Incorporation of the Cook K-Minc Incubator and Media System into the IVF Lab: The Future of IVF

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Introduction

Incubators and culture media are core components of today's In Vitro Fertilization laboratory. Over the past twenty years culture media has evolved from a simple salt solution such as Hams F-10, to the complex sequential culture systems in use today (1-5). In contrast, incubators, an equally important part of the culture system in human IVF, have had relatively little change over the same time period (6). The incubator in use in many IVF labs has an interior chamber volume of approximately 6.5 cubic feet. These incubators were originally designed for large scale culture of somatic cells. Their use in human IVF is a carry over from somatic cell culture systems. The large volume systems have been adapted and equipped with humidification pans to minimize evaporation and osmolarity changes in the culture media. The Forma 3110 is such an incubator (see Figure 1). These incubators maintain a very stable internal temperature and CO_2 concentration if kept closed. However, with the advent of sequential culture and frequent observations of oocytes and embryos, the incubators are opened often. The interior, when exposed to room air is rapidly dehumidified, the temperature drops, and the CO_2 concentration depleted. Once the door is closed the incubator injects CO_2 to restore levels to those present before the chamber was exposed to room air.

Figure 1:

Forma 3110 incubator *K – Minc 1000 incubator*

In contrast the K-Minc 1000 bench top incubator uses a very different system to maintain culture media temperature and pH. The Minc chamber consists of two heated surfaces with a chamber depth of 0.75 inches and a total volume of 0.3 cubic feet. The internal volume is further subdivided into four equal chambers of 0.075 cubic foot volume each. This system places a heated surface directly above and below the culture dish. This is in contrast to the Forma chamber where direct contact is on the bottom of the dish only, i.e. the shelf the dish rests upon with perforations to allow gas flow. The Minc sandwiches the dish between the upper and lower heated surfaces. The surfaces of the Minc are not perforated but form a solid heated surface. The lower surface also contains groves to fit the exact size

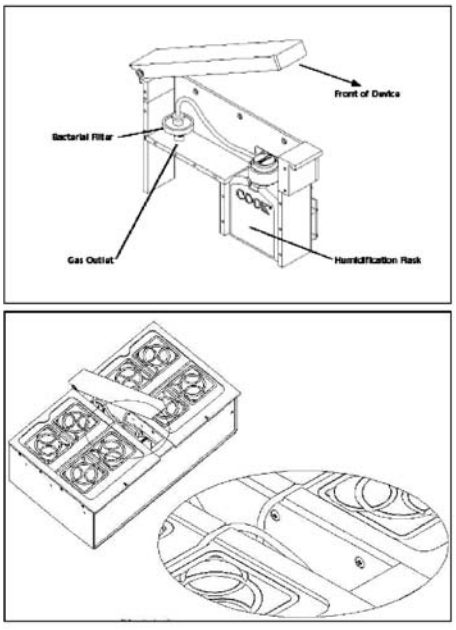
(continued on page 24)

(continued from page 21)

of the culture dish used and allows for direct contact of the dish surface with the lower heated surface. Culture dishes contain a small raised rim on the bottom plate to prevent scratching when moved on a flat surface. This raised rim fits neatly into the groves in the Minc lower heated surface.

The gas delivery system is also different from the Forma. The final concentration of CO_2 is not mixed or regulated by the incubator but is pre mixed in the cylinder by the gas supply company. The gas mixture used in our lab is 6% CO_2, 5% O_2 and 89% Nitrogen. The mixture is certified by our gas supplier. This pre mixed gas enters the rear of the Minc and passes through a 0.2 micron filter (Figure 2). After passing through the filter the gas is humidified by passage through a flask of sterile water. Once passed through the water, the gas is deposited into the 2 chambers of the Minc via a small piece of tubing inserted into a grove in the bottom of the heated chamber surface. There is a constant flow of gas at a rate of 15 ml/minute while the chamber is closed and a purge of 60 ml/minute for three minutes after the lid is opened and closed. The relative humidity of a chamber that has been closed for a minimum of 6 hours ranges from 75% to 80%. The small volume of the chamber allows rapid recovery from exposure to room air (lid opening).

Figure 2: K – Minc 1000 incubator gas delivery and humidification system



Temperature, humidity, and CO_2 inside of the chamber return to pre opening levels rapidly (Table 1).

