TECHNICAL REPORT

THE BENEFITS OF USING THE "IVF CHAMBER" IN THE HUMAN ASSISTED CONCEPTION LABORATORY

INTRODUCTION

Designing, upgrading or optimizing a laboratory for human assisted reproduction technology (commonly referred to simply as "ART" or "IVF" for in-vitro fertilization) is best tackled using a systems approach combined with process control. This can be summarized in the following four basic steps.

- Define all the inputs to the system. These include equipment, consumables and biological materials, as well as operational (technical) procedures and extrinsic factors such as uncontrolled environmental conditions and external (e.g. third-party) influences.
- Define every thing that happens during the entire process, paying special attention to interactive factors.
- Identify and define sentinel indicators for all stages of the entire process so that stage-specific outcomes can be monitored.
- Design strategies to eliminate or correct all suspected adverse influences that can affect equipment, component processes or procedures. Each aspect of the strategy must include not only the perceived remedy (effector) but also a scheme whereby its impact can be monitored.

Clearly, therefore, one must understand how important pieces of equipment operate, and how their operation can be affected by their incorrect use or extrinsic environmental and materials factors. Design specifications and engineering factors can also be important inputs into the analyses, as well as manufacturing standards and QC/QA of components.

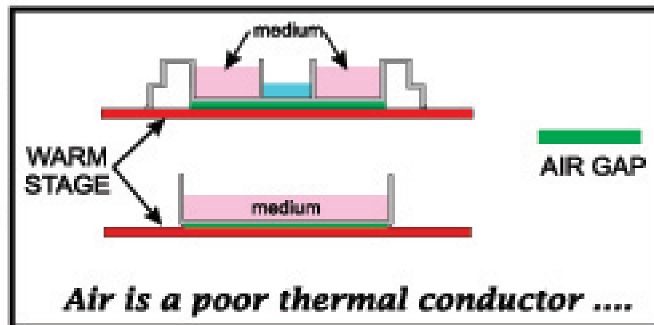
EXTERNAL FACTORS AFFECTING OOCYTES AND EMBRYOS IN VITRO

During all handling of oocytes and embryos they must be protected from extrinsic sources of physical and chemical stress. Such factors can be grouped into three major areas: (1) temperature changes; (2) culture medium osmolarity and pH; and (3) air quality, although they do interact in various ways. The simplest way to consider these factors is that *any* compensation that an oocyte or embryo has to make comes at an energy cost, and hence must be considered as "stress" since the cells' physiological energy budget is dedicated to its normal development.

Temperature

The oocyte in particular is extremely sensitive to alterations in temperature. Cooling causes the spindle to depolymerize, risking aneuploidy of the resulting embryo if not all chromosomes reattach to the spindle when it re-polymerizes as the oocyte warms back up to 37°C [1-3]. In addition, temperature shifts can affect trans-membrane transport and many intracellular metabolic processes. Dogma derived from the early days of bovine embryo transfer states that more frequent shifts in holding temperature, and the greater the magnitude of those changes, the worse the embryo quality (measured in terms of pregnancies achieved). Consequently, human oocytes and embryos must be held as closely as possible at a stable 37°C.

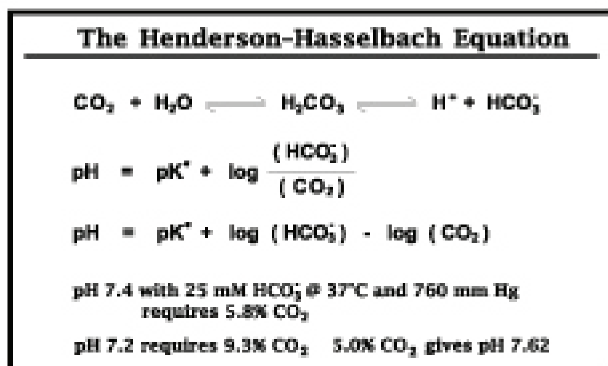
A significant, but poorly-recognized, confounding aspect of temperature control during the microscopic observation of oocytes and embryos in dishes is that the design of all disposable plastic dishes does not allow the base of the dish to come into direct contact with the microscope stage, there is always an air gap (see Figure on right). Because air is a poor conductor of heat, this air gap greatly reduces the efficacy of heated stages (either those attached to or built into the microscope, or the heated work surfaces of the K-Systems cabinets), allowing the medium in dishes to cool below the temperature at which the heated surface is set.



Culture Medium Osmolarity and pH

The composition of culture media used for human IVF have evolved markedly over recent years with the increasingly widespread use of ‘sequential culture media” suites that comprise a range of media and buffers designed to provide optimised support to each stage of the process from oocyte retrieval through fertilization and embryo cleavage to the blastocyst.

