, pH, osmolality and other culture conditions were not affected. This attribute provided a conducive uninterrupted culture condition preventing damaging environmental insults on the cultured embryos or cells.

Materials and methods

Physical features of the phase 2 prototype of the MIC.

The MIC was obtained from Androcryogenics (Malaysia) Sdn Bhd. It is constructed of an air-tight high grade 316 stainless steel embryo incubation chamber of dimensions, 143mmL x 83mmW and 30mmH (Image1: Phase 2 of the MIC prototype.). It is quite small and sits on the palm of the hand. It is a miniature incubation chamber that consists of a detachable base container that accommodates maximum two 60x15mm round or two 4-well dishes, a detachable 316 grade stainless-steel rack to immobile the culture dishes and a 316 grade stainless-steel upper clip-on air-tight lid (fig 1).

The lower base container unit holds the detachable culture dish rack. The rack can be removed for cleaning and heat or steam sterilization. Its height is about 7mm with central circular and superimposed square holders cut into the rack to hold circular culture dishes of size 15mm height and 60mm diameter dishes and 4-well square dishes of 66mm x 66mm with about 14 -17mm height. Appropriate racks are also made for other types of culture dishes or vehicles such as the multi-well dishes, 6- to 100-well dishes or 25-50ml culture flasks (Fig 2).

The incubation chamber has 2 lockable air-tight metal nozzles or control valves that will

Table 1: Pre-clinical trial physical functionality validation tests

Description	Thermometer description	Time in minutes/Loss of temperature in °C				
		0 min	1min	3mins	5mins	10mins
Test 1: Drop in temperature in 4-well culture dish when taken out of incubator at 37°C and placed on cold surface	Thermometer readings	37	34.4	<32*	<32*	<32*
	Loss of temp	0	-2.6	> -5**	> -5**	> -5**
Test 2: Drop in temperature in 4-well culture dish when taken out of incubator at 37°C and placed on warm micro-scope stage at 37°C	Thermometer readings	37	35.7	34.9	34.1	33.5
	Loss of temp	0	-1.3	-2.1	-2.9	-3.5
Test 3: Drop in temperature in round dish taken out of the incubator at 37°C and placed on cold surface	Thermometer readings	37	34.7	<32*	<32*	<32*
	Loss of temp	0	-2.3	> -5**	> -5**	> -5**
Test 4: Drop in temperature in round dish culture dishes taken out of incubator at 37°C and placed on warm microscope stage at 37°C	Thermometer readings	37	35.9	35.3	34.1	34.4
	Loss of temp	0	-1.1	-1.7	-2.2	-2.6
Test 5: Drop in temperature of digital thermometer taken out of incubator at 37°C and placed on cold surface	Thermometer readings	37.2	35.5	31.3	28.5	24.2
	Loss of temp	0	-1.7	-5.9	-8.7	-13
Test 6: Drop in temperature of digital thermometer taken out of incubator at 37°C and placed on warm microscope stage at 37°C	Thermometer readings	37.1	36.1	34.4	33	31.4
	Loss of temp	0	-0.9	-1.2	-1.6	-2.6
Test 7: Drop in temperature of digital thermometer held in MIC taken out of incubator held at 37°C and placed on cold surface	Thermometer readings	37	36.8	36.3	35.9	34
	Loss of temp	0	-0.2	-0.7	-1.1	-3
Test 8: Drop in temperature of digital Thermometer held in MIC taken out of incubator held at 37°C and placed on warm microscope stage at 37°C	Thermometer readings	37.1	37.1	36.9	36.7	35.9
	Loss of temp	0	0 Best out--come	-0.2 Best out--come	-0.4 Best out--come	-1.2 Best out--come

serve as an inlet and outlet incubation gas mixture. The air-tight lid and the leak-proof gas nozzles holds the incubation gas mixture inside the incubation chamber after the valves are locked to maintain the gas mixture at the desired level inside the incubation chamber.

The high quality optical glass plates are attached to its respective lower base container unit and the upper clip-on lid of the incubation chamber and hermetically-sealed so that air-tightness can be assured within its chamber. These glass plates offer the operator the ability to view the developing embryo within the chamber of the MIC under the zoom stereo microscope. Almost, if not all, makes of zoom stereo microscopes can accommodate the MIC and be used to visualize the embryos cultured within the MIC. The glass plates are of the highest optical quality which allows visualization of the embryo/cell/tissue specimen. Each of these four components can be detached to enable routine cleaning or heat or steam sterilization of the incubation chamber [Patent application ref: PI 2023000385 (Ali, 2023)].

The various physical functionality validations tests performed on the MIC are given in left column of Table 1.