In this study we measured the effects of incubation in these two types of systems and culture media by employing a 2 phase approach using sibling oocytes from IVF. In phase 1, we divided sibling oocytes from each IVF/ICSI retrieval between the Minc and Forma incubators - using a single culture media system from Sage Biopharma. In addition we compared recovery times for temperature, humidity, and CO_2 concentration between the incubator systems after removal of a culture dish. In phase 2, we divided sibling oocytes between the Cook media system and Sage media with incubation exclusively in the Minc incubators.

Materials and Methods

Controlled ovarian hyperstimulation for assisted reproduction consisted of one of three protocols. 55% of patients received a late luteal phase leuprolide acetate (Lupron,TAP Pharmaceuticals, North Chicago,IL) down regulation protocol used for young or women with polycystic ovarian disease for their initial cycle. 35% of patients received ganirelix acetate protocol (Anatagon, Organon, Roseland , NJ) used primarily for women over 36 or who had demonstrated some sign of diminished ovarian reserve. While 10% received a micro dose lupron flare protocol for a previous poor response to super ovulation. Pituitary desensitization was achieved with Lupron and /or oral contraceptive pills prior to beginning gonadotropins. On cycle day 2 or 3 after suppression, gonadotropins were given and adjusted based on an individual's response as determined by transvaginal ultrasound and serum estradiol levels. When there were at least six leading follicles measuring 16mm or more in diameter, hCG was administered to trigger ovulation. Follicles greater than 14 mm were aspirated 35 hours after hCG was administered.

At the time of oocyte retrieval, oocytes were aspirated using a rocket pump attached to a Cook 35 cm 17 gauge echotip ovum aspiration needle. Follicle flushing media consisted of warmed, modified HTF media, (MHTF) (Sage Biopharma). Follicles were aspirated into prewarmed, sterile10 ml Falcon snap cap tubes.

Incubators:

The Forma 3110 chamber is surrounded on 5 sides by a water jacket to act as a temperature stabilizer. The incubators are supplied by 100% medical grade

Table 1: Time to recover 90% of measured parameters after opening of incubator for 10 seconds

	K- Minc 1000 % change at opening	K- Minc 1000 – Time (minutes) to recover 90%	Forma 3110 % change at opening	Forma 3110 Time (minutes) to recover 90%	P value
Temperature	1.3	1	31	180	< .01
Humidity	43	12	44	180	< .01
CO_2	33	8	58	120	< .01

a. % change at opening = percent change in value post incubator opening for 20 seconds from reading prior to incubator opening.

b. Time (minutes) recover 90% = The amount of time it took the measured value to reach 90% of the original value prior to incubator opening.

$C0_2$. The CO_2 concentration is set on the unit to 6%. This CO_2 concentration maintains the pH of both the Sage and Cook medias at 7.2 - 7.3. The Forma incubator uses a CO_2 sensor to measure and adjust the interior chamber CO_2 concentration by mixing filtered room air and 100% CO_2 to achieve the set concentration. The gas mixture is circulated inside the chamber via a fan. All CO_2 and room air that enters the chamber are passed through a 0.2 micron filter inside the chamber. In our laboratory, the inner chamber also contains a Coda ultra filtration unit (IVF Online, Ontario, CA) that filters the interior chamber air through a carbon and 0.2 um filter designed to remove volatile organic compounds, chemical airborne contaminants, microorganisms, and particulates (7). The gas mixture is then passed through the water pan to add humidification to the chamber as well as to have the water act as a sink for any particulates that may be in the air. The coda filters are changed monthly per the manufacturer's recommendations. The water pan is changed and cleaned weekly and replenished with sterile water. The relative humidity of a chamber that has been closed for a minimum of 6 hours ranges from 85 to 95%. The chamber CO_2 concentration is monitored daily with a Labotect InControl 1050 infrared CO_2 analyzer (MidAtlantic Diagnostics, Mount Laurel, NJ). The chamber temperature is measured with a certified thermocouple (Labotect, Germany), and the chamber internal humidity concentration is measured with an Abbeon certified hygrometer and a docutemp wireless TRH – 3900 temperature and humidity sensor (DocuTemp Inc., Bedford, MA.). All incubator parameters are recorded daily and noted on the daily QC log. In the case of the docutemp TRH – 3900 wireless sensor the chamber temperature and humidity parameters are recorded by the docutemp software every minute and transferred to the database.

