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I completed my certified training in Obstetrics and Gynecology at Kochi Medical School and Hospital, Japan in 1990. During that training, I studied abroad to the University of Miami School of Medicine, in the Biochemistry and Molecular Biology Department. My area of study was the mechanism of ovulation related to serine protease (Plasminogen, collagenase).

Simultaneously I joined the IVF Programme in the Reproductive Endocrinology Department at the same institution as an embryologist. After completed training as a certified OB & GYN in Japan, I had a position as lab director in a private fertility clinic, the Diamond Institute for Infertility in New Jersey, USA. A period as a lab director enabled me to improve my knowledge and skill for assisted reproductive technology(ART).

For the last 10 years, I have worked as clinical co-director of the Hiroshima HART Clinic, where I am responsible for managing assisted reproduction for 500 cycles per year. My main present interest lies in vitrification for gametes and embryos: I am responsible for the two first reports in the world successful birth from vitrified embryos. Both of these were day 2-3 embryos cryopreserved by vitrification with straw, and the blastocysts cryopreserved by ultra-rapid vitrification using cryoloop technique. My original video for vitrification has been selected to receive Honorable Mention for Technical Achievement in Video: ART Category in 61st ASRM/CFAS annual meeting, Montreal, Canada

Current Positions Held

Vice President of Hiroshima HART Clinic, Hiroshima, Japan

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1995-present	Vice President of Hiroshima HART Clinic, Hiroshima, Japan Scientific Director of Clinical Assisted Reproductive Laboratory, HART Clinic Group
1990-1995	Laboratory Director of Assisted Reproductive Technology,Diamond Institute for Infertility and Menopause, Milburn, New Jersey, USA
1989-1990	Advanced Resident Program, Department of Obstetrics & Gynecology, Kochi Medical School
1988-1989	Research Associate, Department of Biochemistry and Molecular Biology, University of Miami, School of Medicine, Miami, Florida, USA Professor Dr. Jack Frederick Woessner Jr.
1985-1988	Resident Program, Department of Obstetrics & Gynecology, Kochi Medical School, Kochi, Japan Department of OB & GYN, Professor Dr. Yusuke Sagara.
1985-1990	Department of Obstetrics & Gynecology, Kochi Medical School, Kochi, Japan
1985	Medical Board License Examination Medical doctor degree (M.D.)
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Human Blastocyst Vitrification Using Cryoloop Technique

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In assisted reproductive technology (ART) for infertility treatment, cryopreservation of human embryos has proved important for the effective use of supernumerary embryos. In the cryopreservation of embryos, there is a risk of various types of injuries (Kasai, 1996). Among the injuries, the formation of intracellular ice would be the biggest obstacle to cryopreservation. The first strategy to prevent intracellular ice formation was to adopt a long slow-cooling stage. However, it was difficult to eliminate injuries from ice completely. Furthermore, the slow freezing method requires expensive equipment to control the lowering temperature and time consuming effort before embryos are stored in liquid nitrogen. Vitrification is an approach in which injuries related to ice are less probable, because embryos can be cooled directly in liquid nitrogen with relatively high concentration of CPA (Rall and Fahy, 1985). That simplifies the cooling process and does not require the particular equipments, just with a container for LN2. We have shown that vitrification using an ethylene glycol (EG)-based solution with conventional cryo-straws is effective for human embryos at the 4-8 cell stage (Mukaida et al., 1998).

Recent advances in culture systems have made it possible to develop IVF embryos into blastocysts(BLs) easily, and BL transfer has proven effective for increasing the pregnancy rate. Therefore, cryopreservation of BLs has become an important technique. However, the cryopreservation of human BLs using both slow freezing method and vitrification with straws resulted in disappointing survival rates. This is probably because human BLs are much less permeable in cryoprotectant and water, since they shrink more slowly than mouse and bovine BLs in the cryoprotectant solution. This suggests that human BLs are more likely to be injured by intracellular ice.

An innovative strategy to circumvent the problem of intracellular ice formation in less permeating embryos is ultra-rapid vitrification, in which embryos are vitrified with a minimal volume of a vitrification solution using minute tools such as a cryoloop. This is the same concept of EM Grids, cryotop, and open pulled straw system. In this approach, embryos are cooled and warmed extremely rapidly, which can minimize the chance of ice formation. We attempted ultrarapid vitrification of human BLs using cryoloops, and have obtained a high rate of pregnancy (Mukaida et al., 2001).

