Multi-chorionic pregnancies following single embryo transfer at the blastocyst stage: a case series and review of the literature

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Abstract

Objective To report cases of in vitro fertilization-frozen embryo transfer (IVF-FET) with single blastocyst transfer resulting in di- or tri-chorionic pregnancies, and to review the literature on monozygotic, multi-chorionic pregnancies originating at the blastocyst stage. **Design** Retrospective case series and literature review.

Materials and methods All in vitro fertilization cycles (fresh, frozen, autologous, and donor oocyte) performed between June 2012 and June 2017 at the University of California, San Francisco Center for Reproductive Health, were reviewed retrospectively. Cycles with cleavage-stage embryos or transfer of more than one blastocyst were excluded. Cycles were analyzed to determine if clinical pregnancy occurred with the presence of two or more gestational sacs noted on initial ultrasound. An in-depth chart review was performed with further exclusions applied that would lend credence to dizygosity rather than monozygosity such as fetal/neonatal sex discordance, fresh embryo transfer, and natural cycle FET (in which concomitant spontaneous pregnancy could have occurred). Demographic, clinical and IVF-FET cycle characteristics of the resulting patients were collected. Additionally, a review of the English language literature was performed (PUBMED, PMC) using the search words monozygotic twins, dichorionic diamniotic, in vitro fertilization, and single embryo transfer in order to identify cases of DC-DA monozygotic twinning from 1978 to 2017. Resulting articles were reviewed to eliminate all cases of dizygosity and day 3 embryo transfers. We obtained the following data from the literature search: basic patient demographics, type of fertilization, type and day of embryo transferred, number of embryos transferred, gestational ultrasound details, presence of any genetic testing if performed after delivery, and number of live births.

Result(s) Two thousand four hundred thirty-four women underwent fresh or frozen single embryo transfer between June 2012 and June 2017 at the University of California, San Francisco Center for Reproductive Health. Of these, 11 women underwent a single blastocyst transfer with subsequent clinical pregnancies identified as multi-chorionic gestations. Four were in downregulated controlled FET cycles, in which concomitant spontaneous pregnancy could not have been possible. We then reviewed all cases of monozygotic dichorionic-diamniotic (DC-DA) splitting in IVF patients reported in the literature from 1978 to 2017. These eight cases demonstrate monozygotic splitting after the blastocyst stage, which challenges the existing dogma that only monochorionic twins can develop after day 3 post-fertilization.

Conclusion(s) The accepted theory of monozygotic twinning resulting from the splitting of an embryo per a strict post-fertilization timing protocol must be re-examined with the advent of observed multi-chorionic pregnancies resulting from single blastocyst transfer in the context of IVF.

Keywords Monozygotic · Single embryo transfer · Blastocyst

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Introduction

The timing after fertilization at which a single embryo splits into two defines the type of resultant twin gestation. The usual dogma, presented initially by Corner in 1955 and essentially unchallenged for more than half a century, is that DC-DA twins occur if the embryo divides within the first 3 days post-fertilization, monochorionic-diamniotic (MC-DA) twins result from embryo division between days 4 and 8 post-

fertilization, monochorionic-monoamniotic (MC-MA) twins result from embryo division between days 9 and 12 post-fertilization, and conjoined twins occur if the embryo divides 13 days or more post-fertilization [1–3].

DC-DA twins can have either a dizygotic or monozygotic origin, depending on whether they result from implantation of two separate embryos or splitting of a single embryo between days 0 and 3 following fertilization. The resultant gestation is composed of two separate placentas and each fetus develops in its own amniotic sac. While DC-DA twins of both dizygotic or monozygotic origin have a similarly low obstetrical complication rate compared to monochorionic pregnancies, only monozygotic twins have identical genetic information and are considered “identical twins.” [4]

IVF has increased the incidence of all multifetal gestations, most of which are dizygotic due to the intrauterine transfer of more than one embryo at either the cleavage or blastocyst stage [5]. However, IVF has also been shown to increase the rate of monozygotic twinning by two- to twelvefold when compared with the spontaneous occurrence of 0.4% [6–8]. Recent evidence also supports the claim that blastocyst transfer in fresh IVF cycles is associated with a significantly increased likelihood of monozygotic pregnancy compared to cleavage-stage embryo transfers (adjusted odds ratio of 2.78) [9, 10].