The Cook K-Minc – 1000 incubators are supplied by a certified, medical grade tri gas mix of 6% CO_2, 5% O_2, 89% Nitrogen. There is no mixing of gas by the incubator. The gas constantly flows at a rate of 15ml/minute into the chambers through a 0.2 micron filter and a humidification flask of sterile water. The incubator initiates a purge cycle upon opening and closing of the lid that injects the tri gas mixture at a rate of 60 ml/minute for a period of 3 minutes. The humidification flask and tubing are replaced monthly per the manufacturer's recommendations. Temperature and humidity are measured by the TRH-3900 wireless sensor and are recorded at one minute intervals by the docutemp software. The CO_2 concentration is measured at the point of entry into the Minc with the Labotect infrared CO_2 analyzer on a weekly basis. There is no mixing of gas, the CO_2 concentration does not change unless the chamber has been opened or the supply tank has been changed.

Culture dish preparation:

In phase 1 of the study culture dishes were prepared by placing nine, thirty microliter drops of filter sterilized (0.2 micron Nalgene syringe filter) Sage fertilization

media (FM) supplemented with ten percent human serum albumin (HSA) (Sage Biopharma) into Falcon 3110, 60 millimeter culture dishes in a three by three pattern. The drops were covered by overlaying 4 ml of 37°C equilibrated mineral oil (Sage Biopharma) that was washed with FM containing ten percent HSA and gassed overnight. The patient's last and first name, date of birth, and media type (FM, CM, EC) were etched with a diamond marker into the bottom outside of the dish for identification purposes. An additional rinse dish containing 3 ml's of filter sterilized FM supplemented with ten percent HSA (Sage Biopharma) was also prepared for each patient. An additional Falcon, 60 millimeter dish of nine micro drops of Sage culture media (CM) supplemented with ten percent human serum albumin (HSA) (Sage Biopharma) drops was made for cases inseminated via ICSI. Duplicate sets of dishes were made for each patient, one set equilibrated overnight in the Minc and one set equilibrated overnight in the Forma 3110 incubator.

At the time of oocyte retrieval, oocytes were aspirated using a rocket pump attached to a Cook 35 cm 17 gauge echotip ovum aspiration needle. Follicle flushing media consisted of warmed, modified HTF media, (MHTF) (Sage). Follicles were aspirated into prewarmed, sterile10 ml Falcon snap cap tubes. The tubes were placed into a 37°C heating block until the contents were pipetted into a prewarmed, sterile 60 millimeter culture dish observed under a Zeiss dissecting microscope mounted into the surface of a NuAir laminar flow cabinet. The surface of the cabinet was heated to 37 °C to maintain a constant temperature while recovering the oocytes from the follicular aspirates. All recovered oocytes were placed in a new dish containing 5 ml of MHTF supplemented with 10% HSA until the oocyte retrieval procedure was completed. Excess cumulus was removed by cutting it from around the oocyte with the needles of sterile tuberculin syringes. After the cumulus was cut, the oocytes were rinsed in a second dish of MHTF + 10% HSA and recounted. The oocytes were

then evenly divided into rinse dishes of FM + 10% HSA from either the Minc or Forma incubators. The oocytes were rinsed in each media and washed through three micro drops before being placed into groups of three in drops number four through nine. The culture dishes of micro drops containing FM were then placed back into their respective incubators.

In phase 2 of the study all aspects of culture dish preparation was identical to phase 1 except that all cases were incubated in the K-Minc – 1000 incubators. Duplicate sets of dishes of Sage and Cook media were made for each case. Oocytes were divided evenly into each media type at retrieval and inseminated via IVF or ICSI. Culture through out each patient cycle was done in the same Minc incubator.

Insemination:

Oocytes were inseminated with 0.05 x 10^6 motile sperm four to six hours post oocyte retrieval (16). The sperm were prepared using a two layer density gradient (Isolate), Irvine Scientific. Post separation, the motile fraction was washed 2X with sperm wash media, Irvine Scientific. The resultant pellet was resuspended in 0.5 ml sperm wash media. Count and motility was determined using a Hamilton Thorne, Ceros automated semen analysis system (Hamilton Thorne Biosciences, Beverly MA.).

