

The Precise Timing of Embryo Splitting for Monozygotic Dichorionic Diamniotic Twins: When Does Embryo Splitting for Monozygotic Dichorionic Diamniotic Twins Occur? Evidence for Splitting at the Morula/Blastocyst Stage From Studies of In Vitro Fertilization

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There is a long-held credo, as illustrated in *Langman's Medical Embryology* (11th ed., Sadler, 2010), that dichorionic diamniotic (DD) twins develop after embryo splitting in the early stages of embryonic development. However, from our clinical experiences of the examination of data from single-embryo transfers in 16 fertility clinics in Japan and from various reports, the majority of occurrences of DD twins have been found in the blastocyst stages.

■ **Keywords:** blastocyst transfer, monozygotic DD twins, placenta, ultrasonography, embryo splitting time

There is a long-held credo that the timing of embryo division governs the ultimate placental configuration of monozygotic twins (MZTs): within three days of fertilization, dichorionic diamniotic (DD); between four and eight days, monochorionic diamniotic (MD); and after eight days, monochorionic monoamniotic (MM; Benirschke & Kim, 1973; Hall, 2001, 2003; Redline, 2003; Sadler, 2010). The percentage of all twin pregnancies surviving to birth is 25–30% in DD, 70–75% in MD, and 1–2% in MM twins (Hall, 2003; Redline, 2003). Benirschke and Kim (1973) showed a diagram correlating the timing of embryologic events in the first part of zygote development with types of placenta developing when twinning occurs. However, they never thought that a single blastocyst would split into two half-blastocysts, both with inner cell mass (ICM) and trophoctoderm components in the blastocyst stage.

Two-cell embryos have been artificially divided into two embryos which developed into DD twins in sheep, cows, and mice (Tsunoda & McLaren, 1983; Willadsen, 1979; Willadsen et al., 1981). In humans, the blastomeres of a four-cell stage embryo are also flexible and able to develop into blastocysts with ICM and trophoctoderm (Van de Velde et al., 2008). However, we have never observed an embryo spontaneously splitting in half before the blastocyst stage in

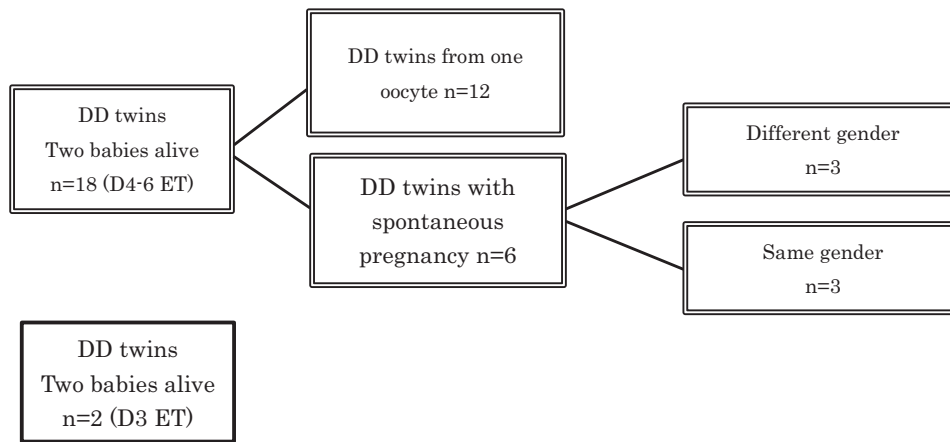
over 30 years of laboratory experience. I would like to show that the majority of occurrences of monozygotic DD twins have been found in the blastocyst stages, at least in vitro, according to various reports and our own data.

Clinical Experience 1

A single, warmed blastocyst (morula stage three hours earlier) was transferred on the fourth day after progesterone supplementation during the luteal phase. This resulted in DD twins — healthy female infants weighing 2,080 g and 2,005 g at gestational 33 weeks 0 days. A λ sign was recognized under ultrasonography at seven weeks gestation, and monozygosity was confirmed by DNA fingerprinting (short tandem repeat). This suggested that there was a 99.9999528% probability that these twins were monozygotic.

RECEIVED 5 January 2013; ACCEPTED 16 April 2013. First published online 23 May 2013.

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**FIGURE 1**

Eighteen cases of two DD twin babies born alive from D4-5 transfer (Kyono, 2012).

