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Factors Associated with Monozygosity in Assisted Reproductive Technology (ART) Pregnancies And Risk of Recurrence Using Linked Cycles

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Abstract

Objective—To evaluate factors associated with monozygosity (number of fetal heartbeats on early ultrasound greater than number of embryos transferred) and the risk of recurrence in subsequent pregnancies using a national ART database

Design—Historical cohort study

Setting—Clinic-based data

Patients—197,327 pregnancies (including 2,824 with evidence of monozygosity) from cycles reported to SART CORS between 2004 and 2010. Adjusted odds ratios and their 95% confidence intervals were computed from logistic regression models.

Interventions—None

Main Outcome Measures—Evidence of monozygosity.

Results—In the univariate analysis, the risk of MZ was increased with ovulation disorders, donor oocytes, GnRHA suppression, assisted hatching (AZH), and day 5–6 transfer, and decreased with higher FSH doses (< 3,000 IU). In the multivariate analysis, the risk of MZ was increased with GnRHA suppression, AZH, and decreased with ICSI and higher FSH dose. The interaction showed that although MZ was more likely with day 5–6 embryos, AZH had a minimal nonsignificant effect, whereas in day 2–3 embryos, AZH had a substantial significant effect. Only one woman had a recurrence of monozygosity in a subsequent ART pregnancy, which is consistent with randomness.

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Conclusions—The risk of MZ was higher with fresh day 5–6 embryos, donor oocytes, GnRHA suppression, lower FSH doses, and AZH (particularly with day 2–3 embryos).

Keywords

monozygosity; GnRHA suppression; blastocyst transfer; assisted hatching

Introduction

“The goal of infertility treatment is for each patient to have one healthy child at a time [1].” The challenges in achieving this goal vary by the factors and circumstances unique to each woman and her situation. Regardless of the choice of ART treatment parameters, the objective remains: maximizing the probability of pregnancy while minimizing the risk of a multiple gestation [1]. Although the incidence of multiples among ART pregnancies has declined by 10–15% over the past decade, depending on cycle type, multiple pregnancy continues to be an acknowledged undesirable outcome of ART, accounting for 30% of all ART live births in 2010 [2, 3]. In an effort to reduce the incidence of multiple pregnancies, the Society for Assisted Reproductive Technology and the American Society for Reproductive Medicine have, since 1998, issued guidelines on the number of embryos to transfer. These recommendations have been periodically revised (most recently in 2012) and adjusted downward as implantation rates have improved, and include advocating for elective single embryo transfer in good prognosis patients [3, 4].

With ART treatment, the primary risk factor for a dizygotic multiple birth outcome is the transfer of more than one embryo [5–7]. The risk factors for monozygotic twinning are less clear. Monozygosity, division of the embryo at an early stage of development, is estimated to occur in 0.42% of all births. The timing at which the embryo splits is a critical factor in subsequent placental development, with the risk of complications being related to the degree of placental sharing. When the embryo divides within four days after fertilization, the twins are dichorionic-diamniotic, indistinguishable from dizygotic twins resulting from two embryos. If the embryo divides between day four and day eight after fertilization, monochorionic-diamniotic twins result. With a split after day eight, the twins are monochorionic-monoamniotic. Conjoined twins result when the split occurs after day 12. The rate of fetal complications is similar in dizygotic and monozygotic twins; however monochorionic placentation poses additional risks, including vascular anastomoses and the potential for development of the twin-twin transfusion syndrome. This complication results in asymmetrical fetal growth and high rates of fetal mortality [8, 9]. In addition, monochorionic-monoamniotic twin pregnancies are at greatest risk for fetal death due to the potential for umbilical cord entanglement [10].

Since the 1980s, monozygotic twinning has been reported with ART [11, 12] and ovulation induction [13], at a frequency ranging from 1.2% to 8.9% [13–21]. Several factors have been implicated in the additional splitting of the embryo with ART, including mechanical manipulation of the zona pellucida, the use of intracytoplasmic sperm injection (ICSI), delayed day of embryo transfer, older maternal age, and GnRHA suppression. The zona pellucida is an acellular area of mucopolysaccharides and specific proteins surrounding the ovum. This layer forms a biological barrier which enables recognition of human sperm, as well as protecting the early cleavage stage embryo. Mechanical manipulation of the zona pellucida by ICSI or assisted hatching might stimulate division of the inner cell mass. Early studies strongly implicated assisted hatching [18, 22], with increased odds ratios ranging from 3.2 to 3.8, depending on the comparison group. A thinner zona pellucida has also been associated with monozygosity with in vitro fertilization [15]; in spontaneous conceptions, older maternal age is associated with monozygosity. Thinner zona pellucida and older

maternal age may be related, with both leading to higher monozygotic rates. GnRHA down-regulation (suppression protocol) has also been associated with monozygosity [15]. Delayed transfer of the embryo at the blastocyst stage (day 5–6) compared to cleavage stage (day 3) may further increase monozygosity due to continued exposure and manipulation of the zona pellucida [23–28]. An acknowledged limitation of prior studies is the extremely low incidence of monozygotic twinning, even after ART [29], particularly with small sample size [30–36]. The objective of this study is to evaluate factors associated with monozygosity and the risk of recurrence in subsequent pregnancies using a national ART database.

