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The beginning of human life under time-laps Cinematography

YASUYUKI MIO

Reproductive Center, Mio Fertility Clinic 1-1, 2-Chome, Kuzumo-Minami, Yonago, Tottoriken, 683-0008 Japan
Kazuo Maeda
Dept. of Obstetrics and Gynecology (emeritus), Tottori University School of Medicine, Nadamachi 3-125, Yonago, Tottoriken, 683-0835 Japan (home)

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Abbreviations:
TLC: time-lapse cinematography
ART: assisted reproductive technology
ET: embryo transfer

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Abstract

Background and Purpose: *The aim of this study was to follow and record the early stages of development of a human embryo using time-lapse cinematography, and to discuss the beginning of human life.*

Materials and Methods: *Human ova were incubated in thermo-stabilized and pH controlled chambers and observed using an inverted microscope fitted with a CCD digital camera. Images were taken at 2-minute intervals for 5–6 days. A total of 30 consecutive images, corresponding to an hour of incubation, could then be replayed in 1 second. This allowed us to watch the development of embryos over 5–6 days in a few minutes.*

Results and Conclusion: *We observed fertilization of an ovum with a single spermatozoon followed by early cleavages, formation of the morula, blastocyst hatching and changes in the embryonic plates and the development of monozygotic twins in 2 of 3 incubated blastocysts. TLC has contributed enormously to our understanding of the morphological mechanisms of fertilization and embryonic development. We would like to suggest that the time at which stem cell-like morula cells form the differentiated blastocyst is the point at which human life begins, and that the ET of blastocyst may increase the risk of monozygotic twins.*

INTRODUCTION

Prior to the 1950s, there was no electronic means of monitoring a pregnancy and determining whether a fetus was alive until the birth of the child. With the introduction of the phono- and electro-cardiogram, cardiotocogram (CTG), Doppler fetal heart detector, real time ultrasound B-mode, and recently 3D and 4D ultrasound, and power Doppler which can confirm the blood flow in the fetal aorta and umbilical cord at 7–8 weeks of pregnancy *(1–5)*, fetal life was shown to begin in early pregnancy.

The discussion over defining the moment at which human life begins was promoted by the advent of assisted reproductive technology (ART) which clarified the process of early embryonic development before embryo transfer (ET). The most fundamental idea,is that life begins with fertilization of the ovum by a spermatozoon. The blastocyst, streak formation, implantation, brain blood flow, fetal movement, perception of the fetus by the mother are just some of the ideas debated as being the beginning of human life.

It is impressive to watch the changes that occur in a fertilized ovum using time-lapse cinematography (TLC) *(7)*. These images may provide evidence that the initiation of human life is a unique feature which distinguishes humans from all other living things. For this reason, we undertook a study of early embryo development using TLC to see if we could provide further material for the debate on the beginning of human life.

MATERIALS AND METHODS

Time-lapse cinematography (TLC) was performed according to Payne *et al. (6)* with some modification by the author YM. The cinematographic procedure was permitted by the Japanese Institution for Standardizing Assisted Reproductive Technology Ethics Committee (JISART Ethics Committee) and was done with the informed consent of parents.

An inverted microscope (IX-71, Olympus, Japan) with Normarski differential interference contrast (DIC) optics (Olympus, Japan) and micromanipulator (Narishige, Japan) was covered by an acryl-resin chamber with an air heater in one corner to maintain an optimal temperature. Embryonic cultures in glass Petri dishes were placed at the center of an acrylic chamber (15 x 15 x 3 cm) set in a water bath on the stage of the microscope. Humidified 5% carbon dioxide (CO2) was infused into the chamber through the water bath at 40 mL/min to maintain a pH 7.45 ± 0.03. A 3 μL sample containing the ovum in culture medium was covered with mineral oil (SAGE, USA). The thermo-plate of the microscope stage was set at 41.8 °C and the temperature surrounding the microscope was kept at 38oC (Figure 1) to keep the sample at 37 ± 0.3 °C. The inverted microscope was equipped with a CCD digital camera (Roper Scientific Photometrics, USA) connected to a PC. Images were displayed using MetaMorph software (Universal imaging, USA).

A single, randomly selected oocyte was prepared for TLC after intracytoplasmic sperm injection (ICSI) or in vitro fertilization (IVF). Digital images of cultured embryos were acquired every 2 minutes using an exposure time of 50 ms. For each embryo, between 2000 and 2500 images were obtained during the observation period (Figure 1) *(7)*. On completion of TLC, embryos at the 2- to 4-cell stage and in good condition were cryopreserved for clinical use.