Blood E2 level was 208 pg/mL, blood progesterone level was 0.2 ng/mL, and endometrial thickness was 11.5 mm. No follicles of more than 10 mm in diameter were detected in either ovary when the progesterone injection was started. We confirmed no ovulation in the patient at that time and no intercourse between the couple during the treatment cycle (Doshida et al., 2012).

Clinical Experience 2

We transferred a single, vitrified-warmed blastocyst, which divided into two half-blastocysts, both with trophoctoderm and ICM components in spontaneously hatching zona pellucida on the fifth day after progesterone administration. Six milligrams per day of oral estradiol was administered from day 1, and 50 mg per day of progesterone was administered from day 14 (blood E2 level: 415.7 pg/mL; blood progesterone level: 0.2 ng/mL; maximum follicular diameter of both ovaries: less than 10 mm; endometrial thickness: 9.1 mm). Two gestational sacs and fetuses with heartbeats showing a λ sign under ultrasonography were recognized at seven weeks of gestation and the patient gave birth to two healthy female infants weighing 1,530 g and 1,388 g at gestational 33 weeks. The pathological examination demonstrated that the DD twin placentas had fused in the third trimester (Shibuya & Kyono, 2012).

Analysis of Data From 16 Facilities

Following these cases, we examined incidence, chorionicity, and amnionicity of MZT pregnancies after single-embryo transfer (SET) in a 16-clinic multicenter study. We asked all 25 centers of the Japanese Institute for Standardizing Assisted Reproductive Technology (JISART) for their cooperation in a study of DD twins following a SET. Sixteen of these centers agreed to support us.

Ultrasonography to examine chorionicity and amnionicity for MZT pregnancy was performed at 7–9 weeks gestation, which is the standard timing in Japan as we refer patients to hospitals for prenatal care at 8–10 weeks.

A total of 15,355 In Vitro Fertilization (IVF) cycles resulting in pregnancy following SET (52,135 cycles) from January 2006 through December 2010 at 16 centers in Japan were reviewed retrospectively. The study was approved by a local institutional review board. Two hundred and seven MZTs (1.4%) were diagnosed after first-trimester ultrasound evaluation. The criteria for determining DD twins were the presence of the twin peak (λ) sign; 27.0% (56/207) of MZTs were DD, 65.2% (135/207) MD, 4.8% (10/207) MM, and 2.9% (6/207) other (see Figure 1 of Kyono et al., 2011). These percentages are reasonably consistent with data of natural conceptions (DD: 25–30%; MD: 70–75%; and MM: 1–2%; Hall, 2001). The numbers of deliveries, ongoing pregnancies, and miscarriages were 37 (66.1%), 9 (16.1%), and 10 (17.9%) in DD; 97 (71.9%), 13 (9.6%), and 25 (18.5%) in MD; 4 (40%), 1 (10%), and 5 (50%) in MM; and 4 (66.7%), 0 (0%), and 2 (33.3%) in others, respectively. In 37 DD twin births, both babies were born alive in 20 cases. In the remaining 17 cases of DD twins, only one baby was born alive. This was due to vanishing (16) and reduction (1) in the first trimester. In 18/20 DD twin cases with both babies born alive, morula to blastocyst stage embryos on days 4–6 had been transferred. Three of these were different-gender twins, thus actually dizygotic. Around 6/18 cases might have occurred due to the combination of a transferred blastocyst and a natural pregnancy, because three cases were different-sex babies, and it is reasonable to assume that the same situation occurred in an equal number of same-sex DD twins. However, these data indicate that at least in the remaining 12 cases, the embryo splitting took place after day 4 (Figure 1; Kyono, 2012). Although there was DNA analysis in only one case in this

multicenter study, the fact that every case was a SET and the λ sign was observed in each case strongly suggests that 12 cases were DD twins. Cases of monozygotic DD twins might be related to extended culture (Kyono et al., 2011).

This examination suggests that the splitting of the transferred embryos took place during or after the blastocyst stage, and that the popular credo stating that the timing of embryo division governs the placental configuration of MZTs must be re-examined as to its veracity, at least *in vitro* and also possibly *in vivo*.