Though the reason for increased risk of monozygotic twinning with IVF is controversial, a number of studies have found associations with factors including oocyte age < 35 years old, extended embryo culture, micromanipulation of the zona pellucida following procedures such as intracytoplasmic sperm injection (ICSI) or assisted hatching (AH), and after fresh embryo transfer [11–14]. The stage of embryo development at time of transfer also appears to be particularly important, as blastocyst-stage embryos are more likely to result in monozygosity than cleavage-stage embryos [13].

The literature on blastocyst transfer resulting in DC-DA monozygotic twins is extremely limited. Since it challenges the accepted doctrine for monozygotic twinning, in which blastocyst transfer should only result in MC-DA or MC-MA gestations, it is necessary to investigate this further. Many DC-DA pregnancies are assumed to result from transfer of multiple embryos or, less commonly, concomitant spontaneous pregnancies [9]. Genetic testing of offspring is rarely undertaken, underestimating the actual rate of DC-DA monozygotic twinning [15]. Here, we report four cases of monozygotic DC-DA twinning following single blastocyst transfer in downregulated, controlled FET cycles.

Materials and methods

Following an expedited institutional review board approval, all in vitro fertilization cycles (fresh, frozen, autologous, and

donor oocyte) performed between June 2012 and June 2017 at the University of California, San Francisco Center for Reproductive Health, were reviewed retrospectively. Cycles with cleavage-stage embryo transfer or with transfer of more than one blastocyst were excluded. The remaining cycles were analyzed to determine the number resulting in a clinical pregnancy, defined as the presence of one or more gestational sacs on initial ultrasound routinely performed at our institution between 5.5 and 6.5 weeks gestational age. We then further identified all patients with two or more gestational sacs noted on initial ultrasound, suggesting possible multi-chorionic monozygotic pregnancies. An in-depth chart review was performed of these patients with further exclusions applied that would lend credence to dizygosity rather than monozygosity. These additional exclusion criteria included fetal/neonatal sex discordance and both fresh embryo transfer and natural cycle FET, in which concomitant spontaneous pregnancy could theoretically have occurred. Demographic, clinical, and IVF-FET cycle characteristics of the resulting patients were collected. Additional obstetrical and neonatal outcome data, as well as any testing confirming zygosity, were analyzed if available.

Controlled ovarian hyperstimulation (COH) was achieved with antagonist-based protocols utilizing a combination of follicle stimulating hormone and human menopausal gonadotropin. The initial dose was individualized per patient based on age, anti-Müllerian hormone (AMH), basal FSH, antral follicle count, and prior ovarian response to COH (if known). Luteinizing hormone suppression was accomplished with the use of GnRH antagonist (ganirelix acetate or cetrorelix acetate) with lead follicle at 12 mm. Either a dual GnRH agonist (4 mg Lupron) plus hCG trigger (1500u) or hCG trigger alone (5000u) was administered when at least two follicles reached 18 mm. Egg retrieval occurred 36 hours after hCG injection.

Recovered oocytes were placed in 0.6 mL Global (Life GLOBAL, LGGG-050) supplemented with 10% Life Global Protein Supplement (LGPS-050) overlaid with 0.3 mL of Lifeguard Oil (Life GLOBAL, #LGUA-500). Oocytes were then incubated at 37 °C (7% CO₂, 5% O₂) and assessed for maturity following cumulus cell stripping in patients undergoing ICSI. The oocytes were then injected 40–42 hours following time of hCG administration with a single sperm depending on medical indication. Zygotes were identified on fertilization check 16–18 hours post-insemination and group-cultured (up to 3) in 50 uL of culture medium Global supplemented with 10% LGPS-050 overlaid with 10 mL of Lifeguard Oil. Morphology of each embryo was assessed using standardized institutional scoring criteria. All embryos were grown to the blastocyst stage.

Assisted hatching was performed on day 3 embryos with the LYCOS Laser Optical System with ZILOS software or SATURN (Research Instruments Viewer) laser system. The RI Saturn active laser system was used to emit a continuous laser beam about three times to make 10–20 µm openings on

clear sections between blastomeres on the zona pellucida. Preimplantation genetic screening (PGS) biopsy occurred on day 5 or day 6. Approximately 5–7 cells were biopsied from the trophoctoderm layer of the blastocyst and transitioned to polymerase chain reaction (PCR) tubes. The cells were sent to a preimplantation genetic laboratory for analysis with labeled DNAs that were hybridized and images obtained through dual laser scanner. Blastocyst biopsy confirmed euploid embryos. In one case, preimplantation genetic diagnosis (PGD) for BRCA status was performed in a similar manner with analysis of the region of concern for BRCA.

