ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Developmental potential of zona pellucida–free oocytes obtained following mild in vitro fertilization

Satoshi Ueno, M.Sc.,[a] Daniel Bodri, M.D., M.Sc., Ph.D.,[b] Kazuo Uchiyama, M.T.,[a] Tadashi Okimura, B.Sc.,[a] Takashi Okuno, M.D.,[a] Tamotsu Kobayashi, M.D.,[a] and Keiichi Kato, M.D.[a]

[a] Kato Ladies Clinic, Tokyo; and [b] Kobe Motomachi Yume Clinic, Kobe, Japan

Objective: To determine the developmental potential of oocytes in which the zona pellucida was damaged and subsequently removed, producing "zona-free" (ZF) oocytes that were cultured until the blastocyst stage.
Design: ZF eggs from cycles with more than one oocytes retrieved (n = 97) were compared with zona-intact (ZI) oocytes originating from the same patient.
Setting: Private infertility clinic.
Patient(s): Infertile patients (n = 135) undergoing minimal ovarian stimulation or natural-cycle in vitro fertilization treatment during 2010–2012.
Intervention(s): ZF oocytes undergoing intracytoplasmic sperm injection (ICSI) fertilization, blastocyst culture, elective vitrification, and subsequent single vitrified-thawed blastocyst transfer (SVBT).
Main Outcome Measure(s): Rate of fertilization, cleavage, and blastocyst development. Live birth rate and neonatal outcome in subsequent SVBT cycles.
Result(s): There were no significant differences in fertilization (77% vs. 77%), cleavage (75% vs. 75%), or blastocyst development rates (39% vs. 32%) between the internally controlled ZF and ZI groups, respectively. Survival after thawing (90% vs. 100%) and live birth rates (37% vs. 36%) per thawed blastocyst were also similar. Newborns originating from all ZF and ZI oocytes had a similar gestational age at delivery (38.3 ± 3.7 wk vs. 39.5 ± 1.5 wk) and birth weight (3,115 ± 946 g vs. 3,010 ± 441 g).
Conclusion(s): Our retrospective comparative study suggests that ZF eggs could be as successfully fertilized and cultured until the blastocyst stage as ZI control eggs without adversely affecting subsequent pregnancy rates and basic neonatal outcome. (Fertil Steril® 2014;■:■–■. ©2014 by American Society for Reproductive Medicine.)
Key Words: Zona pellucida, blastocyst culture, minimal ovarian stimulation, in vitro fertilization

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/uenos-zona-pellucida-free-oocytes-mild-ivf/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

In recent years, increasing interest has emerged in milder approaches to in vitro fertilization (IVF), including minimal ovarian stimulation and natural-cycle IVF (ncIVF) (1, 2). These treatment modalities have become especially widespread in Japan owing to a high proportion of advanced-age infertile women, the increased use of single-embryo transfer (SET) policy (3), and the efforts of specialist centers that have been developing these innovative treatment protocols for more than two decades (4–6). Compared with conventional ovarian stimulation, with the above-mentioned mild approaches the expected egg yield is considerably lower, meaning that usually only a single or fewer than eight oocytes can be retrieved (7). Therefore, in the setting of minimal ovarian stimulation, it is imperative to fully utilize the developmental potential of each harvested egg to avoid the physical and psychologic burden of cycle cancellation for the individual patient.

In 1991 it was first described that human oocytes devoid of their zona pellucida could be successfully fertilized by intracytoplasmic sperm injection (ICSI) and cultured until blastocyst stage. This is of importance because in most centers, eggs with damaged zona pellucida are generally

Received April 17, 2014; revised August 6, 2014; accepted August 18, 2014.
S.U. has nothing to disclose. D.B. has nothing to disclose. K.U. has nothing to disclose. T. Okimura has nothing to disclose. T. Okuno has nothing to disclose. T.K. has nothing to disclose. K.K. has nothing to disclose.
Reprint requests: Satoshi Ueno, M.Sc., Kato Ladies Clinic, 7-20-3, Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan (E-mail: scsosob@gmail.com).

Fertility and Sterility® Vol. ■, No. ■, ■ 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.08.025

discarded (8). A few subsequent case reports described early pregnancies and a single live birth originating from blastocysts cultured from zona-free (ZF) mature oocytes (9, 10). In the latter report, obtained blastocysts were electively vitrified, suggesting that ZF blastocysts could withstand the freezing-thawing procedure and still conserve their developmental potential (10). Although these single case reports were encouraging there are no larger case series available that could lend support to the problem-free culturing of ZF eggs (and cryopreservation of resulting blastocysts) as an option when zona damage occurs in the embryology laboratory. Moreover, data are extremely scarce on neonatal outcome of resulting offspring. Therefore the objective of the present retrospective internally controlled study was to evaluate the reproductive potential of eggs in which the zona pellucida was damaged and subsequently removed, producing ZF oocytes that were cultured until the blastocyst stage. We also report on basic neonatal outcome following transfer of resulting ZF embryos from a single center handling a large number of mild IVF cycles.