Discussion

Although the majority of ART-related multiple pregnancies are thought to be dizygotic (derived from two or more fertilized eggs), numerous reports suggest an increase in MZT pregnancies (Alikani et al., 2003; Aston et al., 2008; Blickstein et al., 1999; Derom et al., 1987; Edwards et al., 1986; Sills et al., 2000). Currently, approximately 1.5–2% of all clinical pregnancies that occur after ovulation induction using exogenous gonadotropin therapy (with or without IVF and embryo transfer) are reported as MZT pregnancies, and the incidence of those is four times higher than that of spontaneously conceived MZT pregnancies which eventuated in live births (Alikani et al., 2003; Derom et al., 1987; Edwards et al., 1986; Schachter et al., 2001; Wenstrom et al., 1993).

Some authors have identified ovarian hyperstimulation (Derom et al., 1987, 2006; Schachter et al., 2001; Sills et al., 2000), assisted hatching (Abusheikha et al., 2000; Alikani et al., 1994; Hershlag et al., 1999; Saito et al., 2000; Schieve et al., 2000; Skiadas et al., 2008; Slotnik & Ortega, 1996; Tarlatzis et al., 2002), and/or extended culture (Behr et al., 2000; Da Costa et al., 2001; Jain et al., 2004; Kyono et al., 2011; Milki et al., 2003; Rijinders et al., 1998; Sheiner et al., 2001; Vitthala et al., 2009; Wright et al., 2004) as possible reasons for monozygotic twinning.

Our aim is to clarify the precise timing of embryo splitting resulting in monozygotic DD twins. First, there have been some reports which suggested that embryo splitting occurred in the blastocyst stages.

There are various reports of atypical hatching leading to twinning in the literature, in particular those of Van Langendonck et al. (2000), Meintjes et al. (2001), and Behr and Milki (2003), but our case was the first that reported successful birth of same-sex DD twins after a single-blastocyst transfer (Doshida et al., 2012). Van Langendonck et al. (2000) reported a case of atypical hatching of a human blastocyst leading to monozygotic twinning. They showed a half-blastocyst in the zona pellucida and a half-blastocyst herniated through the zona pellucida in one blastocyst. After a two-blastocyst transfer, they confirmed one gestational sac and fetus with a fetal heartbeat, and two gestational sacs and fetuses without fetal heartbeats, and it was concluded that this was a dizygotic trichorial triamniotic triplet pregnancy.

Meintjes et al. (2001) reported a trichorionic heterozygotic triplet pregnancy with dichorionic monozygotic twins following transfer of two expanded blastocysts, one of which had two distinct ICMs. We speculate that one blastocyst had two distinct ICMs in one trophoctoderm that formed two half-blastocysts with two ICMs and two trophoctoderms, which developed into monozygotic DD twins. One of the triplets was ongoing at 33 weeks at the time of the original publication.

Behr and Milki (2003) reported a case of atypical hatching of a human blastocyst *in vitro* forming two identical embryos. On day 5, they observed two trophoblast populations sharing an ICM from a single blastocyst. On the afternoon of day 5, the now completely hatched blastocysts of similar size, both of which had a visible trophoctoderm and ICM, connected together. On day 6, the two blastocysts separated completely. However, the blastocysts were not cryopreserved or transferred. Tokunaga et al. (2010) reported monozygotic DD twins (two healthy girls) following a single warmed blastocyst (Grade 5BC) with assisted hatching.

Knopman et al. (2010) reported 98 MZTs confirmed on transvaginal ultrasonography at 7–12 weeks gestation (2% incidence). Only one DD MZT pregnancy was clearly diagnosed in their cohort. However, their diagnosis of DD gestations is limited, because DNA analysis, the gold standard for diagnosing monozygotic siblings, was not performed on the fetuses or children.

Knopman et al. (2010) also reported that they had not seen an embryo splitting in half spontaneously before the blastocyst stage in over 15 years of laboratory experience. In their one case of DD monozygosity, no embryo splitting was noted in a daily microscopic examination during development from zygote to blastocyst.

Second, there are our two cases in which embryo splitting occurred during blastocyst stages. In our two cases, ultrasonography showed a λ sign between two gestational sacs and fetuses with fetal heartbeats at seven weeks of gestation. The diagnosis was a single zygote with two chorions and two amnions. Carroll et al. (2002) reported that the most reliable indicator for dichorionicity was a combination of the λ sign and two separate placentae with a sensitivity and specificity of 97.4% and 100%, respectively. The pathological finding was that the DD twin placentas had fused in the third trimester in the second case. DNA fingerprinting (short tandem repeat) confirmed that the two babies originated from the same zygote in the first case.

