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Embryo culture conditions are significantly improved during uninterrupted incubation: A randomized controlled trial

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ABSTRACT

A parallel group superiority prospective randomised controlled trial was devised to compare the culture characteristics of human pre-implantation stage embryos during uninterrupted culture in a time lapse incubator (TLI) versus the conventional model of interrupted culture in a standard incubator (SI) under low oxygen tension using a single step medium. 221 patients aged 35-and-under, 124 patients aged between 36 and 39 and 86 patients aged 40-and-over years were randomised and cultured either in a SI or in a TLI. Patients in the three age groups were distributed between the TLI and SI in a 1:1 ratio. The development of embryos on days 2, 3 and 5, and the clinical pregnancy and implantation rates were recorded. The fertilisation rate, development of day 2 and clinical pregnancy rates were similar in both treatments but the 8-cell development rate in all age groups combined ($p = 0.016$), blastocyst development rate ($p = 0.0022$) and the implantation rate ($p = 0.0022$) was significantly higher for the uninterrupted culture. These findings demonstrated significant differences between the two incubation groups. It also indicated less efficacious embryonic development with age in both treatments which appeared more pronounced in the conventional incubator. In conclusion uninterrupted culture is superior compared to the interrupted incubation culture system.

1. Introduction

A significant factor in achieving pregnancy using ART treatment is the selection of a suitable embryo for transfer [1–3]. Efficient techniques of embryo culture and the selection of viable embryos for transfer are crucial for successful treatment and to reduce the chance of multiple pregnancies, and its concomitant maternal and neonatal health complications in ART treatment. To reduce health complications there is a need to re-examine old techniques and develop new techniques of embryo culture that yield embryos with higher implantation potential. A commonly used non-invasive technique of embryo selection is to assess embryos based on morphology, which takes into consideration the parameters of morphology assessment such as the presence and degree of fragmentation, number and symmetry of blastomeres, multinucleation and thickness of the zona pellucidae [4–6].

Using conventional incubation procedures, embryo morphological assessment involves brief handling outside the incubator which can subject embryos to potentially harmful fluctuations in environmental conditions such as oscillations in temperature, humidity, pH and light, thereby adversely affecting embryonic development and impacting the viability of the developing embryo [7]. To minimise variations to the culture environment, embryo observation during conventional incubation is restricted to one or two daily observations at specified intervals which limits information acquisition on the progress of embryonic development to a few selected windows. However, time lapse monitoring has the advantage of allowing examination of embryos at all times without the removal of embryos from the incubator and perturbations to their culture medium and milieu. Additionally, time lapse incubation is advantageous as it permits the application of additional kinetic markers for embryo selection [8,9] and enables the development of potentially useful algorithms that may facilitate selection of viable embryo.

However, some questions have been raised regarding the safety of the time-lapse incubator (TLI) on embryonic development. During image acquisition embryos are exposed to light on a more frequent basis than in conventional incubation, and the presence of moving parts and magnetic fields inside the TLI near the culture dish may negatively affect the embryo and call into question the safety of TLIs. The TLI has been shown to not be detrimental to animals and humans in the short term, as Wong et al. showed that the developmental pattern of embryos to the blastocyst stage in a time-lapse system depends on gene expression that is not affected by time-lapse incubation [8]. However, the

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long-term effects of time-lapse incubation have yet to be elucidated. Previous studies examining the safety of TLIs showed no adverse effect on embryo development. Pribenszky et al. [10] performed an investigation of time-lapse incubation on developing mouse pre-implantation embryos and uncovered a strong degree of fragmentation and development to the blastocyst stage. Nakahara et al. [11] suggested that time-lapse observations using an incubator with an integrated optical microscope can be safely utilised in clinical practice. Additionally, Mio et al. [12] showed that cinematography has increased understanding of the morphological mechanisms of fertilisation, development, and behaviour of early stage human embryos. Lemmen et al. [13] used a time-lapse system to study the timing and coordination of events during early development from the zygotic stage to the cleavage stage of embryonic development, while identifying markers linked to good quality embryos and implantation. Kirkegaard et al. [14] and Cruz et al. [15] found that TLIs facilitate embryonic development in cultures to a similar extent as conventional incubators. Holm et al. [16] concluded that time-lapse culture systems allow for detailed observation of the developmental kinetics of bovine embryo. Furthermore, Milewski et al. [17] found that embryonic morphokinetic parameters are associated with developmental and implantation potential and can be considered predictors of embryo quality.