Materials and Methods

The data source for this study was the Society for Assisted Reproductive Technology Clinic Outcome Reporting System (SART CORS), which contains comprehensive data from more than 90% of all clinics performing ART in the US. Data were collected and verified by SART and reported to the Centers for Disease Control and Prevention in compliance with the Fertility Clinic Success Rate and Certification Act of 1992 (Public Law 102–493). The data in the SART CORS are validated annually [2]. Clinic site visits for validation of 2010 ART data were conducted from April through June 2012. During each visit, data reported by the clinic were compared with information recorded in patients' charts. Records for 2,070 cycles at 35 clinics were randomly selected for full validation, along with 135 embryo banking cycles (www.cdc.gov/art2010). The full validation included review of 1,352 cycles for which a pregnancy was reported; of which 446 were multiple-fetus pregnancies. Nine out of 10 data fields selected for validation were found to have discrepancy rates of 3%, including <1% for number of embryos transferred and 2.2% for number of fetal heartbeats on early ultrasound.

The data for this study included cycles reported to the SART CORS between 2004 and 2010, linked by the woman's birth date, first and last names, and social security number (when present). Linkages across clinics also included partner's name and the sequence of ART outcomes. Identifying variables (including names, dates, and social security numbers) were removed and the de-identified analytic file generated. The data were limited to cycles with one or two embryos transferred and one to four fetal heartbeats (FHBs) identified on early ultrasound. Four separate models were developed based on embryo state and number of embryos transferred: 1) fresh-one embryo; 2) fresh-two embryos; 3) frozen-one embryo; and 4) frozen-two embryos. Excluded were cycles designated as research or embryo banking, or which used gestational carriers. The dependent variable was the presence of FHB> number of embryos transferred, as documented on the 4–6 week ultrasound after embryo transfer. Based on prior literature, eight factors were evaluated for their association with monozygosity: year of ART cycle (prior to 2007 versus 2007 and later), diagnosis of ovulation disorders, donor oocytes, GnRHA suppression protocol, assisted hatching (AZH: all or some versus none), intracytoplasmic sperm injection (ICSI, all or some versus none), blastocyst-stage transfer (versus cleavage-stage), and FSH dose (<2000 IU, 2000–2999 IU, and 3000 IU). For frozen cycles, the use of ICSI, day of transfer, and donor stimulation protocol were not available in the SART CORS database and therefore could not be included in these models. The univariate analysis for each model adjusted for woman's age, race and ethnicity, and gravidity; the multivariate analysis, using backwards stepping, adjusted for woman's age, race and ethnicity, gravidity, and all other factors in the model.

Starting in 2007 morphologic measures of each embryo transferred were recorded in the SART CORS database. Models were also fit that included embryo stage and grade, but restricted to cases from 2007 and later (i.e., approximately 65% of cases used in the prior models). Since both the evaluation of grade and stage depend on the day of transfer, the interactions were also included in the modeling.

Adjusted odds ratios (AORs) and their 95% confidence intervals (CI) were computed from the four logistic regression models and adjusted for woman's age (<30, 30–34, 35–9, 40–44, and 45), race and ethnicity (white, Asian, Hispanic, black, other and mixed, and unknown), gravidity, diagnosis of ovulation disorders, oocyte source (autologous versus donor), the use of GnRHA suppression, ICSI, and AZH. The study was approved by the Committees for the Protection of Human Subjects at Dartmouth College and Michigan State University, respectively, and was analyzed using SAS 9.2 software (Cary, NC).

Results

The study population included 197,327 pregnancies among 238,266 women, including 2,824 pregnancies with evidence of monozygosity (FHB>embryos transferred), for an overall rate of 1.4%. The distribution of the study population by number of embryos transferred and number of fetal heartbeats is shown in Table 1. The description of the study population by embryo state and number of embryos transferred is shown in Table 2, and by morphologic stage and grade for day 2–3 and day 5–6 embryos in Table 3. The group with two fresh embryos transferred accounted for 71% of the study population, frozen transfers of two embryos accounted for 15%, and the groups with fresh or frozen embryos in which one embryo was transferred included 9% and 4%, respectively. With one embryo transferred, the rate of monozygosity was 2.15% (2.4% for fresh and 1.7% for frozen); with two embryos transferred, the rate was 1.32% (1.4% for fresh and 0.9% for frozen). Only one woman (out of 238,266 women) had a recurrence of monozygosity in a subsequent ART pregnancy, which is consistent with randomness.