Correct use of all culture media requires the use of special atmospheres that are, at a minimum, enriched in carbon dioxide. Additionally it is now recognized that the human embryo shows better development, and implantation potential, if the oxygen tension is reduced from that in normal air. However, there is great confusion between CO_2 and O_2 concentrations (expressed in volumetric percentage terms) and their partial pressure. Firstly, at higher elevations, not only does atmospheric pressure decrease, but the relative proportions of oxygen and nitrogen (and the other minor gases) also change: for example, whereas there is 20.95% O2 at sea level, at an elevation of 1000 m there is only 18.55% O2 and at 1600 m (e.g. Denver) there is just 17.2%. The same changes affect CO_2, so that if one wishes to achieve a pH of 7.3 in a solution of 25mM bicarbonate at sea level, 6.0% CO_2 is required according to the Henderson-Hasselbach equation (see box on right) otherwise the pH will shift and bicarbonate ions will be lost until the solution reaches a new equilibrium. But at 1600 m above sea level, almost 7.5% CO_2 is required to achieve the correct partial pressure ($ppCO_2$) to maintain the bicarbonate ions in solution and the pH at 7.3.



A major problem with bicarbonate-buffered media is that they take a long time to reach equilibrium, but out-gas very quickly. Recent studies [4] have shown that a 50 ul droplet of medium under oil will out-gas after removal from a CO_2 incubator so that the pH has shifted above 7.45 within 2 minutes – and that after replacing the dish in the CO_2 incubator it will take 35 minutes to re-equilibrate the pH (only about 15 min for Petri dishes containing 5ml of medium). These differences are due to the relative magnitudes of the differential CO_2 contents between the equilibrated medium and air and between the incubator’s atmosphere and the partially out-gassed medium.

Finally in this regard, if culture medium is exposed to air that is not well-saturated with water vapour then there will be evaporative loss from the medium with concomitant increases in medium osmolarity. Evaporation is also higher at warmer temperatures. Whereas culture under oil helps combat this problem, during oocyte retrievals the dishes are typically open without an oil overlay (which would greatly complicate the procedure).

Air

Because of their high metabolic and cell division rates, embryos are highly sensitive to toxic chemicals, thereby making ART laboratories and procedure rooms especially high risk areas for volatile organic compounds (VOCs) and other air pollutants. It is a common misconception that HEPA (high efficiency particulate air) filtration removes gaseous organic and inorganic molecules. HEPA, as its name implies, is a highly efficient system for removing particulates from the air (the standard requires a 99.97% efficiency for a 0.3 um particle), *not* low molecular weight gaseous molecules.

VOCs come from motor vehicle exhausts and industrial emissions (common contaminants of "fresh" air in urban areas), as well as construction materials and paints, cleaning products, pesticides, fixatives and sterilizers, anaesthetic gases, laboratory chemicals, tobacco smoke and cosmetics.

Within the ART laboratory there is evidence that VOC's from the ambient air can be concentrated inside the incubators, thereby exacerbating their deleterious effects upon embryos in culture [5,6]. Consequently, it is now accepted as a standard requirement that careful attention must be paid to the general air supply and additional, point-of-delivery or in-room purification must be employed to eliminate VOCs [5,7-9].

Conclusion

The inescapable conclusion of the above discussions is that an ART laboratory must dedicate a great deal of care and attention in selecting equipment and systems for use with human oocytes and embryos so that the possible deleterious influences of the following extrinsic factors can be minimized as far as possible:

- Temperature fluctuations
- Good quality air
- Maintaining the $ppCO_2$ while media are outside the incubators to maintain the pH of the media
- Minimizing evaporative loss of media to prevent hypertonicity

HANDLING OOCYTES AND EMBRYOS IN THE LABORATORY – BENEFITS OF THE IVF CHAMBER

Oocytes are handled in the laboratory on Day 0 at the time of oocyte retrieval (often called "oocyte pick-up" or "OPU") and again at the time of insemination. Embryos are examined on Day 1 at the zygote stage ("fertilization check") and then typically daily until transfer on either Day 3 (8-cell stage) or day 5 (blastocyst stage) for assessment of their development and quality. Additional observations are sometimes made later on Day 1 to identify embryos showing early entry into first cleavage, a perceived marker for the embryos with highest developmental potential. On each of these occasions dishes are removed from the CO_2 incubator and examined on a dissecting microscopoe and various strategies have been employed to try and protect the oocytes and embryos from external physicochemical variations. These have been summarized in the Table on the following page.

From this analysis it is evident that the IVF Chamber style of workstation provides the best possible protection of oocytes and embryos during handling in the laboratory currently available. The earliest of these units were based upon modified neonatal isolettes (humidicribs) and have been around since the beginnings of clinical service IVF [10]. Indeed, these units actually employed pre-mixed $CO_2/O_2/N_2$ gas mixtures that flowed through a series of culture tubes inside the chamber where fertilization and embryo culture took place, making them fully mobile basis IVF laboratories.

