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Comparison of Clinical Pregnancy from Humidified and Dry Incubation of Embryos in a Tropical Region

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ABSTRACT

The laboratory induced cellular stress on zygotes and embryos in their microenvironment could negatively influence the clinical outcome. One of the core components of an IVF lab is the culture incubator. Incubators can provide a stable and Appropriate culture environment by regulating optimal conditions on parameters such as temperature, gas levels and humidity. Clinical studies which compare incubator characteristics may provide insight to their efficacy. In humid conditions of the tropical climate, the incubators without humidifiers can also be used. Hence this study was done to identify the role of a humidifier in a tropical country. In this multicentre retrospective study, embryos from a total of 787 patients were cultured as two groups -A and B in two different types of incubators- humidified and dry, respectively. 647 patients in group A and 140 patients in group B were examined for the developmental parameters. The embryos were frozen at the blastocyst stage and replaced in subsequent frozen embryo transfer cycles. The resulted pregnancy, miscarriage and the clinical pregnancy outcomes were compared. The data was subjected to statistical validation. There was no significant difference observed in the clinical pregnancy rates between the groups. This study validates the possible use of dry incubators for the in vitro culture of human embryos in tropical climate without aiding any humidification.

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INTRODUCTION

Establishing ideal embryo culture conditions in the ART laboratory is required for a better outcome (Simopoulou *et al.*, 2018). Embryo culture media are commercially available and there is a considerable batch consistency and quality. However laboratory parameters like air quality, temperature, humidity as well as optimal pH levels and osmolality of the culture medium also need to be standardized (Eaton *et al.*, 2012; Esteves and Bento, 2013; Cario Consensus Group, 2019; Swain, 2014; Heitmann *et al.*, 2015). The decision on the number and selection of incubators with the required characteristics for an IVF laboratory plays a crucial role (Swain, 2014; Santos *et al.*, 2015). A multitude of incubator models with several modifications are now available (Holmes and Swain, 2018). The humidified incubators are widely used in IVF labs. The humidity can be supplied by evaporation of water from a reservoir or through the gas inlet

within the incubator. Recently non-humidified, dry benchtop incubators are also available for human embryo culture.

There have been improvisations from conventional C02 incubators to time lapse cinematography incubators including benchtop models with triple gas supply (Armstrong *et al.*, 2019; Kieslinger *et al.*, 2016; Holmes *et al.*, 2019; Wu *et al.*, 2016). The in vivo embryonic development is essentially humid. Original incubators had thermal conductivity CO_2 sensors that required humidity for optimal detection. The recent infrared CO_2 sensors of the incubators do not require humidity. Inlet of humidity in incubator could lead to increased incidence of pathogenic contamination. Traditionally this was minimized by adding copper ions into the embryo culture medium. Oil overlay and air purifying technologies also helped in aseptic cultures. Oil overlay and infra-red CO_2 sensors have enabled the use of non-humidified incubator for the in vitro culture of human embryos (Fawzy *et al.*, 2017; Albert *et al.*, 2018). The osmolality of the micro droplets of culture medium and its stability in non humidified incubation has been studied (Yumoto *et al.*, 2019; Sunde *et al.*, 2016). Small benchtop incubators achieve the control of microenvironmental variables easily (Fujiwara *et al.*, 2007). However, only few studies are comparing the outcome of cultures from non-humidified and humidified incubators in tropical countries. The study hypothesis was "absence of humidification in the incubation chamber does not have any effect on clinical pregnancy in an ICSI set up with new generation dry humidification systems".

MATERIALS AND METHODS

Study design, size, and duration

This retrospective multicentre study was done in de-identified data from 787 cycles of day 5 frozen blastocyst transfers. The study was conducted in 11 University affiliated ART research centers from January to October 2018. The ART center database documented patient's age, weight, height, sperm DNA fragmentation levels, thyroid status, hysteroscopy and ultrasound findings, days of gonadotropin ovarian stimulation, protocol followed, oocytes maturity and the embryo development information. Inclusion criteria were patients age 25-37 years, metaphase 2 oocytes more than 6, less than 30% sperm DNA fragmentation and step down gonadotropin stimulation with fixed antagonist protocol for 11-13 days. Patients with BMI>28, polycystic ovaries, hypo or hyperthyroidism, diabetes, endometriosis, low AMH< 1.5 and with evidence of ovarian hyperstimulation were excluded from the study. A total of 787 embryos were cultured in two different types of benchtop incubators, ESCO MIRI multiroombenchtop incubator (non-humidified) (n1=140) and COOK-K- Minc (humidified) (n2=647) in the period from January to October 2018. The mean age of the patients in the case group (n1=140) was 34.64 and the control group (n2=647) was 34.52 years. The pregnancy and miscarriages were compared in both groups.