Downregulated FET cycles were performed with oral contraceptive started on cycle day 1–3 for 2 weeks. Lupron 10 units once daily was started 6–7 days before last oral contraceptive dose and continued until start of progesterone. Estradiol patch 0.1 mg was placed on cycle day 1 and sequentially increased to 0.4 mg on cycle day 10. Ultrasound was performed to determine endometrial thickness and to confirm the absence of a dominant follicle in the ovaries. All downregulated FET cycles utilized progesterone supplementation intramuscularly which was continued until 13 weeks of gestation. Blastocysts were thawed on the morning of embryo transfer and were considered to have excellent survival if all cells thawed intact. Transfer occurred on day 6 of intramuscular progesterone.

A review of the English language literature was performed (PUBMED, PMC) using the search words monozygotic twins, dichorionic diamniotic, in vitro fertilization, and single embryo transfer in order to identify cases of DC-DA monozygotic twinning from 1978 to 2017. Resulting articles were reviewed to eliminate all cases of dizygosity and day 3 embryo transfers, which could result in monozygotic DC-DA twins. The citations of each article were reviewed for additional monozygotic multichorionic gestations that were not identified using the chosen search words. We obtained the following data from the literature search: basic patient demographics, type of fertilization, type and day of embryo transferred, number of embryos transferred, gestational ultrasound details, presence of any genetic testing if performed after delivery, and number of live births.

Results

Twenty-four thousand five hundred fifty-three IVF and FET cycles were performed between June 2012 and June 2017 at our institution. There were 2434 cycles in which a single blastocyst was transferred, and 1181 (48.5%) resulted in a clinical pregnancy. Eleven of these pregnancies were noted to have two or more gestational sacs on initial ultrasound. Of these, 3 were trichorionic-triamniotic triplet pregnancies, and 8 were DC-DA twin pregnancies. Four patients were identified who underwent a GnRH-agonist downregulated FET cycle (with

mid-cycle ultrasound surveillance documenting absence of ovarian follicular development), which excludes the possibility of concomitant spontaneous pregnancy.

Demographic data for these four patients are provided in Table 1. Two of the 4 identified individuals used an oocyte donor due to age-related infertility; in both cases, the donor was 26 years old at the time of her IVF cycle. One of these two patients also utilized donor sperm due to her same-sex relationship status.

Data from IVF stimulation, subsequent FET cycles, and embryologic data are provided in Table 2. Note that for cases 1 and 2, the IVF information provided is for that of the egg donor cycle. All four patients had one or more described risk factors for monozygotic twinning, including the use of ICSI (4/4), assisted hatching (3/4), and blastocyst transfer (4/4). PGS/PGD was performed in 3/4 patients. Placental pathology was confirmed dichorionic-diamniotic in case 2.

Figure 1 depicts each embryo prior to transfer and the resulting initial ultrasound confirming multifetal gestation. Details on grading of each blastocyst and ultrasound findings can be found in Table 2.

Literature review

A PubMed and PMC literature search of the English language from 1978 to 2017 was conducted using the following terms: single embryo transfer, monozygotic twins, in vitro fertilization, and dichorionic diamniotic. Twenty-two studies were identified and further reviewed to eliminate all cases of dizygosity (15 publications eliminated). Five cases (in addition to our four) were found to have monozygotic multichorionic-multiamniotic gestations following single blastocyst transfer. Two of these described day 3 embryo transfers. Review of the remaining 3 cases including citations revealed an additional 5 monozygotic multichorionic cases that were not identified in the literature search above. A summary of the final 8 cases is provided in Table 3.

In review of Table 3, six of the studies are case presentations while two include a retrospective chart review (Kyono et al. and Knopman et al) [17, 21]. In Kyono et al., the retrospective analysis portion included 15,355 IVF cycles at 16 Japanese centers [17]. Eighteen infants were born alive with day 4–6 morula to blastocyst-stage embryo transfer. The authors cite that “around” 6/18 cases could have occurred from a concurrent natural conception, resulting in 12 patients with splitting of a single embryo after day 4. DNA analysis was performed in only one case and no information was provided on status of fresh, natural cycle frozen, or downregulated frozen embryo transfer. Knopman et al. retrospectively analyzed 4976 pregnancies with a total of 76 confirmed monozygotic pairs identified [21]. Interestingly, only one of these was found to be DC-DA, defined in this study as the number of

