IMPACT OF LABORATORY ENVIRONMENT (Temperature, Humidity and Volatile Organic Compounds) on embryo culture

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Conflict of interest

I declare that i have no commercial or financial interest pertaining to the subject of this presentation and its content

Learning Objectives

- To know the effect of temperature variations on embryo culture in-vitro
- Identify hot spots in the lVF lab
- To know the effects of humidity on embryo culture
- To understand the impact of VOCs on embryo growth and development and sources of VOCs
- Management of VOCs in the IVF Lab

IMPACT OF TEMPERATURE ON EMBRYO CULTURE

temperature

- Focus on temperature in the media, not on the heated stage
- 37^{o}C on a heated stage often means <35^{o}C in the medium, depending on the type of dish
- The media temperature varies with the type of culture media
- Different types of dishes give different temperatures
- Always measure temperature with and without the lid and/or oil overlay
- Use an accurate, sensitive and calibrated thermometer

incubator recovery time

top load incubator

front load incubator

what causes media droplet temp. changes?

- Distance between the base of the dish and the heated stage
- cooling effects of air flow near the dish
- efficiency of direct heat transfer between the heated surface and media within the dish
- volume of media and oil overlay
- position of the droplet within the dish (central or near the edges)
- Ambient temperature in the laboratory environment

Effects of temperature on embryo growth

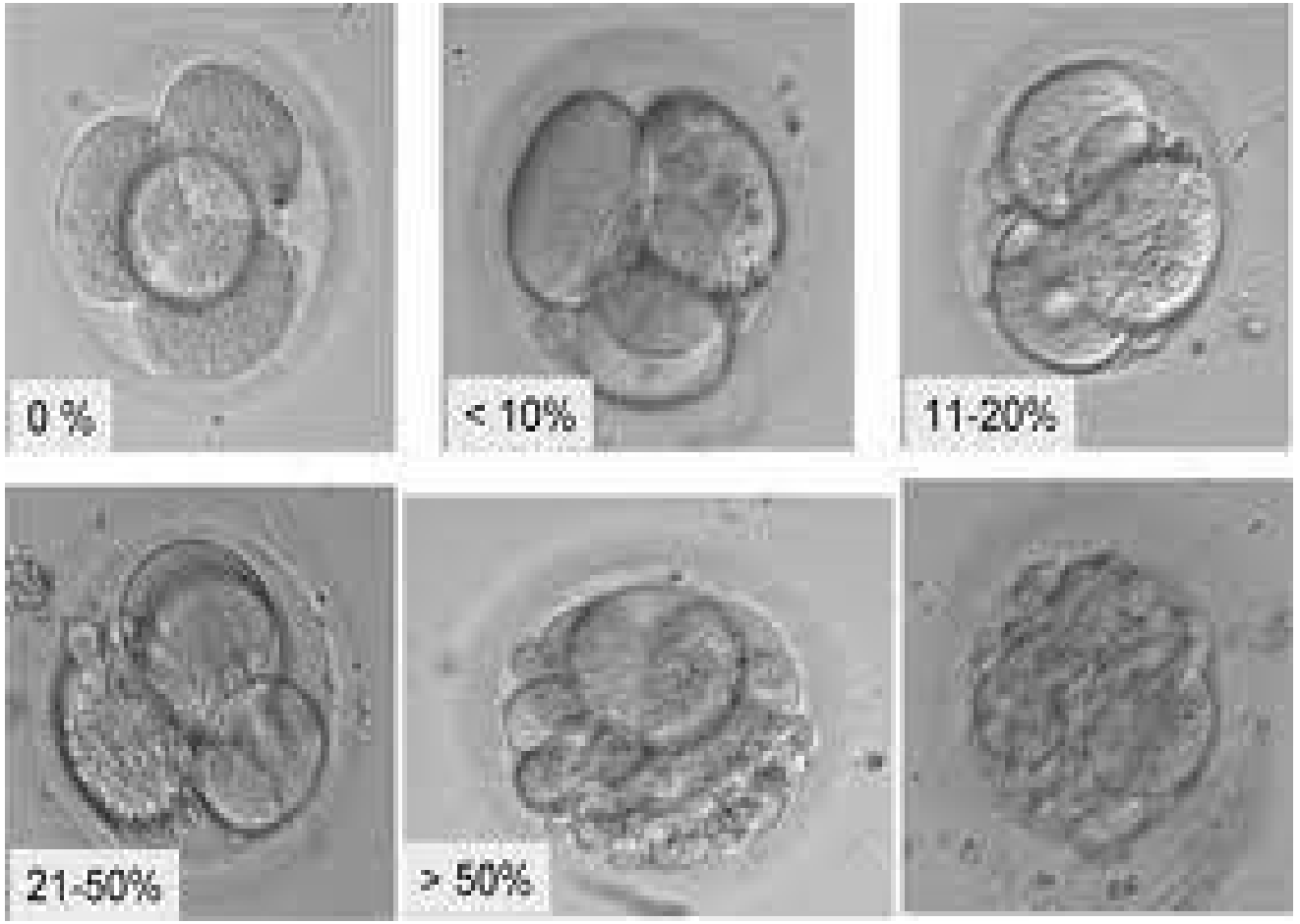
- human microtubules are highly sensitive to temperature
- slight reduction in temp can cause spindle to temporarily disassemble
- lower temperatures causes irreversible damage to the spindle fibres
- prolong exposure of culture of embryo to temperature other than optimal 37^{o}C reduces the ability of fertilization and nullifies the ability of cell division