Effectiveness of various types of IVF work station

Type of work station		Extrinsic Factor / Variable				**Rank**
		Temperature	**CO2 / PH**	**Air**	**Evaporation/Osmolarity**	
Laminar Flow Cabinet	*Configuration*	Heated Stage on microscope	Sometimes a gas “funnel” is put over the dishes while they are *not* on the microscope, there is no protection while on the microscope.	Open to room air throughout unless gas funnel installed, in which case only exposed during observation.	Gas flowing into funnel or box is humidified by bubbling through a water bottle.	**#3**
	Effectiveness	Moderate. Still have air gap between bottom of dish and the heated surface	Up to several minutes of exposure to room air during observation, also during holding if no gas funnel.	Exposed to VOCs and other contaminants in the room air at least during observation.	Moderate effectiveness while under gas funnel or inverted box, *no* protection during observation.	
	Ranking	3	=2	=2	=2	
K-Systems Cabinet	*Configuration*	Heated Stage built into the cabinet working surface	Gas funnel usually installed for when dishes are *not* on the microscope, *no* protection while on the microscope.	Exposed to room air at least throughout the observation period.	Gas flowing into funnel is humidified by bubbling through a water bottle.	**#2**
	Effectiveness	Moderate. Still have air gap between bottom of dish and the heated surface	Up to several minutes of exposure to room air during observation.	Exposed to VOCs and other contaminants at least during observation.	Moderate effectiveness while under gas funnel box, *no* protection during observation.	
	Ranking	2	=2	=2	=2	
IVF Chamber	*Configuration*	Entire chamber warmed to 37ºC	Entire chamber has a CO_2 enriched atmosphere.	Air inlet has 0.22µm filter, an in-line carbon filter can also be installed.	The atmosphere inside the chamber circulates continually over a humidification pan.	**#1**
	Effectiveness	All dishes inside the chamber are held close to 37ºC whether on the microscope chamber or not.	All dishes are held under CO_2 enriched atmosphere	Exposure to room air minimised by semi closed system.	Humidity is maintained high (ca. 80%, i.e. non-condensing)	
	Ranking	1	1	1	1	

Early IVF workstation units used empirically-derived controlled flow rates of CO_2-enriched air to achieve the desired final concentration of CO_2, since pre-mixed 6% CO_2-in-air cannot be used directly as it would be impossible – or at least very expensive – to use it to purge the air from the chamber and then maintain the correct CO_2 concentration during use. Subsequently, models using the Forma Model 3057 or 3063 CH/P external thermal conductivity controller were built but, because of the limitations of thermal conductivity CO_2 controllers (in that they cannot control the CO_2 content correctly unless the gas mixture is at 37°C and humidified), the most modern units have an integral infra-red controller that regulates the CO_2 level inside the chamber independently of temperature and humidity. Humidification of the atmosphere inside the chamber is achieved by circulating it over sterile water in a stainless steel tray located beneath the chamber, adjacent to – but separated from – the electronics compartment. Stainless steel baffles in the tray reduce the risk of spillage during relocation of the chamber if it is being used as a portable workstation.

There was also a change from the "home" modification of old neonatal isolettes to their custom-modification (e.g. the Hoffman IVF Chamber, see ref.11), and then to the custom-designed and -built IVF Chambers from HD Scientific. All materials used in the construction of these units have been carefully selected to prevent any detrimental effects on oocytes and embryos; the principal materials are acrylic, stainless steel and silicone rubber. Uniform temperature and humidity inside the chamber are maintained by a circulating fan, whose motor is located outside the humid environment to prevent corrosion and electrical faults.

In addition to providing a clean environment for handling gametes and embryos, the IVF Chamber also affords substantial protection of the operator from contamination by any inadvertent aerosols or spills. Usual access to the IVF Chamber is through two arm ports with iris seals. While this protection does not reach Class II biohazard standards, it far exceeds the level of protection afforded by a typical laminar flow cabinet. Although normally kept closed, a large door section in the front of the IVF Chamber canopy (which includes the two arm ports) allows ready passage of long catheters, packets of dishes or pipettes, cleaning between cases, etc. Additional features of the IVF Chamber work station are its mobility (a small CO_2 cylinder can be attached to the trolley base so it can be used anywhere) and easy disassembly of the canopy for intensive cleaning. The illumination source for the microscope is an integral fibre-optic light source (using a bulb inside the chamber would cause uncontrolled over-heating) that can be used in conjunction with a wide variety of microscope bases. Flexible canopy design allows any dissecting microscope to be installed into the IVF Chamber. On the latest generation of HD Scientific IVF Chambers all controls are front-mounted and there are test tube racks built into one end of the canopy to allow easy passage of OPU tubes without the scientist having to repeatedly remove his/her hands from the glove ports.