With the informed consent of parents, some embryos not destined for clinical use were further incubated and studied by TLC until they formed blastocysts and hatched. In this study, digital images were taken every 5 or 10 minutes for 5–6 days.

RESULTS

Early stage of embryonic development

The in vitro fertilization of 25 oocytes was observed by TLC. Each ovum was transferred into culture medium in the observation chamber following insemination, and the sperm penetration site of the zona pellucida was detected using a micromanipulator. After penetrating the zona pellucida, the sperm moved across the perivitelline space, attached to the oocyte membrane (Figure 2) and remained there for 40–50 minutes before the sperm head initiated incorporation into the oocyte membrane. The mid-section of the sperm head sank under the oocyte membrane, and finally the sperm head disappeared. The second polar body was extruded shortly after sperm fusion, ca. 2.5 hours after insemination. The fertilization cone appeared concurrent with or shortly after extrusion of the second polar body (Figure 3). Glassy cytoplasmic

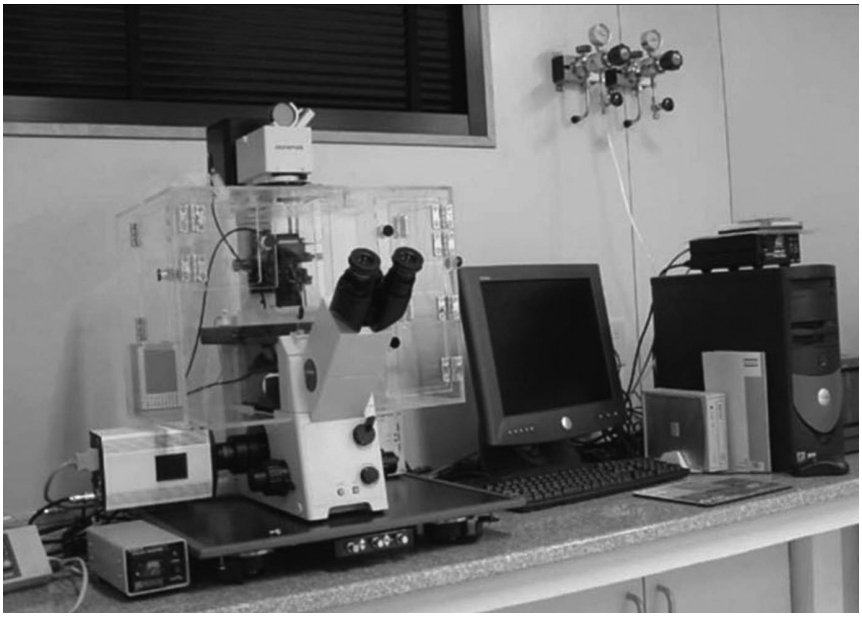

Figure 1. *The time-lapse cinematograph in the Reproductive Center of Mio Fertility Clinic.*

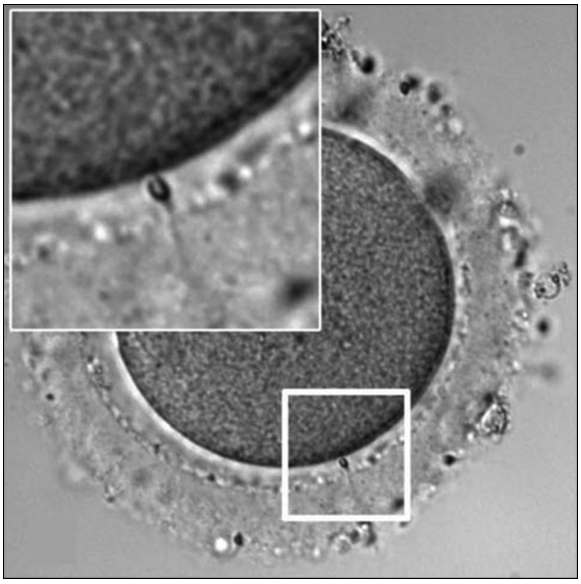

Figure 2. *A sperm approaching the ovum cytoplasm, 1.5 hours after insemination. Upper left square shows its enlarged photo.*