Table 1 Demographic characteristics

	Case 1	Case 2	Case 3	Case 4
Age (years)	43	48	38	36
Gravidity	2	0	0	0
Parity	0	0	0	0
BMI (kg/m ²)	20.1	24.2	22.9	25.1
Ethnicity	Caucasian	Caucasian	Caucasian	Asian/Pacific Islander
Diagnosis	Diminished ovarian reserve	Diminished ovarian reserve, absolute male factor (same-sex relationship)	BRCA1 mutation desiring PGD	Unexplained infertility
Day 3 FSH (mIU/mL)	11	8.7	6.4	7.8
AMH (ng/mL)	Not available	Not available	2.5	2.76
Antral follicle count (AFC)	3	6	30	13
# of prior IVF attempts	4	5	0	1
# of prior transfers	2	7	0	2
Use of donor gametes	Yes (oocyte)	Yes (oocyte/sperm)	No	No

gestational sacs with a fetus greater than the number of embryos transferred. However details surrounding this DC-DA gestation were not provided in the publication.

Micromanipulation techniques (assisted hatching, ICSI, PGS/PGD) were used in 4/8 cases. The authors in 4/8 cases noted division of the blastocyst prior to transfer resulting in DC-DA twins. Van Langendonck reported an unexpanded blast between day 5 and 6 began to hatch prematurely [19]. Instead of hatching from the zona pellucida through a large slit, it herniated instead through a small hole in the zona. Six hours later, the blastocyst had divided into two half-

blastocysts with two trophoectoderm and two inner cell masses (ICM). There was no change in neither the zona thickness nor the overall blastocyst diameter following atypical hatching. Behr reported a case where the blastocyst was intended for cryopreservation but was eventually never frozen due to the noted atypical hatching and subsequently never transferred [20].

Confirmatory testing performed included confirmation of DC-DA placenta on pathology, DNA fingerprinting with short tandem repeats (reported as 99.9999528% probability that twins were monozygotic), or laboratory visualization of the

Table 2 IVF cycle characteristics and pregnancy outcomes

	Case 1	Case 2	Case 3	Case 4
Total days of stimulation	11	10	10	12
Total gonadotropins used (units)	2062.5	2000	2362.5	4125
Peak estradiol (pg/mL)	4687	3559	6123	4094
# of oocytes retrieved	38	35	30	16
ICSI performed	Yes	Yes	Yes	Yes
# of 2PN embryos	19	20	15	8
AH performed	Yes	Yes	No	Yes
PGS/PGD	Yes (PGS)	No	Yes (PGS/PGD)	Yes (PGS)
Stage (day) of blastocyst at vitrification	Expanded (day 5)	Hatching (day 5)	Expanded (day 5)	Hatching (day 5)
Grade of embryo at thaw	Excellent	Excellent	Excellent	Excellent
Endometrial thickness in FET cycle (mm)	11.4	10.0	10.0	7.5
# of gestational sacs	2	2	2	3
# of yolk sacs	1	2	2	3
# of fetal poles	1	2	2	3
# of FHT	1	2	2	3
Pregnancy outcome	Ongoing singleton	2 liveborn females	Vanishing twin, 1 liveborn singleton	Ongoing DC-DA twin gestation, spontaneous reduction of 3rd fetus

Embryos at transfer and first gestational ultrasound

Fig. 1 Embryos at transfer and first gestational ultrasound

presence of two trophoectoderms and two inner cell masses (ICMs) in a single blastocyst [17]. No postnatal confirmatory testing was possible in cases where only one infant was born, pregnancy was ongoing, or no embryo transfer was performed. Two embryos were transferred in 3/8 cases with resultant higher-order multiplets, with DC-DA assessed by ultrasonography. Fresh cycles were performed in 3/8 and unreported in 2/8 cases, resulting in only 3 cases of frozen blastocyst transfer identical to our presented cases.

Discussion

The model of monozygotic twinning was first described by Corner in 1955 and has become accepted as fact, to the extent that it is now often published in textbooks and papers without a citation. Corner's model depicts four stages of embryological splitting along a timeline of development: the 2-cell stage and prior to compaction of the morula (days 0–3, resulting in dichorionic-diamniotic twins), the inner cell mass at the blastocyst stage (day 4–8, monochorionic diamniotic), the bilaminar embryonic disc in the late blastocyst stage (days 9–12, monochorionic monoamniotic), and the primitive streak (> 13 days, conjoined twins [21]. On discussing the initial twinning by separation of the early blastomere, Corner wrote “unless the age arrives of ‘test-tube babies’...this type of human twinning must remain a plausible conjecture” [3]. The theory was quickly accepted due to Corner's prestige, the internal logic of the model, and the convincing nature of his graphic depiction. As Herranz writes, by 1957, the model was repeatedly cited in books and journal articles and “fifteen years after its publication, the model became standard wisdom” [3].

