Clinical Investigation Protocol

Prospective Observational, Comparative and Validation Study using TimeLapse Morphometry MIRI® Imaging Incubator (TiMMI Study)

Device:	ESCO MIRI-TL
Protocol #:	2016-TiMMI-001
Version:	A
Sponsor:	ESCO Medical 21 Changi South Street 1 Singapore 486 777
Date:	June 15, 2016

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Study Title	Prospective Observational, Comparative and Validation Study Using TimeLapse Morphometry MIRI-TL® Imaging Incubator (TiMMI Study) Protocol # 2016-TiMMI-001
Sponsor	ESCO Medical 21 Changi South Street 1 Singapore 486 777
Study Device	MIRI-TL , including: • MIRI-TL incubator • MIRI-TL Viewer • Uninterruptible Power Supply • Accessories CultureCoin dish
Indications for Use/Intended Use	The MIRI-TL is a purpose built embryo culture incubator using a built-in microscope and camera designed specifically for the continuous assessment of embryo development *in-vitro*. As images are digitally obtained and stored, a video can be generated to enable more objective and reliable grading criteria. This technology, in conjunction with the MIRI-TL Viewer allows for non-invasive viability evaluation and assessment allowing for better prediction of embryo developmental potential and implantation success. The MIRI-TL is CE marked for clinical use. The CultureCoin is intended to be used to hold human oocytes and embryos during handling and culture.
Study Purpose	The purpose of this study is to demonstrate and validate the non-inferior or superior safety and efficacy of the MIRI-TL compared to standard big-box incubators used for embryo culture.
Study Design	Prospective, observational, randomized, double-arm

Safety Measures	Adverse events and device malfunctions will be reported and summarized from the start of embryo culture through study exit.

Number of Subjects	Up to 500 patients
Subject Population	Patients undergoing in vitro fertilization treatment who fulfill all eligibility criteria.
Eligibility Criteria	Inclusion Criteria: • At least 18 years of age • Women undergoing in vitro fertilization treatment using their own or frozen oocytes • Fresh or Frozen Embryo Transfer • Fertilization by ICSI • At least 4 diploid (2PN) embryos at fertilization check • Willing to have all inseminated oocytes imaged by Miri • Willing to comply with study protocol and procedures • Willing to provide written informed consent Exclusion Criteria: • Fertilization using surgically removed sperm • History of cancer
Number of Sites	1
Study Duration	Expected study duration is 18 months including enrollment and follow-up. Subjects will be followed until pregnancy outcome has been determined (i.e. date of negative hCG test, 8-12 week clinical pregnancy ultrasound, or date of live birth, if pregnant).
Summary of Data Collection	• Enrollment: Demographics, IVF and pregnancy history • IVF Lab: Embryo morphology • Time-Lapse Imaging Data • Pregnancy test and Live Birth Outcome • Preimplantation Genetic Screening (PGS) Data, if applicable • Frozen Embryo Transfer (FET) Data, if applicable
Study Start	April 2016

1. Introduction

The purpose of this clinical study protocol is to gather information regarding the performance and safety of a novel time lapse incubator compared to standard incubator systems. The MIRI-TL and the Culture Coin are CE Marked and have therefore fulfilled EU safety and performance criteria. In the US, 510(k) submission for both products is planned for Q2/Q3 2016.

As the sponsor of this clinical study, ESCO Medical has the overall responsibility for the conduct of the study, including assurance that the study will be performed according to the clinical investigation plan, applicable US Food and Drug Administration (FDA) and local regulation.

2. Background

The optimal goal of IVF should be the delivery of a healthy singleton baby and healthcare providers may be compelled via legal, financial, and moral obligations to reduce multiple pregnancies.[1-4] The clinical dilemma is how to restrict the number of embryos for transfer to prevent multiple births while at the same time maintaining or improving IVF success rates. The ability to selecting the highest quality embryo that is most likely to result in implantation becomes the key solution, however, this has proven to be challenging.