Stimulation and laboratory culture

All patients had controlled ovarian hyperstimulation (150-300 IU recombinant FSH with or without HMG) for 11-13 days with recombinant HCG (Ovitrelle)/GnRH agonist (Luprolide acetate) trigger when dominant follicle reached 22-24mm diameter. Transvaginal oocyte retrieval was done after 36 hrs of trigger. Oocyte cumulus complexes were collected in HEPES buffered medium and cultured in Vitromed single step medium until denudation. Collected oocytes were exposed to Hyaluronidase enzyme (80IU, Vitromed) for a maximum of 30 seconds and micro pipetting to remove the cumulus complexes. Oocyte maturity and morphological grading were assessed. ICSI was performed under an inverted microscope (Olympus) with a micromanipulator (Narshige) within 4 hrs of oocyte retrieval in HEPES buffered medium overlaid with oil (Vitromed). Oocytes following ICSI were washed and cultured into 20 microlitre droplets of pre-equilibrated single step medium (Vitromed) overlaid with oil (Vitromed). Fertilization assessment was performed at 16-18 hrs after injection; embryos were examined and graded again at 40-42 hrs for cleavage. Embryos were cultured till Day 6 and optimal blastocysts were vitrified using Kitazato medium on both day 5 and 6 of culture. The morphological grading of embryos was based on David Gardner's embryo grading system and the ESHRE (The Istanbul consensus, 2011) guidelines.

Humidified and dry incubation

Our clinics are set up in regions with tropical climatic conditions. The humidified incubator is supplied with premixed gases of 6.0% CO_2, 5.0% O_2 and 89% of nitrogen (MINC model no K-MINC 1000 - Figure 1). The dry incubator is connected with CO_2 and nitrogen supply to have a set mix of 6.0% CO_2, 5.0%O_2 and 89% of nitrogen (Miri-ESCO IPXO-Lithuania – Figure 2). The frozen thawed embryos from fresh cycles were replaced in subsequent cycles at the blastocyst stage. The results were compared from both the groups.

Primary and secondary outcome measures

Figure 1: Benchtop incubator, COOK-K- Minc (humidified)