The cryoloop consisted of a minute nylon loop mounted on a stainless steel pipe inserted into the lid of a cryovial. Both warming and cooling require only two steps each with mixture of ethylene glycol and DMSO as a cryoprotectant. It takes only 10 to 15 minutes to perform either cooling or warming steps. Detailed information is described in our manuscripts (Mukaida et al 2003). Briefly,

the BLs were placed for 2 min in solution I, containing 7.5% dimethylsulfoxide (DMSO) and 7.5% ethylene glycol (EG) in human tubal fluid (HTF) supplemented with 0.5% human serum albumin (HSA), and then placed for 30 seconds in solution II, which contains 15% DMSO, 15% EG, 1% Ficoll 70 and 0.65 M sucrose in HTF/HSA at 37℃. The BLs in solution II were loaded on a small nylon loop (Hamilton Research, CA, USA) and submerged in liquid nitrogen. Warming was carried out by placing the vitrified BLs in 0.33 M sucrose in HTF/HSA for 2 min and then 0.2 M sucrose in HTF/HSA for 3 min. Just after warming, zona hatching was carried out with either acidic tyrode or laser. The BLs with morphologically normal inner cell mass and trophectoderm and re-expanding blastocoel were considered to have survived. Embryo transfer was performed 2-3 hours after warming. The uterine endometrium was prepared with oral estrogen and intramuscular injection of progesterone (50 mg/d). Pregnancy was defined as the presence of gestational sac(s).

Since we started this vitrified BL program (Nov.1999), 439 clinical pregnancies (48.4%) and 559 implantations (32.6%) out of 907 BLs transferred cycles were obtained. 242 babies were born in 208 deliveries. Miscarriage rate is 21.4% (94/439).

However, a major drawback of vitrification is use of relatively high concentration of cryoprotectants, which may affect the embryo and subsequent growth in uterus. Therefore, we reported reproductive and neonatal outcomes, and investigated congenital defects in infants born after vitrification of blastocyst comparing with fresh blastocyst transfer (Takahashi, et al. 2005). Vitrification yields the same high pregnancy and implantation rates as fresh blastocyst transfer and congenital defect rate(1.4%) was similar to fresh blastocyst transfer (2%), proving the method to be safe.

Since the beginning of 2001, we have applied assisted hatching (AHA) of warmed BLs, because cryopreservation including vitrification induces hardening of the Zona pellucida (ZP), which may impair spontaneous hatching after warming. In our experience, assisted hatching (AHA) with acidic tyrode or laser performed at the time of warming, before transfer, significantly improved the implantation and pregnancy rates. Within 10 min after warming seems to be the optimum period to perform AHA, because the blastocele has not fully expanded yet and perivitelline space is present. That makes AHA procedure become safe and easy.

Since 2003, we have also applied artificial shrinkage (AS) of the blastoceoleic cavity of expanded or larger stage of BLs, because the results of our vitrified BL program showed that the efficiency of the vitrification method was dependent on the stage of BL development and was negatively correlated with the size of the blastocoele. We postulated that a large blastocoele might lessen cryopreservative potential due to ice crystal formation during the cooling steps. In order to overcome this problem, shrinkage of the blastoceole was thought to be the appropriate approach. Therefore we analyzed the effectiveness of reducing the volume of the blastocoelic cavity by puncturing with a glass microneedle before vitrification, and obtained statistical increase of survival of expanded and

larger BLs. In our clinical experience, survival rates of expanded and larger BL with AS were significantly increased (76% to 98%) compared to those without AS.

Since September in 2004, we have been applied Laser system* to perform both assisted hatching and artificial shrinkage procedure. Laser system can avoid the exposure of acid and manipulation procedures to embryos. More detailed information and results will be demonstrated and discussed.

* (ZILOS-tk; Zona Infrared Laser Optical System: Hamilton Thorn Bioscience, Beverly MA. USA)

During the lecture, video demonstration of the cryoloop technique will be also shown in order to show the effectiveness and simplicity of this technique.

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