In the late 1990s, improved culture media enabled blastocyst culture, allowing embryos to develop in a petri dish for 2 to 3 additional days. Generally, about 40% of the embryos will form a blastocyst while others arrest in the culture dish during this extended period of incubation. It was broadly reported that blastocyst formation was a key embryo selection criterion for predicting successful implantation. Papanikolaou et al demonstrated in prospective randomized studies that embryo implantation rates and live birth rates were significantly improved with Day 5 transfer, as compared to Day 3 transfer.[5-6] Because of improved implantation and live birth rates, blastocyst culture was widely adopted in clinics, in particular those which utilized single embryo transfer.

Embryos selection based on traditional morphology scores developed in the early 1980s. The morphological criteria are based on multiple 'static' observations of embryos under regular light microscopes. This involved removing the culture dish, which contained the embryos, from the incubator on a daily basis and assessing the embryos based on their morphology and growth. A number of studies have reported observations relating embryo morphology and implantation, including 2-pronuclear score, early cleavage observation, Day 2 or

Day 3 cleaved embryo assessments, and Day 5 or Day 6 blastocyst grading.[7-24]

However, despite exhaustive efforts by clinical embryologists using a regular light microscope to assess embryo quality, it is clear that traditional morphology grading, which only captures static images at a few time points, is of limited predictive value.

Several studies have indicated that using information obtained from time-lapse imaging, which provides a continuous 'movie' of embryo development, may improve embryo selection and ultimately improve clinical pregnancy or implantation.[25-27] This is partly due to the non-invasive and controlled micro environment most purpose built time-lapse incubators provide. The literature has been encouraging as it appears that using time-lapse in the IVF laboratory may have a significant positive impact by providing embryologists with an additional tool to assess and predict which embryo is more likely to lead to a successful pregnancy.

3. Study Objectives

The purpose of this clinical study is to demonstrate and validate the safety and efficacy of the purpose built MIRI-TL incubator and correlate morphokinetic data to improved success rates.

3.1 Primary Objective

- To collect safety and imaging data on the MIRI-TL and CultureCoin.

3.2 Secondary Objectives

- To collect enrollment data: demographics, IVF and pregnancy history
- To collect Day 3, Day 5 and Day 6 embryo morphology data
- To collect pregnancy test and live birth outcome data
- To collect pre-implantation genetic screening (PGS) data (if applicable)
- To collect Frozen Embryo Transfer (FET) data (if applicable)

4. Description of Device

The Miri- TL, manufactured by the study sponsor Esco Medical is a closed-system incubator device with built-in visual monitoring system. This closed system incubation system eliminates any environmental disruption and contamination risk by daily handling. The built-in camera has the ability to record cell division kinetics and morphology of developing embryos which can provide more reliable indicators of embryo quality. This novel time-lapse incubator captures images at five (5) minute intervals and its six (6) separate incubation chambers maximizes temperature and gas stability, providing a safe and secure environment for human embryos.

Figure 1: MIRI-TL

4.1 CultureCoin

The 16-well CultureCoin (**Figure 2**) is a standard size (60mm) embryo culture dish with individual micro- wells aligned in a row in the center of the dish. Each micro-well is designed to accommodate a single patient's oocytes or developing embryos through the blastocyst stage and are culture individually. The 16-well CultureCoin is designed to fit in only one (1) position in one (1) of the individual six (6) incubator chambers, and each micro-well is identified to facilitate embryo tracking (for example, micro-well

Figure 2: CultureCoin

5. Number of Centers and Study Duration

The TiMMI study will include only one investigational site. This site will conduct the study under a single clinical investigation plan.

Expected study duration is 18 months including enrollment and follow-up. Subjects will be followed until pregnancy outcome has been determined (i.e. date of negative hCG test or 8-12 week clinical pregnancy ultrasound, if pregnant).