temperature

- The optimal temp range for human IVF culture is assumed to be between 36.7^{o}C and 37.^{o}C
- Temperature above 37^{o}C may induce the formation of heat shock proteins with detrimental effects on development
- temperature flactuation affects the pH of the culture media

multinucleated embryo

fragmented embryos



Temperature control in IVF lab

- Never rely on the display of your incubator for accurate reading
- Always use an indepentent instrument thermometer or probe to verify your temperature
- Validate and calibrate your heating stages and take into accounts temperature loss for the set point

Temperature control in IVF lab

- Maintain the cold chain of your media
- Temperature at which culture media is stored (mostly +2 to +8)
- Uninterrupted power supply
- Take note of temperature variations in multilayered shelf incubators

Impact of humidity on embryo culture

humidity

- humidity is a characteristic of in-vivo conditions
- humidity of the air in the lab may be reduced if the external environment is very humid to reduce the risk of fungal infections
- humidity should be maintained at 95-98%
- humidity has direct impact on osmolality of media components
- preimplantation embryos develop over the osmolality range of 255-290 mOsm/kg

humid vrs. dry incubator

- Humid incubation results in a shift in osmolality of about 10 mOsm/kg over 7 days. This shift is as high as 56 mOsm over the same period in dry conditions
- Oil preincubation in a humid environment shows no benefit on the rate of osmolality change when the oil is then used in a non-humidified culture environment

humid vrs. dry incubator

- While the type of oil used does influence the rate of osmolality rise, no oil that was tested could fully compensate for the dry incubator conditions

volatile organic compounds (VOCs)

- VOCs are highly volatile hydrocarbon compounds. boiling point usually below 100^{o}C
- Most VOCs are gases at room temperature
- VOCs can be man-made or naturally occuring compounds released from plants

VOCs

- Air cleanliness is classified according to the number and size of particles within a sample of air measured in particles per cubic foot or cubic meter of air
- ISO 14644-1 classifications are defined as ISO class 1 to 9, Cleanest, Ultra-pure air is class 1
- IVF laboratory can be maintained using a positivie air pressure relative to adjacent areas

VOCs (Environmental sources)

VOCs level in the lab should be below 0.5 ppm for acceptable blastocyst development

Sources of VOCs in the IVF Lab

- lab consumables (plastic culture dishes, syringes etc)
- perfumes, hand creams, sanitizers, scented aerosols, aftershaves, paint-thinners etc
- Dry cleaning agents
- new equipments (e.g. incubators) and funiture
- Location of the Lab in an Urban area

VOCs

- significant levels of benzene, toulene, xylenes and styrene were found in and around ART laboratory with the highest concentrations found inside large box incubators

effects of VOCs on embryo development

- significant impairment in fertilization
- degenerated ooplasm/lysing of ooplasm
- decrease in ICM and trophectoderm formation
- reduction in morula and blastocystm formation
- reduced hatching and implantation rates

VOCs control in the IVF Lab

- off-gasing of consumables
- “Burning in” new IVF lab facility and new IVF Equipments especially incubators
- HVAC system
- HEPA Filters remove 99.9% of particles smaller than 0.3um (Laminar flow hoods)
- UV filter air purification system
- KMnO4 can be used to oxidize lower Mw compounds but should always be used with activated carbon

Activated Charcoal and HEPA filtier

Heating, ventilation and air conditioning (HVAC-System)

Conclusion

- The ability to minimize intracellular stress is a significant factor in being able to maintain embryo vialbility in culture
- The more an embryo has to adapt to its environment the more its viability is compromised
- factors contributing to in-vitro stress and affecting the microenvironment of cultured embryos should be dealt with adequate QC, QA and efficient troubleshooting protocols


Thank
you!