For cases inseminated via ICSI, the oocytes were exposed to pre warmed, hyaluronidase solution (Irvine) and pipetted through successively smaller stripper tips (Mid Atlantic Diagnostics, Cook Women's Health) of 300, 150, 130 micron diameter until the majority of cumulus cells were removed. The oocytes were exposed to the hyaluronidase solution for 1 minute or less prior to being rinsed in 3 milliliters of MHTF + 10 % HSA. Oocytes determined to be metaphase II were placed into micro drops of CM + 10 % HSA and incubated an additional 120 minutes prior to intracytoplasmic sperm injection. Oocytes were returned to the Minc or Forma incubator matching where they were placed post oocyte retrieval for culture overnight.

Fertilization assessment:

Oocytes that were inseminated via ICSI were examined 14 to 16 hours post injection for the presence of two pronuclei and two polar body status with an IVF^2 hybrid stereo inverted microscope (Kramer Scientific, Valley Cottage, NY). Oocytes determined to contain two pronuclei and two polar bodies were washed in fresh, preequilibrated CM and placed into incubation.

Oocytes that were inseminated via IVF were examined 16 to 20 hours post insemination and had cumulus removal by aspiration through a 130 micron stripper tip. All oocytes were examined under the IVF^2 hybrid microscope for the presence of two pronuclei and two polar bodies and placed into fresh preequilibrated drops of CM + 10% HSA under oil. Embryo development determination and selection for transfer:

All embryos for each set of sibling patient embryos were examined 24 hours from the fertilization determination and the cleavage stage and embryo grade recorded. The grading system is a modified version of that developed by Veeck, (8). The embryos were examined again 48 hours post fertilization determination when the cell number and grade were recorded. Embryos were selected for transfer based upon embryo quality: embryos with the highest cell number and least fragmentation were preferentially chosen regardless of incubation or media system. In some cases, embryos were cultured for an additional 48 hours and transferred at the blastocyst stage. Grading on day 5 was accomplished using the method of Gardner (9). Excess embryos were cultured to the blastocyst stage and cryopreserved using a modification of the method introduced by Menezo (10).

Statistical Methods:

Statistics were performed using SAS 9.1. Contingency table analysis, with Chi-square tests, was used to test for differences in pregnancy rates between patients implanted exclusively with embryos from K-MINC or FORMA incubators. A t-test was used to compare the mean number of embryos transferred from the two incubator types. The presence of sac formation, relative to the number of embryos transferred was compared between the groups using a logistic regression model. Finally, the rate of fertilization, relative to the number of embryos placed in each incubator type was compared between the groups using a logistic regression model, but with consideration of within-subject dependencies.

Results

Phase 1:

A total of 1189 oocytes were inseminated in 102 IVF/ICSI cycles in 97 different patients. 57 patients oocytes were inseminated via ICSI, 45 via standard IVF insemination. Overall fertilization rates were 5% higher with the Minc 72%, Forma 67%. P <.05. Day 3 embryo grade, Minc 7.5 cells grade B, Forma 6.5 cells, grade B/C, P = NS. Implantation rates were 39% and 32% per embryo for the Minc and Forma cultured embryos P = NS. Clinical pregnancy rates were 64% and 54% per transfer for the Minc and Forma systems respectively. P = NS. The average number of embryos transferred was 2.2 from the Minc system and 2.3 from the Forma system in Phase 1 (Table 2) and 2.4 and 2.3 respectively in Phase 2 (Table 3).

Recovery times were assessed for temperature, humidity, and CO_2 concentration using the Docutemp temperature and humidity TRH-3900 wireless sensor at one minute intervals. CO_2 concentration was measured with the Labotect infrared CO_2 analyzer. The Minc recovered from an opening 15 times faster than the Forma 3110, P = < .01. (Table 1 and Figures 3 and 4).

Phase 2:

In a total of 1115 oocytes inseminated fertilization rates were 84% in the Cook media and 72% in the Sage media, P <.05. Implantation rates were 38% and 32% per embryo transferred from the Cook or Sage media systems respectively P = NS. Implantation rates were 40% and 32% per embryo in the Cook and Sage media cultured embryos P = NS. The clinical pregnancy rates for patients under 35 years old were 65% and 54% per embryo transfer in the Cook and Sage media cultured embryos P = NS. The average number of embryos transferred was 2.4 from the Cook media system and 2.3 from the Sage media system (Table 3).