In the second reported case of DD twins from an embryo that splits in the blastocyst stage, the embryo had been chosen due to its grade being higher than that of the other cryopreserved embryos. When we found the abnormality (two half-blastocysts) in the warmed embryo, we informed the patients, who decided to proceed with the embryo transfer as they did not want to waste the embryo or wait for another embryo to warm. In our laboratory, we measure the pH of

media (7.20–7.40), CO₂ levels (5.5–6.5%), O₂ levels (4.5–5.5%), and temperature (36.5–37.5 °C) every day, and as far as we can ascertain, there were no irregularities. We speculate that this development was neither laboratory-specific nor patient-specific.

It is interesting to note that a human blastocyst has been observed to cleave spontaneously into two complete blastocysts, both with ICM and trophoctoderm components during the hatching process in vitro, a phenomenon that would also result in a DD pregnancy (Behr & Milki, 2003; Shibuya & Kyono, 2012; Van Langendonck et al., 2000). Massip et al. (1982) confirmed this fact in cows using cinematographic and morphometric analysis.

Monozygotic twinning with blastocyst transfer is typically monozygotic, resulting from the division of the ICM within a single trophoctoderm (Chida, 1990; Milki et al., 2001; Mio, 2008). When we diagnose DD twins under ultrasonography at 7–11 weeks gestation, we must discriminate between (1) monozygotic DD twins (two half-blastocysts, both with ICM and trophoctoderm components from a SET), (2) dizygotic DD twins (a SET with a natural pregnancy: Kyono et al., 2009; Sugawara et al., 2010; Van der Hoorn et al., 2011), and (3) dizygotic DD twins (a two-embryo transfer). In MD twins, we must discriminate between (1) monozygotic MD twins (a SET) and (2) dizygotic MD twins (dual-embryo transfer: blood chimerism; Hackmon et al., 2009; Kuhl-Burmeister et al., 2000; Machin, 2009; Miura & Niikawa, 2005; Souter et al., 2003; Williams et al., 2004). We can discriminate by histochemical analysis of placenta, chromosome analysis (blood, skin biopsy specimen, umbilical cord tissue, buccal smear), blood type, enzyme polymorphisms, and human leukocyte antigen types.

The vascular connections can also be expected to lead to the sharing of hematopoietic stem cells, microchimerism between dizygotic twins, and mosaicism if genetic discordance arises between the monozygotic twins. More fetal-maternal microchimerism takes place with complications of pregnancy, such as pre-eclampsia or bleeding, which typically has been seen in twin pregnancies. However, Jang et al. (2010) reported a very rare case of blood chimerism in dichorionic placentas. It is very important for reproductive doctors and obstetricians to exchange information in cases of twin pregnancy.

One possible reason for the common belief that embryo splitting occurs in the two-cell stage is that it is widely known that we can produce monozygotic DD twins by splitting an embryo artificially at the two-cell stage. For example, monozygotic twins have been produced by separation of the blastomeres of two-cell embryos in sheep (Willadsen, 1979), cows (Willadsen et al., 1981), and mice (Tsunoda & McLaren, 1983). However, natural splitting in two-cell embryos has never been observed. We have shown that, without this technique, natural splitting of embryos leading to monozygotic DD twins occurs during the blastocyst stage after day 5 post-fertilization.

Conclusion

In conclusion, previous cases, along with our own reports, suggest that the splitting of transferred embryos took place during the blastocyst stage, and the popular credo related to the timing of embryo splitting outlined above must be re-examined as to its veracity.