The current parallel-group superiority prospective randomised controlled trial aimed to determine the differences in culture conditions and characteristics and development between uninterrupted incubation in a TLI and interrupted incubation in a standard incubator (SI).

2. Materials and methods

2.1. Oocyte retrieval

Controlled ovarian hyperstimulation was performed according to the standard method for short antagonist protocol using recombinant subcutaneous FSH (Merck Serono, Germany). FSH doses were regulated individually according to patient response. Finally, a dose of 5000 IU of hCG (Pregnyl 5000 I.U, Organon, Netherlands) was administrated intramuscularly when three follicles were 17 mm or larger. Ultrasound-guided oocyte retrieval was performed 36 h after hCG administration.

2.2. Oocyte incubation

Oocytes were divided into two groups, with one group injected with ICSI and cultured in a TLI (EmbryoScope™, Unisense Fertilitech, Denmark) and a second group cultured in a SI (CB 150, Binder, Germany). In addition, patients were categorised according to age. The first age group consisted of 221 patients aged 35 years and under (≤35 years old.) randomised into either TLI (Group A_TL) or SI (Group A_SI) treatment. The second age group of patients between 36 and 39 years (36–39 years old.) consisted of 124 patients randomised into TLI (Group B_TL) and SI groups (Group B_SI). Finally, the third age group of 86 patients aged 40 years and over (≥40 y.o.) were likewise randomised into TL treatment (Group C_TL) and SI treatment (Group C_SI). All patients were infertile couples that utilised their own oocytes who did not received donated eggs and consented to having their oocytes randomised into either a TLI or SI. Inclusion criteria other than age were women with regular menstrual cycles, normal uterine ultrasounds taken during their first or second ICSI trial and a body mass index (BMI) between 18 and 30 kg/m^2. Exclusion criteria included males with a total sperm count totalling less than 1 million, males over 45 years of age, patients with any uterine conditions, endocrinopathies or recurrent pregnancy loss, and patients under treatment for any other medical condition.

Couples in the 35 and under and 36–39 age groups were eligible for randomisation if they had 6 or more MII oocytes and couples in the 40-and-over group were randomised if they had 4 or more MII oocytes. Patient charcateristics are given in Table 1a–c. Assisted hatching and biopsy were not conducted for patients admitted for randomisation. The study was performed between November 2014 and February 2015. Oocytes were stripped of their cumulus cells and randomised for individual treatments. Oocytes were randomised by alternating the allocation of a single patient's oocytes to individual treatments (Fig. 1). All oocytes were inseminated by ICSI using an inverted microscope (Olympus IX-71, Japan) and all oocytes were cultured immediately after ICSI either in a TLI or a SI. There were no changes to the method following trial commencement.

After ICSI insemination, oocytes were individually cultured either in

Table 1a
Characteristics of patients below 35 years of age.

Parameter	SI	TLI
No of cases	111	110
Age Mean	29.8	29.8
SD	4.3	4.3
Range	20–35	20–35
BMI Mean	25.6	25.2
SD	2.9	3.4
Range	18–30	18–30
FSH Baseline mIU/ml		
Mean	6.3	6.2
SD	1.8	1.7
E2 baseline pg/ml	26.7	27.8
SD	13.4	12.6
Mean FSH dose	1799	1940
	264	276

Table 1b
Characteristics of patients aged 36 to 39 years.

Parameter	SI	TLI
No of cases	62	62
Age Mean	37.9	37.8
SD	1.0	1.1
BMI Mean	25.4	25.9
SD	2.5	2.8
Range	18–30	18–30
FSH Baseline mIU/ml		
Mean	7.4	8.0
SD	1.3	1.5
E2 baseline pg/ml	28.8	30.1
SD	10.3	10.2
Mean FSH dose	2098	2133
	331	278

Table 1c
Characteristics of patients above 40 years.

Parameter	SI	TLI
No of cases	43	43
Age Mean	42.8	43.1
SD	2.2	2.0
BMI Mean	26.7	26.6
SD	1.9	1.5
Range	18–30	18–30
FSH Baseline mIU/ml		
Mean	8.3	9.0
SD	1.6	1.9
E2 baseline pg/ml	36.5	34.0
SD	8.1	8.2
Mean FSH dose	2405	2543
	306	368