6. Study Design

This is a prospective, non-blinded, observational, randomized, double-arm study.

7. Statistical Methods and Analyses

Up to 500 subjects' embryos will be collected in this ongoing data collection protocol. We aim to recruit 200 patients in the standard culture group, and 300 patients in the MIRI-TL group.

Assuming that 25% of all inseminated oocytes would develop into blastocysts (based on published clinical data), at least 1300 inseminated oocytes (~163 patients) would be needed in each group to detect an increase in blastocyst proportion of 20% with 80% power and a significance level of 0.05 (two-tailed tests).

Assuming that time from insemination to start of blastulation (tSB) follows a normal distribution, and is 96h with a variance of 49h in euploid embryos, and 98h (variance 49h) in aneuploid embryos, ~405 blastocysts (1620 inseminated oocytes = 203 patients) in the MIRI-TL group will need to be biopsied to detect a significant difference in tSB (80% power, 0.95 confidence level, 2-tailed test). Since ~80% of all patients receive PGS at our clinical site, we will need ~255 patients in our MIRI-TL group.

To account for patient dropout and lower than expected oocyte retrieval per patient, we propose to recruit up to 500 subjects in this ongoing data collection protocol: 200 patients in the standard culture (SC) group and 300 patients in the MIRI-TL (MIRI) group.

Embryos with incomplete annotations will be excluded from analysis. All variables will be tested for normality using Shapiro–Wilks test. Continuous data following a normal distribution will be analyzed with Students t-test.

Continuous data not fulfilling the assumption of normality will be analyzed with non-parametric tests such as the Wilcoxon rank-sum test. For categorical data Chi-squared or Fisher's exact test will be used. Two-tailed p-values < 0.05 will be considered significant.

8. Study Protocol

8.0 Patient Randomization

Patients will be randomized to the MIRI-TL group (MIRI) or the Standard culture group (SC) by randomization number generator through www.randomizer.org, using a number set from 1 to 500. Numbers 1-300 will be assigned to the study (MIRI-TL) group while numbers 301-500 will be assigned to the control (Standard Incubator).

8.1 Egg Retrieval and Fertilization-Day 0

Egg retrieval and fertilization follow the standard protocol in place at the clinical site. Insemination, by intracytoplasmic sperm injection (ICSI) will take place on Day 0 (egg retrieval day) following standard protocol currently in place at the clinical site. The fertilized eggs should be free of cumulus cells as much as possible. If the cumulus cells remain in place, they may interfere with the quality of the imaging. Immediately after micro insemination, eggs will be placed in individual wells within a CultureCoin (MIRI group), or a standard culture dish (SC group), and incubated in the MIRI-TL or standard incubator respectively. All eggs will be cultured in the standard fertilization media currently in place at the clinical site. At 20hr after insemination, the MIRI-TL imaging software will be Paused, and all embryos will be examined by the embryologist for successful fertilization (2PN). All normally fertilized eggs will be placed into a new CultureCoin (MIRI group) or standard culture dish (SC group) containing the standard embryo culture media, and incubated in the MIRI-TL or standard incubator respectively.

Once an embryo is assigned a micro-well location, it must remain in the same location throughout the study imaging period. Embryos assigned to the MIRI group will be imaged in the CultureCoin from Day 0 to Day 7. Images of the developing embryos will automatically be captured with the MIRI-TL Microscope. Data collected will include the following:

- Number of eggs inseminated

- Fertilization method
- Total number of fertilized eggs (2PN)

8.2 Day 3 Embryo Assessment

At Day 3, the MIRI-TL imaging software will be Paused and the embryologist will evaluate embryos from both groups per standard procedure. The development stage of the embryos and grading based on traditional morphology criteria will be recorded. It is recommended that the Day 3 morphology assessment is done within 48 hours after the fertilization (2PN) check. ***Per protocol, Assisted Hatching (AH) is allowed if patients have opted for embryo biopsy and PGS.***

After embryo assessment the embryos may be placed back into the MIRI-TL or standard incubator as per their respective groups, and imaging will be resumed for the MIRI group.