Table 2: Results Phase 1. Sage Media, K-Minc 1000 vs. Forma 3110 Incubators

	Cook – K-Minc 1000	Forma 3110	P value
Fertilization rates (FR)	72%	67%	< .05
Day 3 Embryo Grade (EG)	7.5 cells, grade B	6.5 cells, grade B/C	NS
Implantation rate / embryo (IR)	39%	32%	NS
Clinical Pregnancy rate / ET (CPR)	64%	54%	NS
Average number of embryos trans (AET)	2.2	2.3	NS

NS = not significant

Table 3: Results Phase 2. Cook vs. Sage media, K-Minc - 1000 Incubators

	Cook Media	Sage Media	P value
Fertilization rate (FR)	84%	72%	< .05
Day 3 Embryo Grade (EG)	7.5 cells, grade B	6.5 cells, grade B/C	NS
Implantation rate / embryo (IR)	40%	32%	NS
Clinical Pregnancy rate / ET (CPR)	65%	54%	NS
Ave. # Trans. (AET)	2.4	2.3	NS

NS= not significant

Figure 3: Forma 3110 Recovery Parameters

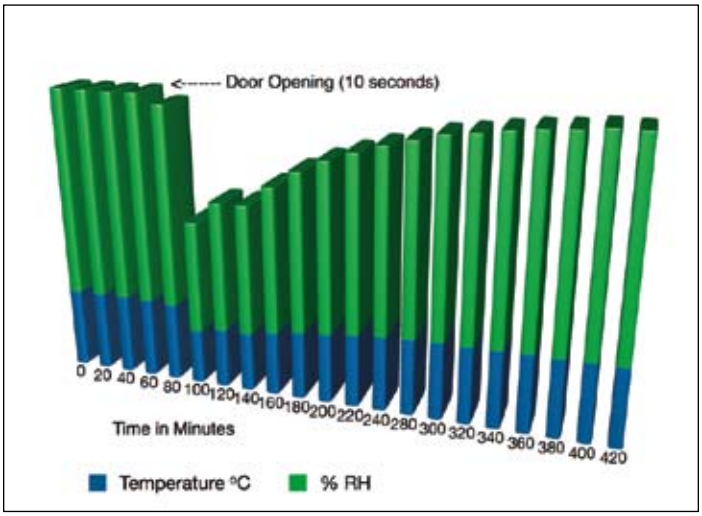
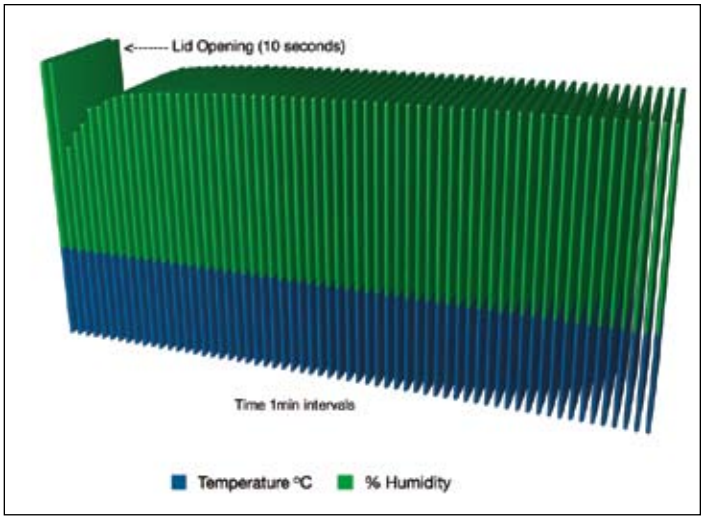


Figure 4: K – Minc 1000 Recovery Parameters



Discussion

Considerable improvement in delivery rates from IVF treatment has occurred over the past decade. We have come a long way since the ground breaking achievements of Steptoe and Edwards (11). Concurrent with these improved delivery rates, stimulation protocols and medications have also steadily improved. Arguably the largest change in IVF in the past decade has come in the laboratory. Laboratory developments such as ICSI and sequential culture methods have steadily increased the quality and quantity of human embryos developed in vitro. One of the few aspects of human oocyte and embryo culture that has not changed considerably is the incubator in which the gametes spend the majority of their time. We introduced the K-Minc –1000 mini bench top to fundamentally change the way our oocytes and embryos are incubated in our IVF laboratory. To my knowledge this was the first incubator designed specifically for human gamete culture.

