

Potential risk of monozygotic twin blastocyst formation associated with early laser zona dissection of group cultured embryos

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Objective: To document the risk of in vitro monozygotic dizygotic twin formation in the implementation of a program of blastocyst biopsy with preimplantation genetic screening (PGS).

Design: Case report.

Setting: Private infertility laboratory.

Patient(s): Prospective PGS patients with intracytoplasmic sperm injection-derived, group-cultured blastocysts over a 3-year period.

Intervention(s): Group culture in Global medium (Life Global) to optimize blastocyst formation of zygotes produced for blastocyst biopsy for PGS ($n \leq 8$ embryos/25 μ L droplet), and laser zona dissection (LZD) of all day-3 cleaved embryos to promote pre-expansion trophectodermal extrusion at the blastocyst stage (i.e., premature hatching).

Main Outcome Measure(s): Blastocyst formation and quality grading on days 5 and 6 of in vitro culture for the vitrified embryo transfer of single or dual euploid blastocysts.

Result(s): Over 3,000 blastocysts were produced in vitro. On two separate occasions, complete trophectodermal amalgamation was observed between two hatching blastocysts. Vitrified single-euploid blastocyst transfers efficiently implanted and established clinical pregnancies similar to dual-euploid blastocyst transfers, without the risk of twin formation.

Conclusion(s): The amazing occurrence of monozygotic dizygotic twin formation has now been documented in vitro, supporting the theory that assisted reproductive technology may facilitate this rare perinatal condition. Furthermore, we have provided clinical evidence that the transfer of a single-euploid blastocyst can optimize a patient's pregnancy success while reducing potentially undesirable conditions associated with monozygotic twin pregnancies. (*Fertil Steril*® 2015;103:417–21. ©2015 by American Society for Reproductive Medicine.)

Key Words: Blastocysts, in vitro derived, monozygotic dizygotic twins

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Monozygotic twins compromise the majority of monozygotic twin pregnancies, and dizygotic twins are extremely rare under natural conditions (1, 2). Monozygotic twin pregnancies are undesirable from the clinical perspective, as they increase the risk

of fetal and neonatal complications (3, 4). Additional complications can transpire in dizygotic individuals as they are susceptible to various levels of chimerism (5–9). The establishment of monozygotic dizygotic twinning has been proposed to occur more frequently in assisted reproduction

technology (ART) cycles (10), as reviewed in six documented cases (11). The latter cases involved multiple-embryo transfers or stimulated intrauterine insemination cycles, thus increasing the risk of embryo-embryo interactions.

Additional monozygotic dizygotic twins have occurred since 2005 with the advent of improvements in blastocyst culture and embryo transfer (ET) success rates. However, the number of fresh/frozen embryos transferred has steadily declined in the United States, from $n = 2.9/2.8$ in 2003 to $n = 2.0/1.8$ in 2012 (e.g., in women 35 to

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37 year old) (12), toward the goal of one embryo per transfer, which would effectively eliminate dizygotic twin pregnancies. In the last 5 to 10 years, vitrification technology has transformed embryo cryopreservation by ensuring a high level of survival and developmental competence equal to fresh embryos. In combination with the preimplantation genetic screening (PGS) of blastocysts, single-embryo transfer (SET) of vitrified, euploid blastocysts can produce repeatable high live-birth rates independent of patient age (<43 year old) (13, 14).

In the establishment of PGS-blastocyst biopsy procedures, programs must decide whether laser zona dissection (LZD) will be performed before blastocyst formation (day 3 or 4) or at the time of blastocyst cavitation (day 5 or 6). We have determined that early rent formation in the zona pellucida of day-3 cleavage embryos produces an ideal premature hatching of trophoctoderm (TE) cells to facilitate easy and immediate biopsy of blastocysts, initiating TE extrusion at the full blastocyst stage. However, the group culturing of induced-hatching blastocysts can position TE cells in very close proximity to one another, creating an unknown risk of chimerism.

MATERIALS AND METHODS

Between 2011 and 2013, oocytes were recovered and intracytoplasmic sperm injection (ICSI) was performed on over 500 perspective PGS cases. All patients were fully informed and consented to the potential risks of blastocyst biopsy and PGS procedures. Patients signed the consent knowing their data could be reviewed, summarized, and published in a deidentified manner as required by the professional organization, the institutional review board, and federal standards. The findings we report were generated in the process of performing standard laboratory ART procedures, including TE biopsies and vitrification, which were developed in conjunction with an approved institutional review board protocol.