References

- Abusheikha, N., Salha, O., Sharma, V., & Brinsden, P. (2000). Monozygotic twinning and IVF/ICSI treatment: A report of 11 cases and review of the literature. *Human Reproduction Update*, 6, 396–403.
- Alikani, M., Cekleniak, N. A., Walters, E., & Cohen, J. (2003). Monozygotic twinning following assisted conception: An analysis of 81 consecutive cases. *Human Reproduction*, 18, 1937–1943.
- Alikani, M., Noyes, N., Cohen, J., & Rosenwaks, Z. (1994). Monozygotic twinning in the human is associated with the zona pellucida architecture. *Human Reproduction*, 9, 1318–1321.
- Aston, K. I., Peterson, C. M., & Carrell, D. T. (2008). Monozygotic twinning associated with assisted reproductive technologies: A review. *Reproduction*, 136, 377–386.
- Behr, B., Fisch, J. D., Racowsky, C., Miller, K., Pool, T. B., & Milki, A. A. (2000). Blastocyst-ET and monozygotic twinning. *Journal of Assisted Reproduction and Genetics*, 17, 349–351.
- Behr, B., & Milki, A. (2003). Visualization of atypical hatching of a human blastocyst in vitro forming two identical embryos. *Fertility and Sterility*, 80, 1502–1503.
- Benirschke, K., & Kim, C. K. (1973). Multiple pregnancy 1. *New England Journal of Medicine*, 288, 1276–1284.
- Blickenstein, I., Verhoeven, H. C., & Keith, L. G. (1999). Zygotic splitting after assisted reproduction. *New England Journal of Medicine*, 340, 738–739.
- Carroll, S. G. M., Soothill, P. W., Abdel-Tattah, S. A., Porter, H., Montague, I., & Kyle, P. M. (2002). Prediction of chorionicity in twin pregnancies at 10–14 weeks of gestation. *British Journal of Obstetrics and Gynaecology*, 109, 182–186.
- Chida, S. (1990). Monozygous double inner cell masses in mouse blastocysts following fertilization in vitro and in vivo. *Journal of In Vitro Fertilization and Embryo Transfer*, 7, 177–179.
- Da Costa, A. L. E., Abdelmassih, S., De Oliveira, F. G., Abdelmassih, V., Abdelmassih, R., & Nagy, Z. P. (2001). Monozygotic twins and transfer at the blastocyst stage after intracytoplasmic sperm injection. *Human Reproduction*, 16, 333–336.
- Derom, C., Leroy, F., Vlietinck, R., Fryns, J. B., & Derom, R. (2006). High frequency of iatrogenic monozygotic twins with administration of clomiphene citrate and a change in chorionicity. *Fertility and Sterility*, 85, 755–757.
- Derom, C., Vlietinck, R., Derom, R., Van Den Berghe, H., & Thiery, R. (1987). Increased monozygotic twinning rate after ovulation induction. *Lancet*, 1, 1236–1238.