Monozygotic twinning has been reported in assisted reproductive technology with a frequency ranging from 1.2 to 8.9% and is typically monochorionic, two ICMs within one trophoectoderm [17, 22]. The East Flanders Prospective Twin Study proposed that only ~20% of all monozygotic pregnancies following assisted reproduction are DC-DA [23]. However, the true number of DC-DA monozygotic pregnancies is frequently underestimated due to an incorrect classification as a dizygotic gestation [21]. DNA analysis, considered the gold standard for confirmation of zygosity, is less frequently utilized due to expense. Several studies have implemented other less expensive, albeit less specific, strategies to differentiate between monozygotic and dizygotic DC-DA twins; these include gender discordance, fingerprinting, and parental questionnaires [24].

Confirmatory testing for monozygosity is important because dizygotic twins have been observed following single embryo transfer [25]. This can be explained by a single embryo transfer with a concurrent natural conception, which can occur in both fresh IVF cycles and in FET cycles without pituitary suppression. In our study, confirmatory testing was not performed, but because these were downregulated FET cycles in which ultrasound monitoring confirmed lack of spontaneous ovulation, we can be confident that the DC-DA twins born in these situations were monozygotic. Additionally, the literature provides three more identical frozen cycles in which blastocyst transfer resulted in DC-DA twins.

Several factors have been shown to be associated with monozygotic splitting. Micromanipulation of the zona pellucida during ICSI, PGS/PGD, and/or assisted hatching, though controversial, has been reported to increase monozygosity in some studies, the mechanism of which is hypothesized to be

Table 3 Literature review of available multi-chorionic monozygotic multiplets

Article author/date of publication	Patient age (years)	Diagnosis	Micromanipulation technique	Embryo transfer type	Embryo(s) at transfer	Embryo day at transfer
Shibuya [1] (Jan 2012)	39	Tubal Factor Infertility	None	Downregulated frozen	Blastocyst	Day 5
Ferreira [16] (Sep 2010)	24	Andrological subfertility	ICSI	Fresh	Blastocysts	Day 5
Kyono [17] (May 2013)	NR	NR	None	Frozen	Warmed blastocyst	Day 4 (morula 3 h before transfer)
Meinijes [18] (Sept 2001)	40	46 X,del (X)(p22.1)	ICSI	Fresh	Expanded blastocysts	Day 5
Van Lagendonck [19] (Nov 2000)	33	Bilateral salpingectomy, recurrent ectopic	None	Fresh	Hatching blastocyst + expanded blastocyst	Day 6
Behr [20] (Dec 2003)	34	NR	ICSI	–	–	–
Tokunaga [17] (2010)	NR	NR	Assisted Hatching	Frozen	Warmed blastocyst	Day 5
Knopman [21] (Jan 2009)	NR	NR	NR	NR	Blastocyst	NR

Article author/date of publication	# of embryos transferred	# of gestational sacs (GS) at 1st US	# of infants born	Monozygotic DC-DA confirmatory testing upon birth	Comments
Shibuya [1] (Jan 2012)	1	2	2	DC-DA placenta	Atypical hatching
Ferreira [16] (Sep 2010)	2	3 → 5 (MZ DC-DA twins, MZ MC-TA triplets)	1	None	–
Kyono [17] (May 2013)	1	2	2	DNA fingerprinting	–
Meinijes [18] (Sept 2001)	2	3 (DZ TC-TA triplets)	Triplet A ongoing	None	2 distinct inner cell masses in 1 blastocyst at transfer
Van Lagendonck [19] (Nov 2000)	2	3 (DZ TC-TA triplets)	NR	NR	Donor oocyte
Behr [20] (Dec 2003)	–	–	–	–	Atypical hatching
Tokunaga [17] (2010)	1	2 (MZ DC-DA)	2	Sexual concordance	–
Knopman [21] (Jan 2009)	1	2	NR	None	No splitting on daily microscopy

NR not reported, MZ monozygotic, DZ dizygotic, TA triamniotic

herniation and subsequent splitting of the inner cell mass [26–29]. It should be noted that in all cases included in our series as well as 4/8 cases described in our literature review, some micromanipulation of the embryo was performed. Both ICSI and AH were performed in all 4 of our cases; ICSI was performed in 3 of the 8 studies in our literature review, and AH was performed in only 1 out of 8. While 3 cases of our 4 included PGS or PGD, none in the literature review used these techniques.

