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MORPHOLOGICAL ASSESSMENT OF HUMAN EMBRYOS AND EMBRYO CULTURE USING EMBRYO SCOPE

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ABSTRACT / INTRODUCTION

Human embryos show a considerable degree of plasticity and can be cultured in diverse set of culture conditions. Embryos are traditionally cultured in a CO_2 incubator which maintains a fairly constant temperature (37^0C) CO2 levels (6-7%) to maintain a pH of 7.3-7.4 in the culture medium. Due to frequent door opening of the incubators during the morphologic assessment of embryos and the resultant fall in temperature and pH of the culture medium which significantly affects the embryo metabolism, a need was felt for a non invasive in-built dynamic monitoring system for the embryo development.The development of the Trigas bench top incubators enables direct heat transfer to the culture dish and a direct purging of the gas mixture (79% N_2,6%$CO_2$and 5%O_2)to maintain constant temperature and pH of the embryo culture medium.

KEYWORDS: Morphology, Embryo, Embryo culture, Embryoscope.

MATERIALS AND METHODS

The time-lapse embryoscope system consists of an incubator with a built in microscope and camera connected to a computer.(Fig 1) It enables to acquire time lapse images of the embryo development and makes it possible to analyze the dynamics of embryo development.[1] This time lapse micro-imagery technology allows the embryologist to use embryo kinetics as potential markers for selection. Human embryos can be cultured in an embryoscope without the requirement of taking out the culture dishes from the incubator for static morphological assessment, thereby allowing the embryos to grow in a constant environment.

Embryoscope monitoring system comprises of a micro sensor which facilitates automatic measurements of the oxygen consumption for each individual Oocyte. It employs an imaging system which uses low intensity red light (635nm) from a single light emitting diode with short illumination of 30 milliseconds per image. The optics of this imaging system comprises of a modular Hoffman's contrast with a 20X objective, providing optimal light sensitivity and resolution for the red wavelength. The digital images are collected by a highly sensitive charge coupled device camera (1,280 X 1,024 Pixels) with a resolution of 3 Pixels /micrometers. It also employs a data collection and recording system with details of the developmental events of individual embryos. The color coding system is used for embryos:- red color signifies degenerating embryos, green color coded embryos are healthy and viable embryos and thus can be transferred whereas blue coded embryos are destined for freezing or vitrification.

During the course of embryo development, embryoscope can be calibrated to maintain a constant temperature and pH of the medium in which the embryos are cultured. The images are taken after every 20 minutes, in seven different planes over a period of 48 hrs. The door of the embryoscope is opened only once i.e. after 72 hrs for transferring the cleavage stage embryos at the 7-8 cell stage. If sequential culture media is used for supporting the embryo development to the blastocyst stage, the media is replenished after 48hrs without taking the culture dishes out of the embryoscope and hence without exposing them to stress. Four different embyoscopes can be connected to a computer, thus enabling the clinical embryologist to monitor the development of a maximum of 288 embryos from the zygote stage to the cleavage stage without even entering the IVF laboratory. (Fig 2)