Using MCO-5M mini Sanyo/Panasonic tri-gas incubators (5% O₂/5.3–6.0% CO₂), we group-cultured up to eight embryos per 25-μL droplet of LG medium (Global; Life Global) supplemented with 7.5% synthetic protein supplement under Ovoil (Vitrolife) until blastocyst biopsy. Embryos were initially evaluated on day 3, at which time LZD was performed using a 1,410-nm diode laser (Zylos; Hamilton Throne). The laser was set at 600 mHz with a 250-ms pulse duration to deliver two to three sequential ablations to breach the zona pellucida (ZP) with a 10–12-μm furrow. Early rent formation in the ZP (35–50 μm long) was performed to promote early TE herniation, beginning at the full blastocyst stage. The same laser settings were used for biopsying the TE using a combination of LZD (2–10 pulses) and mechanical separation (refer to our 2012 video from the American Society for Reproductive Medicine annual meeting) (15).

Evaluation of blastocyst formation and morphologic grading of blastocysts was performed on days 5 and 6, before biopsy by use of Gardner classifications (16). The Gardner grading of blastocysts was modified to account for premature hatching: 3 = 10% or less TE herniation (full blastocyst), 4 = 10%–50% TE extrusion (expanded blastocyst), and 5 ≥ 50%

TE hatching (hatching blastocyst). In year 1, the orientation of blastocyst herniation was recorded to assess the location of the inner cell mass (ICM): fully extruded (hatching ICM first), partially extruded (ICM at the ZP opening), or not extruded (ZP enclosed). After blastocyst biopsying, most blastocysts were individually cryopreserved using microSecure vitrification (μS-VTF) (17) with non-dimethyl sulfoxide containing VTF solutions (Innovative Cryo Enterprises LLC), before subsequent transfer of one or two euploid blastocysts in a vitrified embryo transfer (VFET) cycle. Differences in pregnancy outcomes were statistically determined by chi-square analysis, and *P* < .05 was considered statistically significant.

Based on blastocyst assessments and other known averaged occurrences (e.g., fertilization rates and blastocyst production rates), statistical modeling was performed to predict the risk of monochorionic dizygotic twin or conjoined twin formation under natural ovulation or ET conditions.

RESULTS

In a preliminary investigation of more than 300 hatching blastocysts, the orientation of the ICM with respect to TE herniation revealed 14% herniating all (8%) or part (6%) of their ICM first, while 86% retained their ICM within the ZP upon hatching. There were no observed problems associated with LZD-induced blastocyst trapping, as fully hatched blastocysts are commonly observed on day 6, and high implantation and live-birth rates of single or dual VFET of euploid blastocysts has occurred (see Table 1). The implantation of twin fetuses was significant after dual ET (DET), with no monozygotic twin formation after SET. Although one of the DET twins was initially diagnosed as sharing a single yolk sac (at 6 to 8 weeks), by 13 weeks a detailed ultrasound confirmed it was dichorionic dizygotic. It is interesting that implantation success was more efficient after SET, at 17.3% higher (*P* < .05), with no differences observed in clinical pregnancy outcomes.

A unique observation was made, however, after the first year: two prematurely hatching day-5 blastocysts had merged together (Fig. 1A, case A). These blastocysts had completely fused (i.e., amalgamation) to form a single blastocoel cavity

TABLE 1		
Efficiency of applying day-5 and day-6 blastocyst biopsy with preimplantation genetic screening technology in VFET cycles implementing single-embryo transfer (SET) or dual-embryo transfer (DET) of euploid blastocysts.		
Parameter	Euploid SET	Euploid DET
No. of VFET cycles	42	36
Mean age	36.6	36.5
No. of embryo ET	42	72
Clinical pregnancies (%)	88.1	88.8%
Live-birth rate (%)	71.4	86.1
Implantation (%)	88.1	70.8 ^a
Twin pregnancies (%)	0.0	44.4 ^a
Note: ET = embryo transfer; VFET = vitrified embryo transfer.		
^a Column values within rows are different (<i>P</i> < .05).		
Schiewe. Monochorionic dizygotic blastocysts. Fertil Steril 2015.		

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