Results

The outcome of the physical functionality validation tests are given in Table 1. When dishes are cultured devoid of the MIC, the drop in temperature when placed on both cool and warm surfaces was dramatic, dropping below 32°C within 3 mins. In comparison, however, if these dishes were held within the MIC, the loss in temperature was less, especially when placed over warm surface of the heated stage of the microscope.

The MIC held the temperature of culture dishes longer when placed on even cool surface and much longer on warm surface. Therefore loss of temperature was very minimal if the MIC was used, especially on warm surfaces the loss was insignificant.

The humidity level inside the MIC remained constant at 99% for 5 days. This meant the MIC

was air-tight, and would maintain the incubation gas mixture unaltered at 5% CO₂ in air for 5 days, therefore pH would remain unaltered. The phenol red color of the media held in the MIC was similar to control.

Discussion

It is universally recognized that when the embryo culture dish is taken out of the incubator for routine microscopic examination, the embryos will suffer damage due to a rapid alteration to its climate-controlled incubation system resulting in detrimental environmental insults which could damage the embryo to varying degrees of harm from minimal or potentially hazardous maximal, the latter occurs if the embryos are kept outside of the incubator for far too long.

This alteration to controlled-climate conditions result in a drop in culture temperature, loss of incubation gas and humidity, the time-dependent progressive increase in the pH of the culture medium due to loss of carbon dioxide incubation gas mixture, and to changes in partial pressure of oxygen in the culture medium, changes in its osmolality, exposure to light, exposure to volatile organic chemicals (VOCs) and free radicals (Higdon et al., 2008; Swain 2012, 2014, 2019; Awonuga et al., 2013; ESHRE guidelines, 2015; Vienna Consensus, 2017; Nguyen et al., 2018; Swearman et al., 2018; Mortimer et al., 2018; Mortimer, 2023).

These damages may not occur when the embryos are cultured are held within the MIC. The present findings indicate excellent climate-control by the MIC. The drop in temperature in the chamber of the MIC where the culture dishes are held is insignificant with no or insignificant loss at 1 minute, 0°C, -0.2°C, -0.4°C and -1.2°C at 3, 5 and 10 minutes respectively. This drop in temperature is unlikely to damage the culture conditions and the embryo. This attribute is expected to help increase clinical pregnancy and cryopreservation rates. The MIC could be very useful in teaching/academic settings as it allows longer microscopic examinations thus assist in training workers without or insignificant damage to the embryos.

Unlike the dynamic and well-controlled in vivo environment of the embryo, the in vitro environment is less controlled. The incubators are built to provide almost optimal climate conditions that favor embryo development. It has been reported that reduction in exposure of human embryos outside the incubator enhances embryo quality and blastulation rate (Zhang et al., 2010). Although the incubator supposedly offers climate-controlled condition where all the physical factors required for the development of embryos are maintained at an almost optimal condition, however it is well recognized fluctuation that affects this controlled-climate environment occur when the incubator door is opened (Nguyen et al., 2018) which causes an interruption in the incubation process which is detrimental for embryo development (Alhelou et al., 2018).

Furthermore subtle fluctuations are known to occur within the incubator concomitant to changes in laboratory ambient temperature (Alhelou et al., 2020) but this change within the incubator is not displayed by the incubator. Indeed Fujiwara et al. (Fujiwara et al., 2007) reported that the mini-incubator took approximately 5 minutes to recover its temperature after a 5-second door opening/closing procedure, whereas the conventional incubator took 30 minutes. Additionally, the mini-incubator's recovery of oxygen tension was significantly better at 3.0 ± 0 minutes than it was in the conventional incubator (7.8 ± 0.9 minutes). This finding is completely logical which indicates incubators with smaller volumes recover faster.

The MIC described in the present report basically appears completely capable of circumventing the issues related to minor interruptions in incubator temperature that occurs during door openings or due fluctuating ambient temperatures. This is because the embryos cultured inside the MIC are protected from such perturbations since the MIC has the capability to hold its gas contents unperturbed and internal chamber temperature unaffected at 37°C even when taken out of the incubator for short periods of time. The MIC held the temperature of culture dishes longer when placed on even cool surface and much longer on warm surface. With the use of the MIC the development of the embryos or cells held within

its chamber can be viewed through its viewing port without having to take the dishes out of the MIC. It is very small in size and sits on the palm of the hand making it very convenient and manageable during embryo culture procedures.

Conclusion

In conclusion, embryo and cell culture dishes held in the MIC remained almost unperturbed when taken out of incubator for routine microscopic examination. The loss of temperature and alterations to the incubation gas mixture and the overall culture conditions within the MIC is very minimal and insignificant. This would prevent environmental insults that could result in harmful changes in pH, cold shock and other alterations to the culture conditions that could occur if the culture dishes were exposed to the environment during routine microscopic examination. The MIC enables uninterrupted embryo culture which is beneficial for human embryo and cell development in vitro.

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