Influence of different oocyte insemination techniques on early and late morphokinetic parameters: retrospective analysis of 500 time-lapse monitored blastocysts

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Objective: To determine how standard IVF vs. intracytoplasmic sperm injection (ICSI) fertilization influences early and late morphokinetic parameters during prolonged embryo culture.
Design: Five-hundred expanded blastocysts that were monitored in a time-lapse monitoring incubator were analysed retrospectively. Early (pronuclear fading [PNf], t2–t9) and late (start of blastulation, expanded blastocyst) morphokinetic variables were scored according to published consensus criteria.
Setting: Private infertility clinic.
Patient(s): A total of 209 consecutive infertile patients (mean ± SD age, 38.4 ± 4 years; range, 28–47 years) undergoing 238 natural IVF/minimal ovarian stimulation cycles during 2012–2014.
Intervention(s): Minimal ovarian stimulation, oocyte retrieval, fertilization with standard IVF or ICSI, prolonged embryo culture in a time-lapse monitoring incubator.
Main Outcome Measure(s): Differences in morphokinetic parameters according to insemination techniques.
Result(s): In total, 29% and 71% of the whole cohort was fertilized with standard IVF and ICSI, respectively. During early cleavage stages (PNf to t4) there was a statistically significant delay (+1.5 to +1.1 hours) among IVF-fertilized embryos. By contrast, at the expanded blastocyst stage IVF-fertilized embryos showed faster development (−3.3 to −4.1 hours). After normalizing to the time point of PNf, differences in cleavage-stage parameters disappeared, but those at all blastocyst stages increased even further in favor of IVF-fertilized embryos (−3.2 to −5.7 hours).
Conclusion(s): The observed 1.5-hour time difference between standard IVF- and ICSI-fertilized embryos is an artificial phenomenon. At the blastocyst stages, however, genuine timing differences arise between IVF- and ICSI-fertilized embryos, possibly related to their different quality. Normalization to a common time point permits the joint analysis of IVF- and ICSI-fertilized embryos, thus increasing the size of studied cohorts. (Fertil Steril® 2015;104: 1175–81. ©2015 by American Society for Reproductive Medicine.)
Key Words: Time-lapse monitoring, blastocyst culture, ICSI, single-embryo transfer, in vitro fertilization

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Received May 9, 2015; revised July 19, 2015; accepted July 29, 2015; published online August 22, 2015.
D.B. has nothing to disclose. T.S. has nothing to disclose. J.Y.S. has nothing to disclose. M.K. has nothing to disclose. R.K. has nothing to disclose. S.K. has nothing to disclose. T.M. has nothing to disclose.
Presented as an oral presentation at the 31st Annual Meeting of the European Society of Human Reproduction and Embryology, Lisbon, Portugal, June 14–17, 2015.
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Fertility and Sterility® Vol. 104, No. 5, November 2015 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2015.07.1164

In recent years time-lapse monitoring (TLM) of human embryos during IVF treatment has emerged as a promising, noninvasive tool to enhance classic morphology-based embryo selection and possibly even improve overall clinical outcome (1–3). Sophisticated incubators with built-in cameras and dedicated software were developed, allowing the

accurate measurement of numerous morphokinetic variables, proposed to be used in models that aimed at predicting blastocyst formation, aneuploidy status, and the chance of implantation (4–6). Time-lapse variables, however, are also affected by a number of external factors, including laboratory and culture conditions (fertilization method, oxygen concentration, culture media) as well as patient- (obesity, smoking) and treatment-related characteristics (gonadotropin dose, stimulation protocol) (7–14).

Among these factors the influence of different oocyte insemination techniques (standard IVF vs. intracytoplasmic sperm injection [ICSI]) has only been investigated by a handful of studies to date, yielding somewhat contradictory results (8, 9). Although it was already known that by circumventing early fertilization events ICSI zygotes developed faster, from existing time-lapse studies it was unclear whether this difference in timing persists beyond the early cleavage stages. This has considerable implications if mixed cohorts of IVF-/ICSI-fertilized embryos were to be analyzed together. On the other hand most TLM predictive models published to date, by design, involved ICSI-fertilized embryos exclusively, and no models have been developed to date for IVF-fertilized or mixed cohorts (4–6).