Our study consisted of two phases, both of which included the introduction of this new type of incubator. Only after extensive culture of sibling oocytes and embryos in our control culture media (Sage Biopharma) did we introduce a new sequential culture system that was designed to work in conjunction with the incubator system. We experienced an overall increased fertilization rate, embryo quality and clinical pregnancy rates over the two phases of our study.

In phase one of the study we initiated use of the K-Minc 1000 mini bench top incubators. We used sibling oocytes to act as their own control. The oocytes were divided between the Forma 3110 incubators and the Minc incubators at oocyte retrieval. There have been similar studies done by Matsuko et al (12) that compared results culturing in the Minc and Hamatso incubators that showed improved results in the Minc. A study by Higdon et al. (13) comparing single and triple gas Forma incubators, demonstrated improved results in the triple gas system. We present a comparison of two basic incubation types, CO2 in air (20% O2) in the Forma and low O2 (5%) in the Minc. The other difference is the chamber size which directly contributes to recover time.

Culture dishes of Sage media supplemented with 10% HSA from Sage Biopharma were set up the day prior to the oocyte retrieval and gassed overnight in each incubator system. We experienced higher fertilization with both IVF and ICSI in the oocytes incubated in the K-Minc 1000 low oxygen incubators. We also had higher average cell numbers and less fragmentation on day 3 of culture in embryos cultured in the Minc system. The implantation rate per embryo and the clinical pregnancy rate per embryo transfer were also higher in embryos cultured in the Minc incubators.

There are several major differences between the two incubator systems; interior chamber size and volume, gas delivery and humidification method and oxygen concentration of the atmosphere inside of the incubator chamber. The chamber size has an inverse

relationship to the recovery time of the incubator. The larger the space needing humidification and gas concentration adjustment the longer it takes to get back to the conditions that existed in the chamber prior to its opening and exposure to room air. This in demonstrated in Figure 3 which demonstrates the recovery parameters of the Forma 3110. Figure 4 shows the recovery parameters of the K-Minc 1000. In Figure 3 the Forma was opened, the inner glass door was opened and finally the small glass door to the shelf containing the culture dish was opened. A culture dish was removed and all doors were closed. This sequence was measured to take 10 seconds. When measured with the docutemp wireless TRH – 3900 temperature and humidity sensor the starting temperature inside of the Forma was 35.7°C and the relative humidity was 93%. After the door opening the temperature dropped to 35°C and the humidity to 89%. Twenty minutes later the temperature had dropped to 24.5 °C and the humidity to 50%. It took a full 320 minutes or 5.3 hours from the time of the door opening for the conditions in the chamber to come back to 35 °C and 91% humidity. CO_2 concentration, as measured with the Labotect In Control unit went from 6% to 2.5% after the door opening. The CO_2 concentration did not return to 6% for 120 minutes or 2 hours. While the temperature, CO_2 concentration, and humidity levels are compromised the culture media that contains the oocytes and embryos is exposed to these undesirable conditions. This may potentially affect the pH of the media (14,15). Furthermore, it has been shown that human gametes are susceptible to damage from temperature fluctuations that may result from door openings (16). Both of these conditions may be mediated somewhat by the common practice of overlaying culture drops with equilibrated mineral oil. This oil overlay is not an airtight seal and therefore allows any fluctuations in atmosphere to affect the culture conditions in the dish. How the oocytes and embryos deal with this stress is currently a topic of great interest. It is known in animal models that gametes have the ability to regulate internal pH but human oocytes lack this ability (14,15,17). The inability to regulate internal cellular pH in human oocytes and embryos makes the incubation conditions critical to the preservation of the oocytes and embryos. In the event that the external media pH is not optimal for the cell, it must come at a cost to other metabolic processes

taking place in the gametes (18). This regulation, as well as fluctuations in temperature, produce "stress" on the oocytes and embryos leading to lower fertilization and cleavage rates, and ultimately lower implantation and pregnancy potential.

