Evaluation of the safety of time-lapse observations for human embryos

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Abstract

Purpose To assess the effects of light from an integrated optical microscope and evaluate the safety of time-lapse observations using a built-in microscope incubator.

Methods We prospectively compared the fertilization rate and embryonic morphology after intracytoplasmic sperm injection between embryos cultured with time-lapse observations every 15 min in an incubator with an integrated optical microscope and embryos with intermittent observations (once a day) in conventional incubators.

Results No significant differences were observed in the fertilization rate (57.5% vs. 57.5%) or the rate of excellent-good cleavage embryos (36.0% vs. 36.0%).

Conclusions These results suggest that time-lapse observations using an incubator with an integrated optical microscope may therefore be safely utilized in clinical practice.

Keywords Built-in microscope incubator · Embryo grade · ICSI · Time-lapse observation

Capsule The fertilization rate and the embryo grade were similar in regard to the use of conventional observations and time-lapse observations using a built-in microscope incubator.

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Introduction

There is a need to identify viable embryos with the highest implantation potential in order to increase in-vitro fertilization (IVF) success rates and reduce multiple pregnancy rates. For these reasons, many criteria for embryo selection have been proposed in the field of assisted reproductive technology (ART) [1–7]. Among of these criteria, morphologic features have been used extensively in selecting good-quality embryos. However, selection for transfer should not be based on cell number and morphology solely on the day of transfer [8].

Time-lapse observations using an incubator with an integrated optical microscope may minimize the changes in the culturing environment by integrating the culture, observation, and time-lapse recording of cells into one system. In comparison, culturing with intermittent observations requires the removal of embryos from the incubator for observation. Time-lapse observation has also advanced the understanding of the morphologic mechanisms of fertilization, development, and behavior of early human embryos [9, 10]. Therefore, the automatic capture of time-lapse images will provide us with useful information for embryo selection. However, embryos cultured in an incubator with an integrated optical microscope are periodically exposed to light when the digital images are obtained. Light exposure is an unnatural stress to embryos and might affect embryo development. Ottosen et al. observed light-induced retardation in the cleavage of mouse and rabbit embryos [11]. Takenaka et al. also reported that light is one of the physical factors of the embryonic environment and that its effects should not be ignored [12]. To our knowledge, little is known about the effects of light on human embryos during time-lapse observation, and these effects should be assessed prior to

Table 1 Influences of light on fertilization, embryo quality and cleavage rate

	Time-lapse (<i>n</i> =146)	Conventional (<i>n</i> =146)	<i>p</i> ^a
Fertilization (%)	84 (57.5)	84 (57.5)	0.906
Excellent and good quality embryo (%)	30 (36.0)	30 (36.0)	0.872
≥ 4-blastomeres embryo on day 2 (%)	51 (60.7)	54 (64.3)	0.750

^a Chi-square test

clinical use. Therefore, we prospectively compared the embryonic morphology after intracytoplasmic sperm injection (ICSI) between embryos cultured with time-lapse observation in an incubator with an integrated optical microscope and embryos with intermittent observation in conventional incubators.

Materials and methods

All ICSI cycles performed between April 2007 and September 2008 at Nagoya University Hospital that fulfilled the criteria were included in this study. The ovarian stimulation protocol has been described previously [13]. Briefly, recombinant FSH (Follistim; Schering-Plough Corp., Kenilworth, NJ) or highly purified urinary FSH (HMG “Kowa”; Kowa Pharmaceutical Co. Ltd., Tokyo, Japan) was administered with pituitary desensitization using nafarelin acetate (Nasanyl; Pfizer Japan Inc., Tokyo, Japan, *n*=44, long protocol) or cetrorelix acetate (Cetrotide; SHIONOGI & Co., Ltd., Osaka, Japan, *n*=40, flexible antagonist protocol). Follicle growth was monitored by transvaginal ultrasound. When two or more follicles reached the size of 18–20 mm in diameter, 10,000 IU of human chorionic gonadotropin (Gonotropin; ASKA Pharmaceutical Co., Ltd., Tokyo, Japan) was administered and oocytes were retrieved 35.5 h later. ICSI was performed 4–5 h after oocyte retrieval and this day was designated as day 0.

To assess the effects of light from an integrated optical microscope, we randomly selected 1–4 oocytes for time-lapse observation using an incubator with an integrated optical microscope after ICSI and reserved the same number of oocytes for conventional observation. All embryos were cultured in Embryo GPS dishes (SunIVF, Guelph, ON, Canada) with Global medium (LifeGlobal, USA). The oocytes selected for time-lapse observation were monitored in the SANYO In vitro Live Cell Imaging Incubation System (MCOK-5 M; Sanyo Co., Ltd., Osaka, Japan). The monitoring using a built-in microscope incubator started just after ICSI. During culture in the SANYO In vitro Live Cell Imaging Incubation System, digital images of the cultured embryos were obtained every 15 min with a 0.1 W white light emitting diode (LED) that was lit only at the time of exposure (80 msec for each capture in three different focuses). The digital images were collected on a computer using MTR-4000 software

