Optimising Embryo Culture

- Journal Review -

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'There is only one thing that is truly important in an IVF laboratory: everything' Cairo Consensus Guidelines on IVF Culture Conditions

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OUTLINE

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OUTLINE

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08 MICROMANIPULATION

OUTLINE

01. INTRODUCTION

- Never ending process of optimising embryo culture in the IVF lab.
- Unrestricted development of IVF over the past 40 years has lead to multiple culture systems
- Old vs New
- What constitutes best practice?

GOAL: To Identify how best to operate within a framework of quality to achieve best practice and optimise the developmental competency of all gametes received and embryos obtained

02. TEMPERATURE

ADHERANCE TO RANGES

Inappropriate temperatures during cell handling and culture will compromise cell function and development which could lead to more adverse IVF outcomes

EQUIPMENTS

Should meet set points and routine assessments be performed and record in dataloggers

MAINTENANCE AT 37℃

Current culture media have been formulated for use at approximately 37℃. Alterations can affect cell's metabolisim and pH

EFFECTS

An oocyte as a result of cooling temperature can cause spindles to disorganize thereby displacing chromosomes

03. HUMIDITY

THE RATIONALE

Create an environment to reduce evaporation. In vivo state is moist so imitating this in vitro appears to be physiologically correct

THE SCIENCE

Evaporation can lead to increase in culture osmlality. Ideal range for mammalian embryo development 250 - 290 mOsm/kg

THE SETPOINT

Optimum level of incubator humidity has yet to be determined

THE PRACTICE

Dish preparation should ideally be individualized with oil overlay placed immediately after culture droplets

04. CO_2 & pH CONTROL

IMPORTANCE

Maintainance of intracellular cytoplasmic pH (pHi) is an energy requiring process for gametes and embryos. pHi depends on pHe (extracellular pH)

01

FACTORS

Correct pH setting of culture medium depends on the relationship between levels of bicarbonate in the culture medium and CO_2 settings of the incubator

02

IDEAL RANGE

pHe Range: 7.2 to 7.3

03

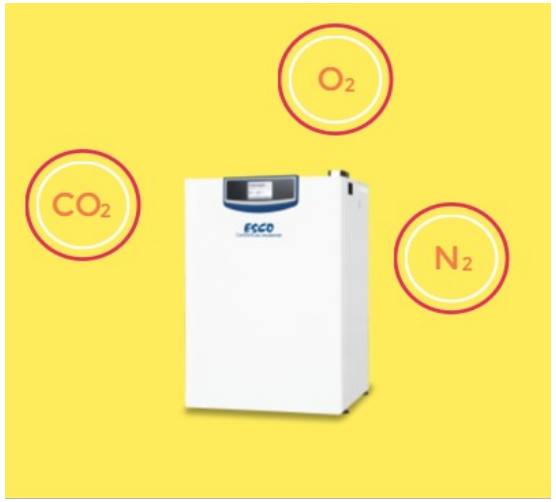
04 REMEMBER

Cellular Development versus Homeostasis. What do I want my embryos to be doing.

05. OXYGEN TENSION

IN VIVO

- Atmospheric/Ambient $O_2 \approx 21\%$
- Reported O_2 levels in the reproductive tract is between 2-8%



IN VITRO

- Studies have shown O_2 levels of 5-6% as being optimal for embryos.
- Atmospheric O2 levels lead to increased production of ROS increasing oxidative stress on the embryo
- Tri-gas incubation for O_2 is it worth it?

06. DESIGN & ENGINEERING OF WORKSTATIONS

MIMICRY

IVF processes are governed by the biology of gametes and embryos thus the equipment selection and lab design should be engineered to meet correct biophyical and biochemical conditions for each step in the IVF process

LAB DESIGN

The distance between workstations and incubators should be as short as possible

07. MAINTAINING CULTURE ENVIRONMENT IN INCUBATORS

Incubators are designed to control certain parameters during culture such as pH, temperature,humidity and air quality

NIST

National Institute of Standards and Technology: A non-regulatory body that develop and promote measurement standards and technology to enhance productivity

MAINTENANCE

- Optimum set points and tolerances
- Routine Monitoring
- Validated quality management program for regular routine monitoring
- Data captured with dataloggers
- Gas supply should be filtered before entering incubator
- Backup supply is a must
- MInimal disturbance of incubator environment

08. MICROMANIPULATION

EQUIPMENT

- Use heated stage or warming plate to maintain the temperature as close to 37℃ as possible throughout duration of procedure
- Rigs should not be located in areas of active air flow
- Oocytes are sensitive to the blue green end of the visible light spectrum so avoid those wavelengths as much as possible
- Lowest intensity of light
- Vibration reduction

GAMETE PREP

- Remove all traces of seminal plasma (endogenous toxin)
- Complete removal of cumulus oophorus and corona radiata cells to minimize exposure to stress
- Limited number of eggs per dish to enhance results
- Hyaluronidase exposure is known to reduce fertilization and adversely affect developmental stages subsequently

09. CULTURE MEDIA BUFFERING & pH

COMPOSITION

Culture media should contain a minimum of proteins, carbohydrates, amino acids with glutamine (Dipeptide form), and antibiotics

SEQUENTIAL

Based on the principal of the in vivo needs of the embryo which from day 0 to 3 is pyruvate and then from day 3 to 5 switches to glucose, lactate and amino acids

SUPPLEMENTATION

Supplementing media with exogenous proteins (ie Growth Factors) beyond the manufactures recommendation could change the composition and performance

SINGLE STEP

Based on the in vivo principal of a continous sustained environment for development and growth

10. USE & MANAGEMENT OF MEDIA

COLD CHAIN

- Culture media products must be kept at refrigerated temperatures according to manufacturer's instruction (2-8℃)
- 2 possible failures: Freezing and Overheating

STORAGE

- Culture media must be kept away from light and stored at temperates as noted above
- Expirary dates should be clearly noted and products used in order of batch/lot numbers
- Appropriate refigeration can be achieved with clinical grade “pharmacy” refigerators which reduce fluctuations

Thank YOU