The CO2 Incubator: the masterpiece for IVF Laboratories.

The conditions in which human embryos can be cultured after In Vitro Fertilization (IVF) has been extensively descrided.But most authors emphazised on the composition of embryo culture media , its preparation or their quality screening bioassays.

As a matter of facts , most of the IVF textbooks or any others contributions do not give any precision about the type of incubator which can be used for this purpose and how it can be used. My purpose is to provide technical counsselling about the culture systems in term of apparatus

The buffer system of the culture medium determines the use of a CO2 incubator:

Most embryo media utilize a bicarbonate /CO2 buffer system to maintain physiological pH of

around 7.2 to 7.4 in the medium .Of course the inclusion of sodium bicarbonate in usual concentration requires the use of a gassing incubator to maintain a certain CO2 atmosphere.

A sodium bicarbonate buffer dissociates as follows.

$NaHCO_3 + H_2O <------> Na^+ + HCO_3^- + H_2O <------> Na^+ + H_2CO_3 + OH^- <-------> Na^+ + OH^- + H_2O + CO_2$

(The relative humidity of 95% can be assured by adding distilled water with an anti fungi /algae solution like Barrycidal .)

This gassing system is of great advantage, because it is a physiological buffering system in the fluid surrounding mammalian cells.As main drawback , the pH rises tremendously when exposed to air , leading to rapid intracellular damages or necrosis of the embryos.

Layering the embryo culture media with equilibrated paraffin oil prevents from rapid gas exchanges as well as from air contaminants when culture dishes are taken outside the incubator for fertilization assement or embryo scoring.An other way would be to use a phosphate buffered medium which does not require CO2 environment to maintain its Ph in air.Regrettfully this system is not advised for embryos as it has been recognized as detrimental for their development in vitro.

Which gas phase should be used ?

The two active gases used in embryo cultures systems are oxygen and carbon dioxide.

A majority of IVF centers use a CO2/bicarbonate gassing system. Many studies lead on animal embryos have shown that reduced oxygen levels (5-7%) is far superior in supporting embryo development than the 20 % in air . This could be explaned by a consequent decreased levels of superoxide radicals known to be toxic and responsible for development arrests in the mouse embryo.Even one hour exposure in 20 % O2 could significantly reduce embryonal development.So even if the littearure do not provide many

references about the effects of high oxygen levels on human preimplanation embryo , it seems advisable to use a reduced oxygen tension (around 5%).

At the beginning of IVF, for 20 years and still now some operators were using a combination of CO2 /O2 .This possibility works very well but is still a heavier system than a CO2 in air gassing system.Endeed the incubator has to operate a mixture with the help of a processor unit which can be a supplementary source of problems. More , the change of gas tanks are occuring at different time which oblige the operator to manage the gas supplies and of course to forsee more tanks as security reserve.

Since some years the general tendancy is to provide only CO2 (99,9% purity for medical use) in air at the rate of 5 to 6 % which is quite sufficient for embryo cultures not tresspassing 48 hrs.For late embryo transfer, ig. at the blastocyst stage , a higher tension of O2 could be needed due to metabolic changes and thus a special gas mixture required .

Parameter controlling:

Incubators whatever they are, shouldbe controlled at regular interval.

External measuring systems are required for the temperature and the CO2 contain.

A calibrated thermometer hanging in the air and in oil container not too near from the glass door should be readible from outside.Variation from 0,5-1° C are acceptable.For CO2 , Drä ger cartridges are advised, because more precise as the Ferryte solution.

Anyway the use of capnographs apparatus like the ones from Datex Company for anesthesiology are preferable as they continously measure the CO2 concentration.

Controls for Temperature and CO2 have to be performed after overnight gassing without door opening .

Care and Maintenance:

Considering the warm and humid atmosphere provided by the incubator it is quite normal that after some weeks, appear some fungis on the shelves or growth of algea in the water bath. According to the IVF frequency , the operator should clean regularly the incubator with ethanol 70° after removing the water bath with the hand pump.A desincfection spray (Barrycidal) not containing formaldehydes compounds (used in many hospitals) can complete the desinfection procedure in all unaccessible corners of the incubator.The shelves and all removable pieces should be removed and cleaned separatly through brushing.Let the incubator undergoing the desinfection for a couple of hours. After drying and evaporation of desinfection media, the shelves can be remounted and the incubator started again.The water bath should be performed only with distilled water supplemented with some drops of Barrycidal .After 24 hrs of stabilization (without door opening and for not infra red "IR"controled incubators) CO2 and temperature should be checked out and adjusted if needed.

At last let control your incubator once a year by the customer service .

Safety rules & devices:

In order to assure a continous gassing for the embryos ir is much important to use two CO2 tanks linked to a **gas tank monitor**.In case of a gas leak or empty tank during night or week end, the monitor will dispath to the secon d full tank. This device is also equiped with a loud alarm warning the immediate

neighbourhood.A similar alarm system can be connected to a modem unit to transmit the emergency message to a free telephone line.

Concerning the power supply, it is primordial to assure a continous current supply to the incubator.To assure this ,it is advisable to connect the incubators to a **emergency power supplier** or to an extern electric current generator. In case of short interuption, the parameter setting should controlled.

At last, for evident reasons of security , it advisable to let mount safety locks on the incubator. Since Human embryos are considered as a part of a personn and consequently protected by the german embryo protection law; nobody except the authorized technician or IVF biologist responsible for the laboratory should have access to the incubator.

Which incubators are suitbale for IVF?

Copper inside housing or not ?

There is at the moment no clear evidence that copper residues could be released directly into the IVF culture plates.As it has been well documented that copper ions are toxic and deleterious for invertebrates cells, cooperhousing should be avoided for human IVF.If it is not the case a layer of paraffin oil onto the culture medium could probably protect the culture.

Anyway the germ free status of the incubator can be assured by regular desinfection procedures (depending on the door opening frequency a day)

Water mantel incubator or not ?:

Incubators built with a water mantel system show a higher temperature stability as others mainly after door opening.This system is of course more complicated (heavier incubators) and needs regular water adjustement of the mantel .(closed system with no easy control of the water purity)

Need of a special inbuilt or added air-contaminant filter ?

Many filtration systems for heavy metals or other possible contaminants have been commercialized.After some years , it has been shown from user side that these expensive and care consuming system do not improve any results in the IVF lab .(i.e.no significant higher pregnancy rates).

Twin incubators sets:

it is advisable to use two incubators units. One is only used for culture of human oocytes before insemination and after insemination up to the fertilized stages ie. 24 hrs culture after oocytes recovery.The second one is used only for development of ferilized oocytes into preimplantation embryos up the 2-8 cells stages (ie. 24hrs more in culture) or more in case of blastocyst culture.This disposition allows many advantages :

-The door opening frequency will be reduced

-In case of breakdown, immediately the embryo can be transferred into the second incubator.

-For care & maintenance,one incubator can be turn off , the other still functionning.On the next day it's the other way round.

The new generationof CO2 incubators:

In the field of IVF, where human gametes or embryos are particularly sensitive to the culture environment and its variations , it is very important to have a **reliable** , **constant** , **safe** and **simple** CO2 gassing system. The new generation of Co2 incubators offer such skills.

The new IR controlled CO2 incubators like **Atmos TI** from KB Biosystem company have a rapid CO2 recovery time (<1mn) ,a small gassing chamber and it's care and maintnance are quite easy to perform (no humidity required).It's setting panel is well protected and easy to clean..As it's size is particularly small , it can be used in IVF dependencies like , Cryolab or ICSI lab where big incubators (120l) often do not have any place.

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