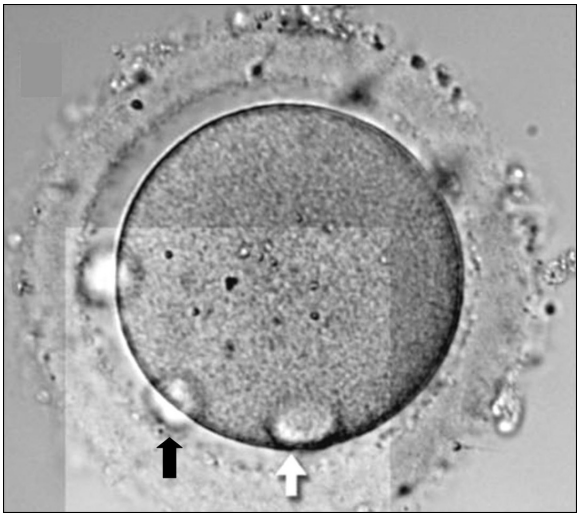

Figure 3. *The second polar body (black arrow) was extruded 2.5 hours post-insemination after which the fertilization cone (white arrow) appeared.*

flares appeared prior to male pronucleus formation, which may represent a visual manifestation of the sperm aster. The male and female pronuclei abutted ca. 2 hours after their appearance in the cytoplasm. Following abutment of the pronuclei, cytoplasmic organelles were drawn from the cortex towards the center, and a halo appeared in the peripheral ooplasm. The halo disappeared before syngamy. Three hours after syngamy, the first cleavage occurred over a period of 30 minutes (Figure 4). The second cleavage occurred ca. 10 hours after the first one (Table 1) *(7)*.

Hatching of the blastocyst

Hatching is the release of an expanded blastocyst from the shell-like zona pellucida, enabling further development and implantation of the blastocyst into the endometrium. With TLC, we observed embryos undergo cleavage from the 2 cell stage into a morula, the cell masses that then develop into a blastocyst. Occasionally, a morula cell was seen to form multiple small cytoplasmic fragments (Figure 5). However this fragmentation did not affect the development of the morula into a blastocyst. Hatching occurred when a small hole formed in the zona pellucida through which the blastocyst was extruded without injury (Figure 6).

TABLE 1

Sequence of phenomena in the fertilization analyzed by time-lapse cinematography (7).

Phenomena in 25 oocytes	Hours after the insemination (mean ± SD)
Sperm penetrated	1.5 ± 0.2
Sperm incorporated	2.4 ± 2.2
2nd polar body extruded	2.5 ± 1.2
Cone appeared	2.5 ± 0.5
Flare appeared	3.7 ± 0.7
Cone disappeared	4.5 ± 0.8
Flare disappeared	5.5 ± 0.5
Male pronucleus formed	6.6 ± 0.3
Female pronucleus formed	6.8 ± 0.3
Both pronuclei abutted	8.8 ± 0.7
Halo appeared	9.0 ± 0.5
Halo disappeared	19.2 ± 1.1
Syngamy	24.8 ± 1.0
The 1st cleavage commenced	27.3 ± 1.0
The 2nd cleavage commenced	37.2 ± 1.2

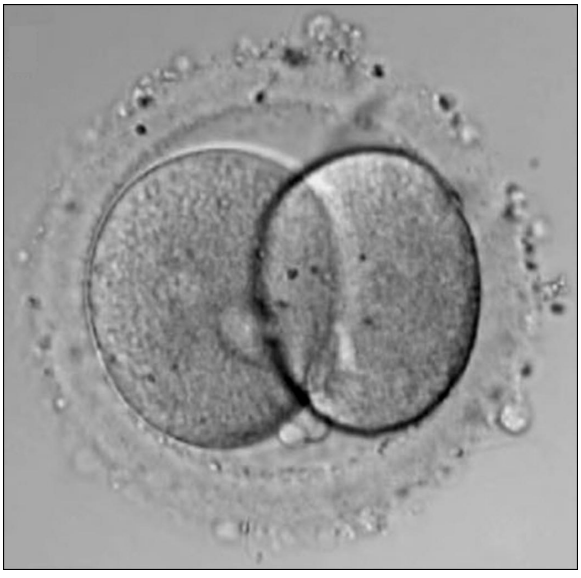

Figure 4. *Two cells developed after the first cleavage ca. 28 hours after insemination.*

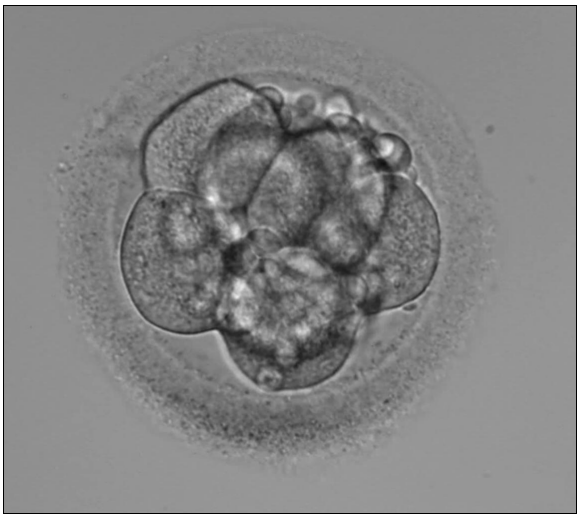

Figure 5. *Cytoplasmic fragmentation occurred in some morula cells, 2 days after the insemination.*