- Doshida, M., Toya, M., Araki, Y., & Kyono, K. (2012). A review of dichorionic diamniotic twin pregnancies by the single embryo transfer. *Journal of Assisted Reproduction and Genetics*, 29, 1–4.
- Edwards, R. G., Mettler, L., & Walters, D. E. (1986). Identical twins and in vitro fertilization. *Journal of In Vitro Fertilization and Embryo Transfer*, 3, 114–117.
- Hackmon, R., Jormark, S., Cheng, V., Green, C. O., & Divon, M. Y. (2009). Monochorionic dizygotic twins in a spontaneous pregnancy: A rare case report. *Journal of Maternal-Fetal and Neonatal Medicine*, 22, 708–710.
- Hall, J. G. (2001). Twins and twinning. In D. L. Rimoin, J. M. Connor & R. E. Pyeritz (Eds.), *Emery and Rimoin's principles and practice of medical genetics* (Vol. 1, 4th ed., pp. 501–513). New York: Churchill Livingstone.
- Hall, J. G. (2003). Twinning. *Lancet*, 362, 735–743.
- Hershlag, A., Paine, T., Cooper, G. W., Scholl, G. M., Rawlinson, K., & Kvapil, G. (1999). Monozygotic twinning associated with mechanical assisted hatching. *Fertility and Sterility*, 71, 144–146.
- Jain, J. K., Boostanfar, R., Slater, C. C., Francis, M. M., & Paulson, R. J. (2004). Monozygotic twins and triplets in association with blastocyst transfer. *Journal of Assisted Reproduction and Genetics*, 21, 103–107.
- Jang, J. H., Jung, H., Kim, J. H., Park, W. S., & Kim, S. H. (2010). Blood chimerism in a dizygotic dichorionic pregnancy. *Korean Journal of Laboratory Medicine*, 30, 521–524.
- Knopman, J., Krey, L. C., Lee, J., Fino, M. E., Novetsky, A. P., & Noyes, N. (2010). Monozygotic twinning: An eight-year experience at a large IVF center. *Fertility and Sterility*, 94, 502–510.
- Kuhl-Burmeister, R., Simeoni, E., Weber-Matthiesen, K., Milde, A., Herwartz, C., Neppert, J., & Suttorp, M. (2000). Equal distribution of congenital blood cell chimerism in dizygotic triplets after in vitro fertilization. *Human Reproduction*, 15, 1200–1204.
- Kyono, K. (2012). New mechanism of twinning: A mini-review from the facts confirmed practically (Japanese). *Journal of Japan Society of Assisted Reproduction*, 15, 13–19.
- Kyono, K., Nakajo, Y., Doshida, M., Toya, M., & Araki, Y. (2009). A birth of twins — one boy and one girl — from a single embryo transfer and a possible natural pregnancy. *Journal of Assisted Reproduction and Genetics*, 26, 553–554.
- Kyono, K., Shiotani, M., Watanabe, H., Oka, C., Takahashi, K., & Hayashi, N. (2011). When is the actual splitting time of the embryo to develop monozygotic dichorionic diamniotic (DD) twins following a single embryo transfer? *Fertility and Sterility*, 96(Suppl.), S275.
- Machin, G. (2009). Non-identical monozygotic twins, intermediate twin types, zygosity testing, and the non-random nature of monozygotic twinning: A review. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*, 151C, 110–127.
- Massip, A., Mulnard, J., Vanderzwalmen, P., Hanzen, C., & Ectors, F. (1982). The behavior of cow blastocyst in vitro: Cinematographic and morphometric analysis. *Journal of Anatomy*, 134, 399–405.
- Meintjes, M., Guerami, A. R., Rodriguez, J. A., Crider-Pirkle, S. S., & Madden, J. D. (2001). Prospective identification of an in vitro-assisted monozygotic pregnancy based on a double-inner-cell-mass blastocyst. *Fertility and Sterility*, 76(Suppl. 1), S172–S173.
- Milki, A. A., Hinvkley, M. D., Grumet, F. C., & Chitkara, U. (2001). Current IVF and spontaneous conception resulting in a quadruplet pregnancy. *Human Reproduction*, 16, 2324–2326.
- Milki, A. A., Jun, S. H., Behr, B., Giudice, L. C., & Westphal, L. M. (2003). Incidence of monozygotic twinning with blastocyst transfer compared to cleavage-stage transfer. *Fertility and Sterility*, 79, 503–506.
- Mio, Y. (2008). Dynamic morphological analysis of human embryonic development using timelapse cinematography. *Acta Obstetrica Et Gynaecologica Japonica*, 60, 389–398.
- Miura, K., & Niikawa, N. (2005). Do monochorionic dizygotic twins increase after pregnancy by assisted reproductive technology? *Journal of Human Genetic*, 50, 1–6.
- Redline, R. W. (2003). Nonidentical twins with a single placenta — Disproving dogma in perinatal pathology. *New England Journal of Medicine*, 349, 111–114.
- Rijinders, P. M., van Os, H. C., & Hansen, C. A. (1998). Increased incidence of monozygotic twinning following the transfer of blastocysts in human IVF/ICSI. *Fertility and Sterility*, 70, S15–S16.
- Sadler, T. W. (2010). *Langman's medical embryology* (11th ed., pp. 106–108). Philadelphia, PA: Lippincott Williams & Wilkins.
- Saito, H., Tsutsumi, O., Noda, Y., Ibuki, Y., & Hiroi, M. (2000). Do assisted reproductive technologies have effects on the chemotherapy of monozygotic twinning? *Fertility and Sterility*, 74, 178–179.
- Schachter, M., Raziell, A., Friedler, S., Strassburger, D., Bern, O., & Ron-El, R. (2001). Monozygotic twinning after assisted reproductive technologies: A phenomenon independent of micromanipulation. *Human Reproduction*, 16, 1264–1269.
- Schieve, L. A., Meikle, S. F., Peterson, H. B., Jeng, G., Burnett, N. M., & Wilcox, L.S. (2000). Does assisted hatching pose a risk for monozygotic twinning in pregnancies conceived through in vitro fertilization. *Fertility and Sterility*, 74, 288–294.
- Sheiner, E., Har-Vardi, I., & Potashnik, G. (2001). The potential association between blastocyst transfer and monozygotic twinning. *Fertility and Sterility*, 75, 217–218.
- Shibuya, Y., & Kyono, K. (2012). A successful birth of healthy monozygotic dichorionic diamniotic (DD) twins of the same gender following a single vitrified-warmed blastocyst transfer. *Journal of Assisted Reproduction and Genetics*, 29, 255–257.
- Sills, E. S., Moomjy, M., Naninovic, N., Veeck, L., McGee, M., Palermo, G. D., & Rosenwaks, Z. (2000). Human zona pellucida micromanipulation and monozygotic twinning frequency after IVF. *Human Reproduction*, 15, 890–895.
- Skiadas, C., Missmer, S. A., Benson, C. B., Gee, R. E., & Racowsky, C. (2008). Risk factors associated with pregnancies containing a monochorionic pair following assisted