8.3 Day 5, 6 and 7 Embryo Assessment

The embryologist will evaluate the embryos using traditional morphological grading. It is recommended that the Day 5 morphology assessment is done within 48 hours after the Day 3 morphology assessment.

8.4 Embryo Biopsy and Preimplantation Genetic Screening Data - if applicable

Pre-implantation Genetic Screening (PGS) is not required in this study. If the physician and the patient have previously decided to perform PGS in the IVF cycle, the outcome data will be collected. Embryo biopsy and PGS will be performed per standard procedure. Transfer procedures and disposition of embryos not selected for transfer will be determined as per the standard protocol in place at the time of the study.

8.5 Embryo Transfer

Embryo(s) will be selected for transfer based on the current standard protocol in place at the clinical site. Data pertaining to the embryo transfer procedure will include the following:

- Day and date of transfer
- Total number of embryos transferred
- Luteal phase support

8.6 Outcome: Pregnancy Test

After embryo transfer, each subject will be followed to determine if a chemical pregnancy has been achieved by undergoing the clinical sites' standard serum pregnancy testing procedure. Data will be recorded and include the following:

- Date of the serum pregnancy test
- Quantitative hCG level

Each subject with a positive serum pregnancy test result will continue to be followed. If the result of the pregnancy test is negative, the subject will have completed the study and be exited at that time.

8.7 Outcome: Pregnancy Confirmation Ultrasound

A clinical pregnancy is typically verified by the detection of a fetal heartbeat via vaginal ultrasound. The clinical site verifies clinical pregnancy at approximately 5-8 weeks gestation. The results of the test will be recorded and include the following:

- Date of ultrasound
- Result of initial pregnancy ultrasound
    - Number of intrauterine gestational sacs
    - Number of fetal heart beats

If the result of the ultrasound is negative, that is, if no fetal heartbeat is detected, then the subject will have completed the study and be exited at that time.

8.8 Outcome: Ongoing Pregnancy Ultrasound

The clinical site verifies the viability of the pregnancy (ongoing pregnancy) at approximately 8-12 weeks gestational age via vaginal ultrasound. Data will be recorded and include the following:

- Date of ultrasound
- Result of ultrasound
    - Number of gestational sacs
    - Number of fetal heartbeats
- Pregnancy outcome

If the result of the ultrasound is negative, that is, if an ongoing pregnancy is not confirmed, then the subject will have completed the study and be exited at that time.

8.9 Frozen Embryo Transfer (FET) Cycles – if applicable

If the patient does not get pregnant from the fresh embryo transfer, data from blastocysts previously frozen in the fresh cycle may be thawed for a FET for the duration of the study. Endometrial preparation for FET cycles will be performed per the clinical sites standard protocol. Data pertaining to the stimulation protocol, pregnancy, and outcome data will be collected.

8.10 Protocol Deviations

A protocol deviation occurs when a clinical investigator and/or study site personnel does not conduct the study according to the clinical investigation plan. All deviations are recorded on the **Protocol Deviation** case report form. Data collected will include the following:

- Date of deviation
- Type of deviation
- Description of deviation
- Corrective action

The Investigator must maintain accurate, complete, and current records relating to the clinical study. This includes source documents showing the dates and reasons for each deviation from the clinical investigation plan.