Extended culture has also been described in association with MZT. The goal of elective single embryo transfer at the blastocyst stage is to improve the pregnancy rate while reducing the risk of multiples; however, it has simultaneously led to an increase in the risk of MZT [30, 31]. Blastocyst transfer has been shown in several studies to significantly increase the monozygotic twinning (MZT) rate, estimated at 1.7% which is 4.25 times higher than the natural pregnancy MZT rate of 0.4% [27]. A proposed theory for this observation is potential hardening of the zona pellucida with extended culture media, causing pinching or splitting of the ICM when hatching. An alternate theory is that extended culture can cause changes in cell-to-cell adhesion and therefore splitting of the ICM [32].

With the advent of in vitro fertilization, direct observation of human embryonic development has become possible. While artificial removal of individual blastomeres from a human 4-cell embryo has been shown to develop into separate blastocysts, embryologists in IVF labs have never witnessed an embryo spontaneously splitting before the blastocyst stage, as would be expected with DC-DA monozygotic twinning in the first 3 days of embryonic life [17, 21, 33]. In accordance with this phenomenon, none of the embryos in our sample of patients were noted to have splitting prior to the blastocyst stage. However, as described in several of the studies found in our literature review, the human blastocyst (beyond day 3) has been seen to split spontaneously into two blastocysts during the hatching process [20]. The resulting monozygotic DC-DA twin gestations, as well as the others described in which splitting prior to transfer was not observed, challenge the notion that only monochorionic gestations result from the blastocyst-stage embryo. Therefore, this popularly held credo of chorionicity based simply upon the day of embryonic development must be challenged.

Several models have been proposed to replace Corner's overly simplistic version. Lopez-Moratalla offers the possibility that monozygotic twinning is the result of an extended fertilization process, where mitosis of the fertilized egg occurs before polarization by calcium ions, creating two separate zygotes [34]. Subsequent division and polarization could generate two genetically unique zygotes. Herranz builds on this theory with the proposal that all monozygotic twinning occurs with the first division of the fertilized egg, where twin zygotes

are produced instead of blastomeres [3]. Additionally, the idea of splitting is instead changed into abnormal fusion of the membranes within the zona pellucida, or fusion of the embryonic bodies with conjoined twins. The theory is further described where if no fusion occurs, both zygotes follow their own courses to the blastocyst stage and hatch as two independent blastocysts to implant and develop into DC-DA twins. However, if the trophoectoderm fuses, permitting the fusion of the independently developing blastocysts, monochorionic gestations would then be possible [35]. It is clear that there must be continued scientific discussion on the proposed mechanism underlying monozygotic DC-DA twinning.

A strength of our study, unlike several of the published studies included in our paper, is that it includes only down-regulated frozen embryo transfer, with confirmed lack of mid-cycle ovulation preventing the possibility of dizygotic DC-DA gestations. Three of the cycles reviewed in the literature reported fresh embryo transfers while 1 did not report fresh or frozen status. Additionally, none of the resulting infants in our four cases had confirmatory DNA analysis due to cost. The numbers in our study are unfortunately too small to perform statistical analyses. However, taken in consideration with the overall number of published cases since the advent of IVF, the reality that a zygote has never split spontaneously into two zygotes prior to the blastocyst stage is striking.

Conclusion

We reviewed 4 cases found among 24,553 IVF cycles conducted at our institution where a single blastocyst transfer resulted in a monozygotic multichorionic gestation. In addition, a literature review identified an additional 8 cases in which a single embryo transfer resulted in confirmed monozygotic multichorionic multiples. Without question these reports are underestimates as zygosity is not often confirmed in multiple gestations, particularly when more than one embryo is transferred. Further investigation into the etiology of monozygotic splitting, as well as the effect of IVF techniques on embryonic division, is warranted. Finally, the ubiquitous acceptance of Corner's theory of embryonic division created prior to the age of "test-tube babies" must be reevaluated with the vast experience from modern in vitro fertilization. Further theories underlying the mechanism of monozygotic DC-DA gestations must be proposed and analyzed scientifically to ultimately reduce the increased risk of monozygotic multiplets associated with IVF technologies.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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