Standard safety features include a safety thermostat that is independent of the temperature controller and will prevent accidental over-heating in the unlikely event of controller failure. Finally, the IVF Chamber comes standard with two pipette holders suspended from the inside of the canopy roof, making it equally suited for either right- or left handed operators; pipettes hang vertically, away from contact with any other surface.

The only common negative comment voiced by some users regarding IVF Chambers is that the operator must keep his/her elbows against their sides with the forearms extended horizontally forwards. However, this is a simple matter of posture readjustment, compared to the usual elbows-extended position when working in a laminar flow or K-Systems cabinet – and a very small price to pay for the enormous improvement in environmental control that the work station provides, and which translates into improved embryo quality and clinical outcomes (see ref.12 and Appendix A).

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EFFECT OF CULTURE CONDITIONS ON EMBRYO CLEAVAGE RATES AND MORPHOLOGY

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Introduction

Fluctuations in pH and temperature can have adverse effects on oocytes and embryos in vitro, so control of the gas environment and temperature during routine oocyte and embryo manipulations are essential. Another aspect of the culture conditions which may affect embryo development is glucose in the culture medium. While studies have shown that glucose is inhibitory to cleavage-stage embryo development, these studies began with fertilized oocytes. Therefore, the value of glucose in the insemination medium remains in question, given that spermatozoa require glucose as an energy source during capacitation.

During a period of change in our laboratory, many of the procedures and culture conditions were changed sequentially. The aims of this study were to investigate the effect of (a) glucose in the insemination medium; and (b) the manipulation of oocytes and embryos in a controlled environment; upon embryo cleavage rates and morphology.

IVF Chamber

Materials and Methods

Oocytes collected for IVF / ICSI procedures were placed in the culture medium for the first 20 to 23 hours of culture. After the fertilization check, all zygotes were transferred into P1 medium. Embryo cleavage stage and morphology were assessed 70 to 72 hours after retrieval. Results for each Group were compared by χ^2 analysis.

All oocyte and embryo manipulations were carried out in either a laminar flow hood with a heated stage, or in an IVF chamber, which had an humidified 37°C, 6% CO_2 atmosphere.

Inclusion criteria

Criteria for inclusion in the study were:

Female age < 38 years

Ejaculated spermatozoa used for insemination

No "failed" fertilization (< 25% fertilization)

Culture medium

"P1" = P1+SSS (10% (v/v)) (Irvine Scientific)

"P1+glucose" = "P1" containing 2.8 mM glucose

Breakdown of Groups

Group 1	P1 medium	Laminar flow hood
Group 2	P1+glucose	Laminar flow hood
Group 3	P1+glucose	IVF Chamber

Embryo Scoring System

A multiparametric system was used to assess embryo quality. The factors considered were:

cell number

cell size and shape

membrane appearance

cytoplasm appearance and colour

degree of fragmentation

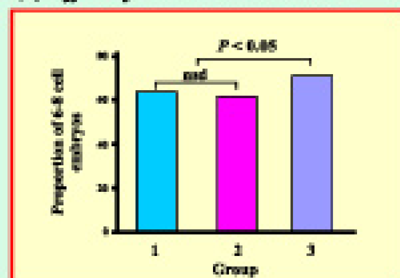
Embryos were scored out of a maximum of 20, with a score of 18 or above considered "good".

Results

(i) Patient distribution

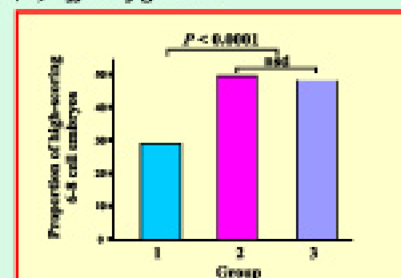
Group	No. patients	No. embryos
1	74	541
2	75	541
3	53	436

(ii) Effect of external environment



Gametes and embryos manipulated in the IVF chamber had a significantly faster rate of cleavage than those in the laminar flow hood (Group 3 versus Groups 1 and 2).

(iii) Effect of glucose



When gametes were exposed to 2.8mM glucose during the first day of culture, the quality of the resultant embryos was significantly higher.

Discussion

Because the cleavage rate was significantly improved by the use of the IVF chamber, while the embryo morphology was significantly improved by the addition of glucose to the P1 medium during the first 20 to 23 hours of culture, these results suggest that embryo cleavage rate and morphology are controlled separately. The positive influence of incubation of gametes in glucose-containing medium indicates that a different medium should be used for fertilization than that used for culture of early cleavage stage embryos.