- reproductive technologies. *Human Reproduction*, 23, 1366–1371.
- Slotnik, R. N., & Ortega, J. E. (1996). Monoamniotic twinning and zona manipulation: A survey of U.S. IVF centers correlating zona manipulation procedures and high-risk twinning frequency. *Journal of Assisted Reproduction and Genetics*, 13, 381–385.
- Souter, V. L., Kapur, R. P., Nyholt, D. R., Skogerboe, K., Myerson, D., Ton, C. C., . . . Glass, I. A. (2003). A report of dizygous monochorionic twins. *New England Journal of Medicine*, 349, 154–158.
- Sugawara, N., Hukuchi, H., Maeda, M., Komaba, R., & Araki, Y. (2010). Sex-discordant twins despite single embryo transfer: A report of two cases. *Reproductive Medicine and Biology*, 9, 162–172.
- Tarlatzis, B. C., Qublan, H., Sanopoulou, T., Zepiridis, L., Brimbizis, B., & Bontis, J. (2002). Increase in the monozygotic twinning rate after intracytoplasmic sperm injection and blastocyst stage embryo transfer. *Fertility and Sterility*, 77, 196–198.
- Tokunaga, M., Shibahara, H., Shimada, K., Hirano, Y., Suzuki, T., Takamizawa, S., . . . Suzuki, M. (2010). Monozygotic DD twins following single blastocyst transfer. *Journal of Mammalian Ova Research*, 27, S60.
- Tsunoda, Y., & McLaren, A. (1983). Effect of various procedures on the viability of mouse embryos containing half the normal number of blastomeres. *Journal of Reproduction and Fertility*, 69, 315–322.
- Van de Velde, H., Cauffman, G., Tournaye, H., Devroey, P., & Liebarers, I. (2008). The four blastomeres of a 4-cell stage human embryo are able to develop individually into blastocysts with inner cell mass and trophectoderm. *Human Reproduction*, 23, 1742–1747.
- Van der Hoorn, M. L., Helmerhorst, F., Claas, F., & Scherjon, S. (2011). Dizygotic twin pregnancy transfer of one embryo. *Fertility and Sterility*, 95, 805e1–805e3.
- Van Langendonck, A., Wyns, C., Godin, P. A., Toussaint-Demyelle, D., & Donnez, J. (2000). Atypical hatching of a human blastocyst leading to monozygotic twinning: A case report. *Fertility and Sterility*, 74, 1047–1050.
- Vitthala, S., Gelbaya, T. A., Brison, D. R., Fitzgerald, C. T., & Nardo, L. G. (2009). The risk of monozygotic twins after assisted reproductive technology: A systematic review and meta-analysis. *Human Reproduction Update*, 15, 45–55.
- Wenstrom, K. D., Syrop, C. H., Hammitt, D. G., & Van Voorhis, B. J. (1993). Increased risk of monozygotic twinning associated reproduction. *Fertility and Sterility*, 60, 510–514.
- Willadsen, S. M. (1979). A method for culture of micromanipulated sheep embryos and its use to produce monozygotic twins. *Nature*, 277, 298–300.
- Willadsen, S. M., Lehn-Jensen, H., Fehilly, C. B., & Newcomb, R. (1981). The production of monozygotic twins of preselected parentage by micromanipulation of non-surgically collected cow embryos. *Theriogenology*, 15, 23–29.
- Williams, C. A., Wallace, M. R., Drury, K. C., Kipersztok, S., Edwards, R. K., Williams, R. S., . . . Zori, R. T. (2004). Blood lymphocyte chimerism associated with IVF and monochorionic dizygous twinning. Case report. *Human Reproduction*, 12, 2816–2821.
- Wright, V., Schieve, L. A., Vahgratian, A., & Reynold, M. A. (2004). Monozygotic twinning associated with day 5 embryo transfer in pregnancies conceived after IVF. *Human Reproduction*, 19, 1831–1836.