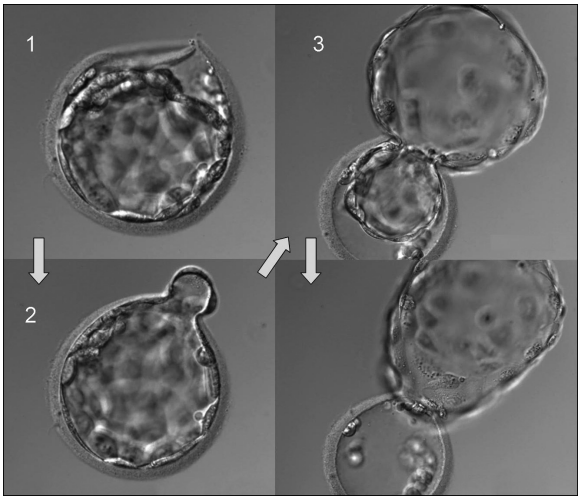

Figure 6. *These 4 photos illustrate the hatching of a blastocyst through the hole in the zona pellucida.*

Formation of monozygotic twins from blastocysts

The embryonic plates of two of three *in-vitro* incubated blastocysts divided into two pieces. A strand formed between the two cell masses was seen to expand and eventually break resulting in two embryos from one blastocyst. Since the trophoblasts were a single mass, the twins are monozygotic. As two of three blastocysts developed into twins, the incidence was very high (Figure 7).

Safety of the time-lapse cinematography procedure

We compared the fertilization rates and embryo quality between the study groups and sibling groups of oocytes after ICSI and IVF as a preliminary experiment. There were no significant differences in the fertilization rate and embryo quality between the two groups. Following TLC, 4 embryos at the 2 or 4 cell stage, were transferred into the uterus of patients and developed into normal and healthy neonate confirming the embryos were unharmed by the procedure.

DISCUSSION

Time-lapse cinematography (TLC) enabled us to compress the prolonged course of early embryonic development into a short movie. The changes that occur in the hours after fertilization are clearly defined by visual analysis. This technique has dramatically improved the morphological and chronological analysis of reproduction and therefore has made important contributions to assisted reproductive technology (ART). Furthermore, it may resolve issues arising from the discussion on the beginning of human life.

TLC revealed the various biological characters exhibited by the developing embryo. These include the capacity of the fertilized oocyte, a single cell, to develop into a human being, the pluripotential morula cells from which some cells can be removed for prenatal diagnostic analysis without affecting the development of the fetus. The fragmentation and apparent death of a cell within the morula appeared to have no effect on the development of the blastocyst. This suggests that morula cells have a stem cell-like character with the potential to differentiate into any tissues and organs. These cells differentiate to create an inner cell mass (embryoblast) surrounded by cells of the trophoblast thus forming the blastocyst and eventually a human fetus and placental cells. The images obtained in this study suggest the point at which cell differentiation begins in the human embryo is when the blastocyst develops from the stem cell-like morula cells. Therefore we propose that human life begins when the blastocyst is formed.

In addition, TLC revealed a high incidence of monozygotic twins in blastocysts after prolonged *in vitro* incu-

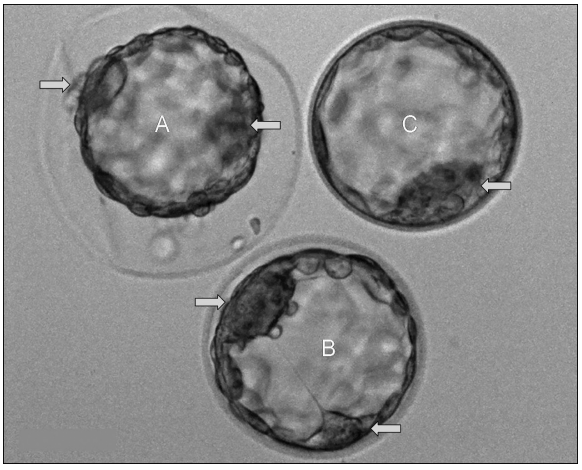

Figure 7. *Embryonic masses in blastocysts are indicated by white arrows. Each two embryonic masses in the two blastocysts represent monozygotic twins.*

bation. This finding is supported by clinical studies which found an increased incidence of monozygotic twins in patients who received a blastocyst *(8–11)*. Therefore ET of incubated and hatched blastocysts may increase the risk of monozygotic twin pregnancy.

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