(Sanyo). The embryos were continuously evaluated using a built-in microscope incubator for 72 h after ICSI to determine pronuclear formation and cleavage divisions. Other embryos that had not been selected for time-lapse recording were cultured in the same incubator without microscope as the control group. The oocytes in the control group were removed from the incubator once a day for evaluation of fertilization and cleavage status using an optical microscope outside of the incubator on day 1 (between 18 and 21 h after ICSI), day 2 (between 42 and 45 h after ICSI), and day 3 (between 66 and 69 h after ICSI). Oocytes were classified as fertilized if two pronuclei (PN) were present. Abnormally fertilized oocytes (1PN or 3PN) were excluded. All embryos were cultured at 37°C under a 5% CO₂/95% air atmosphere. All cultured embryos were evaluated according to the morphologic evaluation by Veeck [14]. Depending on the woman’s age and the embryo quality, one or two embryos were transferred. The procedures of embryo transfer and luteal phase support have been described previously [15]. Clinical pregnancy was defined as the presence of a gestational sac on transvaginal ultrasound examination. This study was approved by the ethics committee of Nagoya University Hospital.

Results

The primary causes of infertility in the couples included male factor (37.9%), tubal factor (25.8%), endometriosis (19.7%), and unexplained (16.7%). Out of the 146 oocytes used in this study, 84 (57.5%) oocytes in time-lapse observation and 84 (57.5%) oocytes in conventional observation were fertilized normally and cleaved on day 2. In the time-lapse group, 30 embryos (36.0% of fertilized oocytes) developed as excellent or good quality embryos (Grade 1 or 2, respectively) by the modified Veeck’s morphological classification. Fifty-four embryos (64.0% of fertilized oocytes) developed as fair or poor quality embryos (Grade 3 or 4, respectively). In the conventional observation group, 30 oocytes (36.0%) developed as excellent or good quality embryos (Grade 1 or 2) as classified by the modified Veeck’s morphological classification. Fifty-four oocytes (64.0%) developed as fair or poor quality embryos (Grade 3 or 4). The rates of embryos with 4 or more blastomeres on day 2 (between 42 and 45 h after ICSI) were 60.7% in the time-lapse group and 64.3% in the

conventional observation group, respectively. There were no significant differences in embryonic morphological grade or cleavage stage between the embryos which were cultured in time-lapse observation and the control groups (Table 1). Embryo transfer was performed to 68 women out of 84 women; the mean age was 36.8 y, recruited into the study. The pregnancy rate per patient performed ET was 6/68 (8.8%). Three women were pregnant with embryos from the conventional observation and two were pregnant with embryos from the time-lapse observation. Two embryos (each one embryo from the time-lapse observation and the conventional observation) were transferred at the same time to the other one pregnant woman.

Discussion

Embryo selection criteria based on routine assessment of embryo morphology are not always associated with a higher implantation rate or pregnancy rate [16–18]. Therefore, other criteria should be considered in the identification of embryos with a good prognosis for implantation or pregnancy. The early cleavage status of an embryo has recently been used as an additional criterion in embryo selection [4, 6, 19, 20]. Time-lapse observation may allow us to assess early cleavage embryos more precisely. Therefore, we might select good quality embryos based on the cleavage status with time-lapse observation more precisely than with conventional intermittent observation. Moreover, time-lapse observation using an incubator with an integrated optical microscope may minimize alterations in the culturing environment by integrating the culture, observation, and time-lapse recording of embryos into one system in comparison to intermittent observation, which requires the removal of embryos from the incubator for observation. However, embryos cultured in time-lapse observation are periodically exposed to light when digital images are obtained. Mio et al. recently reported that the clinical pregnancy rate (per embryo transfer cycle) after time-lapse observation was not significantly different from observed pregnancy rates without time-lapse observation [10]. However, they did not compare the quality and morphology of embryos between time-lapse observation and conventional intermittent observation. In the current study, embryos under time-lapse observation were exposed to LED lighting approximately 300 times, and we demonstrated no significant differences in the fertilization rate of ICSI, the cleavage rate, or the morphological grade of embryos in a prospective manner with internal controls (i.e., oocytes obtained from the same patients). Our results suggest that there is little effect on embryos from exposure during time-lapse observations; therefore, we can safely use time-lapse observation for research and clinical use. We did not

include oocytes for conventional IVF, which were to be taken out the incubator for denudation of their granulosa cells on day 1.

In summary, this study is the first to assess the safety of time-lapse observation. Time-lapse observations using an incubator with an integrated optical microscope are therefore considered to provide us with significant information in regard to embryo development, including the time of pronuclear formation, time of the first cleavage, time of compaction, and cleavage synchronicity. This process may be a useful tool for assessing embryo viability and quality in clinical use. Further study with a larger number of patients is therefore necessary to assess the usefulness of time-lapse observations and the pregnancy rate.

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