MATERIALS AND METHODS

Participants and Study Design

The retrospective study period was from May 2010 to August 2012 (2 years 4 months), including all patient cycles (n = 135) performed at Kato Ladies Clinic, Tokyo, Japan, involving the retrieval of an oocyte where the ZP was found to be damaged, resulting in an egg that was fertilized with ICSI and cultured without its envelope (ZF eggs). In those study cycles where more than one egg was retrieved (n = 97), the outcome of the ZF group was compared with the rest of the zona-intact (ZI) oocytes obtained from the same patient. The outcome of those study cycles (n = 38) where only a single ZF egg was obtained was also presented separately without any control group. The fate of all vitrified blastocysts was followed until December 2013 (allowing for subsequent vitrified-thawed embryo transfer cycles). All resulting pregnancies were followed until delivery. Institutional Review Board was obtained for the present study (IRB approval no. 13-21).

Minimal Stimulation and Natural Cycle IVF Protocol

All patients undergoing IVF treatment at our center received detailed information about the proposed treatment option and provided written informed consents. Additionally those patients who had ZF eggs were also informed accordingly and agreed to their use. A clomiphene citrate (CC)–based minimal stimulation protocol was used in the majority of cycles, whereas unstimulated natural-cycle IVF was used in a smaller proportion of cases. Details of the CC-based minimal stimulation protocol were described previously (5). Briefly, CC (50–100 mg/d) was administrated orally with an extended regimen from cycle day 3 until the day before inducing final oocyte maturation. hMG or recombinant FSH was added in the form of injections (50–150 IU every other day) or nasal spray to obtain one to four mature follicles. Monitoring involving ultrasound scans and hormonal profiles (E_2, LH, and P) was usually started on day 8 and continued every other day until triggering day. Ovulation triggering was performed with a GnRH agonist, buserelin (Suprecur, 600 μg), administered in a nasal spray form. In the natural-cycle IVF protocol, the only pharmaceutical intervention consisted of inducing the final oocyte maturation with a GnRH agonist. Monitoring consisted of ultrasound scans and hormonal profiles (E_2, LH, and P) and was usually started in the morning of day 10–12 according to the patient's cycle length. When the leading follicle reached 18 mm with a concomitant E_2 level $\geq$250 pg/mL, oocyte retrieval was scheduled. Oocyte retrieval was usually performed 30–34 hours after triggering, though in some cases where the start of the LH surge was detected it was performed 26–30 hours after triggering (11).

Oocyte Retrieval and Fertilization

Oocyte retrieval was performed without general anesthesia with the use of a 21–22-gauge fine needle (Kitazato) with an aspiration pressure of 300–330 mm Hg. Follicular flushing was not used during retrieval. Oocyte maturity was checked immediately after egg retrieval. In our study cases, we did not observe any "intrinsically" absent ZP. Rather the ZP was found to be fractured and the oocyte was protruding through its ruptured envelope (Fig. 1). Denuding was performed with a hand-drawn pipette with the use of a hyalorunidase (Sigma Chemical) containing in-house–made human tubal fluid medium with HEPES. Despite the gentle removal of surrounding cumulus cells the oocyte escaped spontaneously and was cultured further without its envelope. To avoid polyspermy, all ZF eggs were fertilized with the use of ICSI performed 5 hours after oocyte retrieval, whereas ZI eggs could be fertilized with conventional insemination or ICSI. During the ICSI procedure, the oocyte was handled gently with extra care to avoid any damage to its oolemma. The location of meiotic spindle was confirmed beforehand with polarized light microscopy (IX-Robopolar; Olympus). Supplemental Video 1 (available online at www.fertstert.org) shows observation of the spindle and the ICSI procedure performed on a ZF oocyte. Fertilization assessment was done 16–20 hours after insemination. From day 1 to day 3, normally fertilized two-pronuclei (2PN) zygotes were cultured in drops of 20 μL Quinn Advantage Protein Plus cleavage medium (Sage) covered by mineral oil in Falcon 1008 dishes (Becton Dickinson Labware). Following this, the embryos were transferred to Quinn Advantage Protein Plus Blastocyst medium (Sage) from day 4 to day 6. ZF embryos were always cultured individually to avoid the risk of chimera formation. All embryos were cultured at 37°C under the gas phase of 5% O_2, 5% CO_2, and 90% N_2 with 100% humidity in water-jacket small multigas incubators or dry desktop incubators (Astec). The liquid nitrogen was produced by a N_2 generator system in a 10,000-class clean room environment.

Embryo Culture, Frozen-thawed Cycles, and Embryo Transfer Procedure

During the study period, only SETs were performed in our center, and an exclusive SET policy was strictly observed.