Recently it was demonstrated that available published TLM models are largely center-specific and could not be easily transferred to different settings (15, 16). As a consequence, each IVF unit is encouraged to develop its own predictive model based on its own data, which reflect its local laboratory practices, including the proportion of ICSI- and IVF-fertilized treatment cycles. According to the most recently published registry data from the United States and Europe, the ICSI procedure was performed in approximately two-thirds of all treatment cycles (almost identical in both regions at 67%–68%) (17, 18). In Europe, however, where country-specific data were also available, there was a large variation among countries in the proportion of ICSI cycles, ranging from 21% to 96% (17).

The aim of the present retrospective analysis was to determine how standard IVF vs. ICSI fertilization affected early and late morphokinetic parameters during each step of the prolonged embryo culture in a large sample of time-lapse monitored blastocysts. Furthermore, we have also tested whether standardizing to a common time point after fertilization could diminish the observed differences and thus permit the joint analysis of mixed embryo cohorts.

MATERIALS AND METHODS

Study Patients

All consecutive infertile patients who underwent treatment between October 2012 (the acquisition of a single TLM incubator) and December 2014 at our center (Kobe Motomachi Yume Clinic, Kobe, Japan) were eligible for inclusion in this retrospective analysis. Inclusion criteria were as follows: [1] fertilized oocytes that underwent prolonged embryo culture in a time-lapse incubator; [2] embryos that had developed into expanded blastocysts and were electively vitrified (or transferred in fresh state). Institutional review board approval was not required for the present study because in our center all patients undergoing IVF treatment were informed about and gave consent to the anonymous use of their data for retrospective analyses. A flowchart depicts the number of gametes/embryos in the IVF- and ICSI-fertilized groups from oocyte fertilization up until the expanded blastocyst stage (Fig. 1).

Natural Cycle IVF and Minimal Ovarian Stimulation Protocols

Clomiphene citrate (Serofene, Merck Serono) or letrozole (Femara, Novartis) based minimal stimulation was used in the majority of cycles, whereas unstimulated natural cycle IVF or other mild stimulation protocols using low-dose hMG (Ferring) represented only a smaller proportion of cases. Details of the clomiphene citrate–based minimal stimulation and natural cycle IVF protocols were described previously (19, 20). Patients were not selected according to their age, and this treatment option was offered over a wide age range.

Oocyte Retrieval and Fertilization with Conventional IVF or ICSI

Transvaginal ultrasound–guided oocyte retrieval was performed without anesthesia using a very thin 21–22 G needle (Kitazato Medical). Mature (metaphase II) oocytes were inseminated by conventional IVF or injected by ICSI. Subsequently injected oocytes were immediately placed in pre-equilibrated slides (EmbryoSlide, Unisense Fertilitech), whereas inseminated oocytes were first cultured at 7.3 pH, and 5% O_2, 5.5 %CO_2 atmosphere in a conventional, tri-gas, water-jacket incubator (Astec) in Quinn's Advantage Fertilization Medium (Sage) and transferred to a TLM incubator next morning if fertilization was confirmed.

Prolonged Embryo Culture in a TLM Incubator, Elective Vitrification, and ET

In our center elective blastocyst culture, vitrification, and subsequent vitrified–warmed single blastocyst transfer are routinely practiced in cases with previous failed cleavage-stage embryo transfer cycles (19). Prolonged embryo culture was performed in a time-lapse incubator (EmbryoScope, Unisense Fertilitech) according to previously described methodology (4). Briefly, normally fertilized two pronuclei zygotes were incubated at 37°C and 7.2 pH in a 5% O_2, 5.5% CO_2 atmosphere and cultured individually until day 2–3 in Quinn's Advantage Cleavage Medium and subsequently until blastocyst stage to day 5–7 in Quinn's Advantage Blastocyst Medium. During cleavage stage, morphologic selection was performed by classic criteria according to consensus criteria (21). Embryos that reached the blastocyst stage by day 5 or 6 were eligible for elective vitrification as soon as they expanded to a size of at least 160 μm. Expanded blastocysts were graded into five categories (A–E), depending on female age and time to reach blastocyst expansion as per previously published criteria of our group (22). Almost all expanded blastocysts were electively vitrified using the Cryotop vitrification method (Kitazato Medical) as described previously (23, 24).