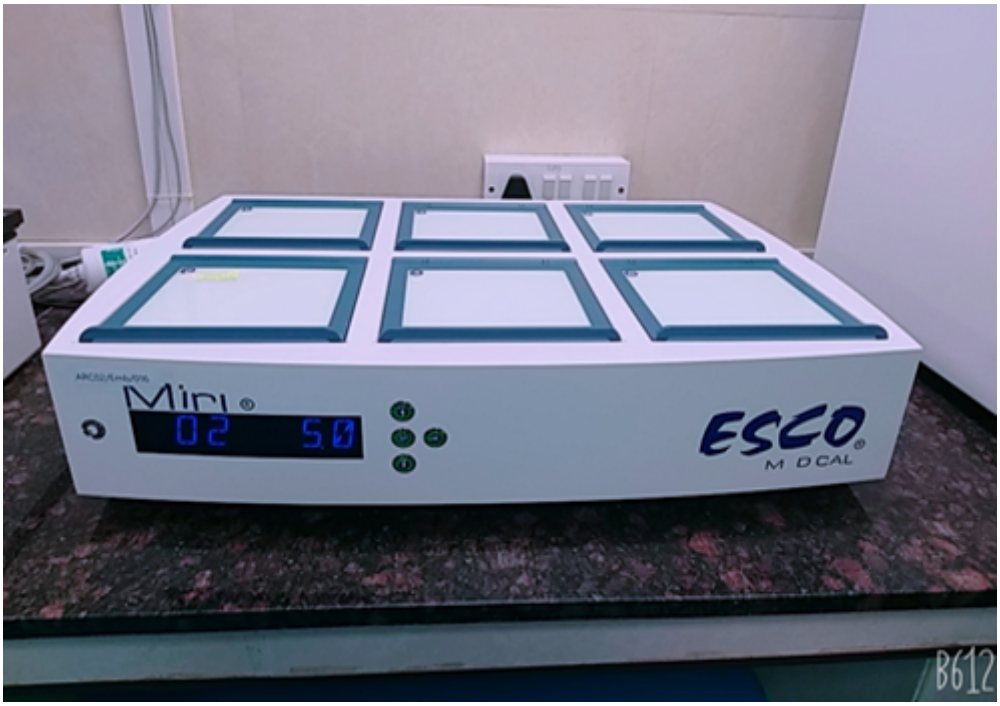

Figure 2: ESCO MIRI multiroom benchtop incubator (non-humidified)

Table 1: Results from Embryos that were cultured in humidified incubator for 140(n1) patients and in dry incubator for 647(n2)

Embryo transfer Outcome	Embryo Culture Method			Statistical Analysis		
	Non Humidified Incubator (n1=140)	Humidified Incubator (n2=647)	Total (n=787)	Odd's Ratio (Confidence Interval)	Z statistic	P value
Live Birth	71	324	395	1.0258(0.7118-1.4783)	0.137	0.8913
No Pregnancy	66	316	382	0.9342(0.6481-1.3470)	0.364	0.7155
Biochemical Pregnancy	1	6	7	0.7686(0.0918-6.4351)	0.243	0.8082
Miscarriage	2	24	26	0.3762(0.0879-1.6107)	1.318	0.1876

The primary outcome was the clinical pregnancy rate, defined as a pregnancy visualized by ultrasound and demonstrating a normal fetal heart rate (number of clinical pregnancy/number of embryo transfers). The implantation rate (no of sacs/no of embryos transferred), the miscarriage rate (number of miscarriages/number of clinical pregnancies) and the live birth rates (number of live births/number of embryo transfers) were also compared between the groups.

Ethical Approval

All collected data was examined and approved by the appropriate ethics committee (Ethical Code: SMC/IEC/2016/01/241) and have therefore been performed in accordance with the ethical standards laid down in the Updated Revised Declaration of Helsinki (2008).

Statistical Analysis

Descriptive and inferential statistics was used to find significant levels of differences. Mean implantation rates, clinical pregnancy rates, miscarriage rates and carry home baby rates were calculated by taking percentages. Odd's ratio, Z statistic and p-value were calculated (Table 1). Statistical analysis was done using MEDCALC (Belgium). Confidence interval was kept at 95% and a p-value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Embryos were cultured in a humidified incubator for 647(82% Group A) patients and dry incubator for 140(18% - Group B). 324 patients (50%) achieved positive pregnancy in group A and 71 patients (50.7%) in group B. 6 patients(0.9%) and 1 patient(0.7%) had biochemical pregnancy and 24 patients (3.7%)and 2 patients(1.4%) had a miscarriage from group A and B respectively.

Of all the ongoing pregnancies, 294 patients (45.4%) were from group A and 68 patients (48.5%) from group B. The pregnancy, miscarriage and the clinical pregnancy rates were compared using the Chi-square test. The statistical value obtained was non significant, which indicates the low evidence to support any of the groups. There was no Statistically significant difference between both groups.

Limitations, reasons for caution

The size of the population is smaller, particularly with the dry incubator culture. Moreover, there were no sibling cultures performed. Despite using the same set of equipment, there was no clear evidence that all the labs have the same environment.

CONCLUSION

The results from the study suggest dry incubators are as efficient as humid ones for the production of in-vitro embryos for a successful outcome. The possible mechanism associated is the faster equilibration of the culture components at incubation. Embryos cultured in humidified and dry incubators do not show any significant difference in terms of clinical pregnancy upon transfer. Benchtop incubators without humidifiers are simple and economical. So it can definitely be employed for the routine embryo culture in tropical countries. However, non humidification method can be considered suitable for the IVF treatment without significantly affecting implantation and carry home baby rate. A larger study may be required to confirm the current find-

ings. Furthermore, prospective sibling studies can be carried out to compare and find the effect of humidification on pregnancy outcome in the same set of patients with similar clinical characteristics.

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