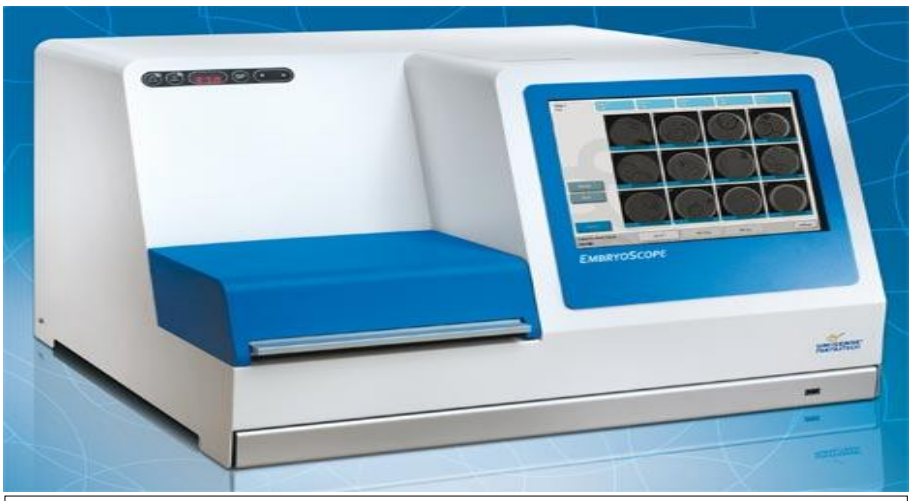

Fig 1.The Embryoscope

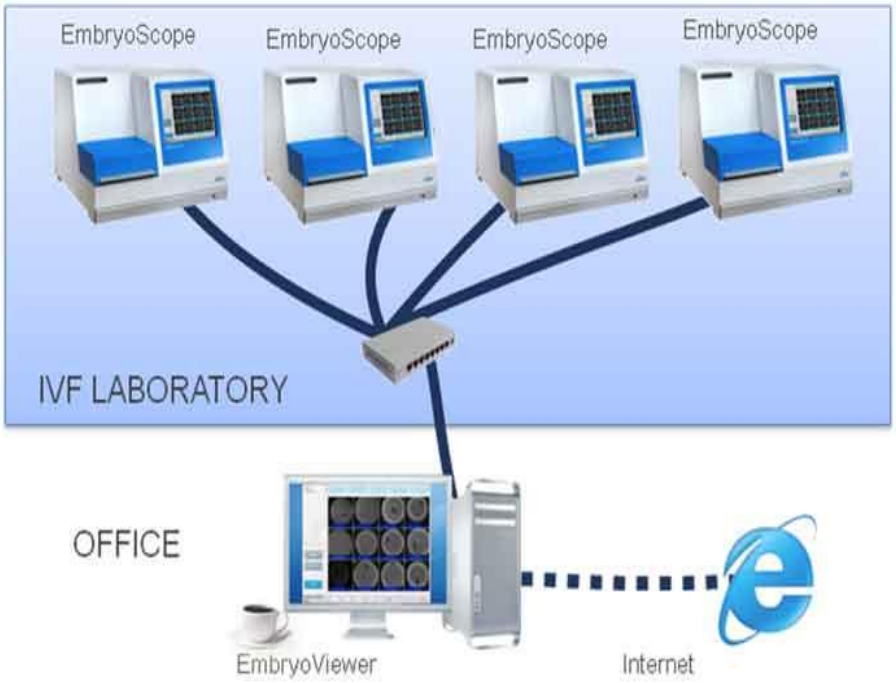

Fig 2: Four Embyoscopes of IVF Laboratory connected to a computer.

Table1: Events registered in time-lapse analysis using an Embryoscope.

S N.	Parameter	Description
1	Cell stages	Time points for each cell division until compaction
2	Embryonic stages	Time points and duration of compaction, morula and blastocyst stages
3	Blastocyst contractions	Time points, duration and extent of blastocyst contractions

DISCUSSION

Embryoscope is a better alternative to the standard incubator for the culture of human embryos. This time lapse imagery may potentially improve the selection of most viable embryos for transfer. It provides a wealth of information on the cleavage rates and synchrony at the early developmental stages.[2, 3] Additional information is also obtained in the form of compaction, cavitations, blastocoel formation and hatching. Detailed data on the developmental trajectory, available through time lapse imagery enables detection of significant differences in embryo growth that may be missed by conventional static scoring system of morphology assessment. In addition, the embryo respiration rate as determined by embryoscope provides a simple, fast, non-invasive and consistent measurement without affecting the embryos. This may significantly improve the selection of the embryos before transfer and can be a step towards a single embryo transfer.

Time lapse imagery can minimize the disturbances in the embryo culture environment by integrating the incubation and observation of embryo development into single equipment. Embryo culture in a standard incubator requires intermittent observations by removing the culture dishes from the incubator. The dynamic processes of embryo development using the time lapse technology provides vital information for embryo selection. It is a unique non-invasive method of monitoring early embryogenesis in humans. In short, embryoscope provides an adequate environment for human embryo culture without affecting the embryo quality or viability.[3]

Embryo selection based on routine morphological assessment is not always associated with higher implantation and pregnancy rates. This led to exploring new non-invasive and valid methods of assessing embryo vitality which can result in high implantation and clinical pregnancy rates. The combination of optimal incubation conditions for early developing embryos and a time-lapse embryo monitoring system give a wealth of information on embryo development. This technology can help in assessing their quality for transfer and freezing. Embryos cultured in an embryoscope are exposed to light when digital images of various developmental stages of embryos are acquired. This light exposure leads to stress to the embryos which can affect embryo development. The application of time-lapse imagery observation will promote the development of non-invasive methods for the assessment of embryo viability which can be applied to a clinical setting. The data obtained using an embryoscope will not only assess embryo morphology and chronology of events of early embryonic development but also help in understanding physiological events at this crucial stage of human development. Time-lapse embryoscope monitoring can be applied safely to human embryos, thereby enabling the clinical embryologist for improved embryo selection for transfer.[4] Embryoscope gives unprecedented information about the first cell divisions that can be used to identify embryos that will develop into expanding blastocysts.timing and cytoplasmic loss in compact stage embryos assists in predicting their ability to develop into optimal blastocysts. The cytoplasmic fragments and developmental delay in compact stage morulas do not affect the blastocyst rate[5] Marcos Meseguer et al retrospectively analyzed the data and concluded that culturing and selecting embryos by Embryoscope significantly improved the relative probability of clinical pregnancy .The elevated clinical pregnancy rate was attributed to a combination of stable culture conditions and the use of morphokinetic parameters for embryo selection.[6] DrNeilsRamsing, CSO of M/s UnisensFertilitech a/s, Denmark has codeveloped this technology with Dr Marcos Meseguer from IVI clinic,Spain

who has published data for more than 12000 IVF cycles using the embryoscope.[2, 6] In conventional IVF, embryo morphology is observed and evaluated at certain time points during embryonic development. The crucial decision on which embryo to transfer is based primarily on developmental stage and blastomere symmetry at the day of transfer, taking into account embryo morphology evaluation at earlier observation points.[7] Embryoscope significantly increasesthe number of morphologic observations available tothe embryologist for assessing embryo quality.[8,9]

CONCLUSION

Embryoscope is a novel approach for human embryo culture which holds the promise of morphological assessment of embryo viability noninvasively without disturbing the environment of the embryo culture medium. This new technology can prove a boon for in vitro culture of human embryos in the laboratory and holds great promise in the field of assisted reproductive technology.

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