8.11 Device Incidents

Any feedback in regards to the Miri- TL should be reported to Esco Global as soon as possible. Feedback may consist of any alleged deficiency related to the physical characteristics, identity, quality, durability, reliability, safety, effectiveness, or performance of the MIRI-TL and/or CultureCoin. Engineering and Quality will investigate device deficiencies and perform an evaluation to determine and document the root cause of any device deficiency. Reports will be filed in the MIRI-TL Study project file and/or in document control. The following data will be collected:

- Date and time incident first observed
- Associated Device(s)
- Description of incident
- Troubleshooting
- Outcome

8.12 Adverse Events

While the overall IVF procedure may involve several visits and procedures, the first time the subject or user has any encounter with the MIRI-TL and CultureCoin is when the embryos are first loaded onto the MIRI-TL for imaging. Adverse

events for the subject or user will be reported from the point of embryos being placed in the MIRI-TL through study completion for each subject. An adverse event will be followed until resolved or stabilized at a level acceptable to the investigator.

Events well documented to be associated with ART/IVF procedures (as outlined in each clinic's consent for IVF procedures) will not be collected as adverse events under this protocol. Other common non-study or non-device related, minor health complaints will not be collected as adverse events (for example: colds, sprains, headaches).

The investigator should follow the reporting requirements of the reviewing IRB/REB for all adverse events (including serious and unanticipated adverse device effects). Documentation of any IRB and Esco Global notifications should be maintained in the site's clinical study files. It is recommended that acknowledgement of receipt from the IRB be maintained in the study file as well.

Adverse events will be reported to Esco Global and data collected will include the following:

- Date of adverse event
- Type of event and description
- Action/treatment taken
- Severity of event
- Seriousness of event
- Investigator's assessment as to the relationship of the AE to the MIRI-TL
- Investigator's assessment as to the relationship of the AE to IVF/ART procedures

Investigator's assessment if the event is a potential Unanticipated Adverse Device Effect (UADE)

Source documentation for adverse events must be collected and filed with the subject's medical chart. Refer to Section 10 – Adverse Event Definitions and Reporting for additional information. Because study procedures are limited to the noninvasive collection of clinical data, no Data Safety Monitoring Board will be established for this study.

8.13 Study Exit

Study Exit data should be collected at the time a subject is exited from the study. A subject will be considered to have exited from the study for any of the following reasons including, but not limited to:

- Subject completed follow-ups required by the clinical protocol
- IVF cycle not completed or cancelled
- Subject requested to be withdrawn
- Subject terminated IVF treatment for personal reasons
- No availability of culture slots in the MIRI-TL
- Investigator requested that subject be withdrawn to protect the welfare of the subject
- Subject was lost to follow-up
- Subject was not compliant
- Other (specify)

A subject may elect to withdraw from the study at any time. The subject should notify the investigator if she wishes to withdraw from the study. The investigator and research staff should encourage all subjects to return for required follow-up visits. The investigator is free to withdraw a subject at any time if, in the investigator's opinion, it is in the best medical interest of the subject. A subject so withdrawn will be treated according to standard of medical care. The sponsor reserves the right to terminate this study at any time. Subject data will be included in the analyses up to the time that consent was withdrawn or up to the time that the study was terminated.

8.14 Subject Confidentiality

Subject confidentiality will be maintained throughout the study to the extent permitted by law. That is, every attempt will be made to remove subject identifiers from study documents. For this purpose, a unique subject identification code (subject number) will be assigned and used to allow identification of all data reported for each subject. A log of subject initials and the corresponding subject ID will be maintained to ensure that the information can be tracked back to the source data. All subject study records and files shall be kept in a secure location with limited access. Access shall be granted only to those individuals who are recorded as authorized study staff members.

Study data may be made available to third parties, e.g., in the case of an audit performed by regulatory authorities. The identity of a subject will never be disclosed in the event that study data are published.

9. Potential Benefits

In this study, there is no direct benefit to subjects who participate. However, the data collected in this study may lead to more effective methods of visualizing, monitoring, and selecting the most viable IVF embryos by a physician or embryologist, to maximize the chances of implantation and successful pregnancy

outcome for future patients.

Subjects who successfully complete all study procedures will receive a video of the embryo which was transferred or embryo(s) which were cryopreserved. No other reimbursement will be provided for the IVF cycle.

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