In contrast, as demonstrated in figure 4, the starting temperature and humidity in the equilibrated Minc chamber was 37.4 ºC with 75% relative humidity as measured with the docutemp wireless TRH – 3900 temperature and humidity sensor. The lid of the Minc was opened, a culture dish was removed and the lid was closed. The totaling opening time was 10 seconds. Within one minute the temperature dropped to 36.9 ºC and the relative humidity to 43%. Three minutes later the temperature had recovered to 37.3 ºC and the humidity to 49.2%. It took twelve minutes for the relative humidity to reach 70% and fifty eight minutes to reach 73%. The temperature did not change. In summary, it took the temperature three minutes to recover and the humidity twelve minutes. The CO_2 concentration took eight minutes to return to 6% from a low of 2% after the lid opening as measured with the Labotect In Control unit. The recovery time of the K-Minc – 1000 chamber parameters was 1 minute for temperature and twelve minutes for the relative humidity. Compared to the Forma, the recovery of all parameters is much faster. This undoubtedly puts less stress on the oocytes and embryos.

The initial drop in temperature in the Forma 3110 represents a 31% change. The time to recover 90% of the temperature was 180 minutes. The initial drop in humidity in the Forma represents a 44% decrease. The time to recover 90% of the humidity level at the time of opening was 180 minutes. The initial drop in CO_2 concentration amounts to a 58% drop. The time to recover took 120 minutes. The time to 90% recovery of the temperature and humidity parameters in the Forma was 180 minutes.

The initial drop in temperature in the K-Minc 1000 amounted to a 1.3% change. The time to recover to 90% of the initial temperature was 1 minute. The initial drop in humidity in the Minc chamber equaled a 43% decrease. The time to recover 90% of the initial humidity took 12 minutes. The initial drop in CO_2 concentration equaled 33%, and the time to recover was 8 minutes. These results are represented in Table 4.

It took the Forma chamber conditions fifteen times longer to recover than the Minc. The improved culture outcome parameters in Minc cultured oocytes and embryos must be at least partially explained by this huge discrepancy in recovery times. The major confounding factor besides the chamber size and recovery superiority that the Minc showed over the Forma was that it also was operating on a low oxygen gas concentration. There have been numerous publications (18-23) touting the superiority of low O_2 in terms of embryo quality and pregnancy outcome. There have also been some that show no difference and Higdon (13) showed a lower fertilization rate under low oxygen conditions.

In order to overcome this issue in the study we initiated phase 2. In phase 2 we used 1115 sibling oocytes from 98 cases of IVF and ICSI, divided into Sage and Cook media only cultured in the triple gas Minc incubators. The overall fertilization rate in the Cook media was 84% vs. 72% in the Sage media . The embryo grade on day 3 was an average of 7.5 cells grade B for the Cook media and 6.5 cells, grade B/C with the Sage culture media. Implantation rate per embryo was 40% with the Cook media and 32% with Sage . The overall clinical pregnancy rates were 64% per embryo transfer in the Cook media system and 54% with the Sage system (Table 3). This phase of the study

Table: 4. The Percent Change and Time to recover 90% of measured parameters after opening of the incubator

	Minc K- 1000 - % change at opening	Minc K- 1000 – Time (minutes) to recover 90%	Forma 3110 - % change at opening	Forma 3110 – Time (minutes) to recover 90%
Temperature	1.3	1	31	180
Humidity	43	12	44	180
CO_2	33	8	58	120

represents an argument to the potential pitfalls of phase one of the study. While all incubation took place in the triple gas Minc incubators the Cook media system gave better fertilization, embryo quality, and implantation rates per embryo. This may be partially explained by the fact that the media was originally designed for the Minc as described by Mortimer (24). The formulations of the Cook media have been changed slightly since the Mortimer study but the base components are very similar. Petersen (22) was able to demonstrate that post thaw human embryos did better in low oxygen than those cultured in 20 % oxygen. Quinn showed that low oxygen concentrations were beneficial to mouse embryos (20). Feil (23) demonstrated that in mouse, culturing under low oxygen resulted in subsequent improved fetal and placental development. Biggers (25) summed it up in his paper on general embryo culture conditions saying that low oxygen and minimal stress to gametes and embryos is by far the most sensible and productive way to optimize conditions. This is exactly what we have demonstrated in our laboratory with the introduction of the Cook K-Minc – 1000 incubators and media system. Given the increase in fertilization rates, embryo quality, and pregnancy rates, as well as, the space savings from the smaller footprint of the incubator, we believe that a culture system incorporating triple gas (low O_2), a mini bench-top incubator (such as the K Minc 1000), along with compatible culture media, is the way of the future in human IVF laboratories. ■

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