The results of the models are shown in Table 4. In the univariate analysis, the risk of monozygosity was increased with ovulation disorders (AOR 1.22 to 1.30), donor oocytes (AOR 1.25 to 1.32), GnRHA suppression (AOR 1.09), AZH (AOR 1.50), and day 5–6 transfer (AOR 1.59 to 2.33), and decreased with higher FSH doses (< 3,000 IU, AOR 0.68). In the multivariate analysis, the risk of monozygosity was increased with suppression protocol (AOR 1.11) and AZH (AOR 1.50), and decreased with ICSI (AOR 0.89) and higher FSH dose (< 3,000 IU, AOR 0.70). The interaction showed that although monozygosity was more likely with day 5–6 embryos, assisted hatching had a minimal nonsignificant effect (AORs 1.77 to 3.29 with AZH and 2.43 to 3.36 without AZH), whereas in day 2–3 embryos, AZH had a substantial significant effect (AOR (AORs 2.05 to 2.48 with AZH and 1.00 without AZH).

When the models included morphologic stage, there was a reduced probability of monozygosity when the transfer was on days 2–3 compared to expanded blastocyst on days 5–6 (AORs 0.66–0.72 for one embryo transferred and 0.28–0.56 for two embryos transferred). Similarly, when the models included morphologic grade, there was a reduced probability of monozygosity on days 2–3 compared to a grade of good on days 5–6 (AORs 0.59–0.71 for one embryo transferred and 0.28–0.43 for two embryos transferred). These differences were primarily associated with day of transfer rather than stage or grade within day of transfer, although earlier stages had lower AORs than later stages within the same day of transfer. When both stage and grade were entered simultaneously, the effect of grade became nonsignificant (data not shown).

Discussion

These findings indicate that the risk of monozygosity after ART is elevated by several factors, including ovulation disorders, donor oocytes, fresh embryos, GnRHA suppression, assisted hatching, and blastocyst transfer. Differences in monozygosity with two embryos transferred may be an artifact of a higher rate of embryo loss; additional embryo splits may

be hidden by corresponding embryo losses. Therefore, the rate estimated when one embryo is transferred provides a lower bound for monozygosity with ART. Our findings confirm previous reports that monozygotic twinning is associated with assisted hatching [15, 22, 26] and blastocyst transfer [23–28].

Birthweight and length of gestation discrepancies have been reported in ART versus spontaneous conceptions, even when limited to singleton pregnancies [37–40]. An estimated half of this difference may be determined by factors present during the first trimester [41, 42]. Some of this excess risk reflects the residual effect of fetal loss, and is directly proportional to the starting number of fetuses [43–48]. Bukowski et al [42] demonstrated that smaller-than-expected fetal size before 12 weeks gestation with in vitro fertilization or intrauterine insemination ART pregnancies was significantly associated with reduced length of gestation, in proportion to the deficit. In twin gestations, this deficit in first trimester size, as smaller-than-expected crown-rump length, is associated with significantly greater incidences of structural or chromosomal anomalies [49], growth restriction and perinatal morbidity [50], and prematurity [51–53].

Fetal loss, or degenerating implantation sites, may in turn adversely affect placentation and subsequent risks for adverse pregnancy outcomes [54, 55]. Twins conceived with ART have significantly higher rates of marginal or velamentous cord insertions, and single umbilical arteries compared to twins conceived spontaneously [55]. Additionally, a recent meta-analysis of nine studies reported that compared to dichorionic twin pregnancies, monochorionic twin pregnancies have a threefold greater risk for stillbirth throughout the third trimester [56].

We can only speculate on the mechanisms by which clinical factors resulted in the monozygosity seen in this study. Of the factors that were associated with monozygotic twinning, ovulation disorders, use of assisted hatching, use of greater amounts of FSH, and transfer of two versus one embryo suggest the possibility of monozygosity being associated with embryos of compromised morphologic quality. Ovulation disorders, such as polycystic ovarian syndrome, often result in a large number of embryos, but these embryos have a propensity to poor quality [57]. Similarly, assisted hatching is more frequently clinically recommended for patients with poor quality embryos including those of older patients, patients with fragmented embryos or prior fertilization failure, and others with zona pellucida abnormalities [58]. Transfer of two rather than one embryo may also be more frequent in situations where embryo morphology is less than optimal. Since compromised morphology might include more than appearance and be related as well to a greater likelihood of genetic abnormality [59] it is possible this could also correlate with monozygosity.

Assisted hatching has previously been suggested as a cause of monozygotic twinning, although this observation has not been seen consistently and may depend on laboratory technique and method of hatching [58]. Theoretically, twinning could result from an embryo being squeezed through a hole in the zona pellucida, particularly if that hole was too small to allow the embryo to hatch appropriately. There are numerous methods for assisted hatching, including use of acid Tyrode's solution, laser assisted hatching, and partial zona dissection; the risk of twinning may differ by method [58]. Unfortunately, the SART CORS database does not contain any information on the method used for hatching or size of the hole generated, nor does it contain any information on the experience of the embryologists performing the procedures. A recent analysis by Knopman et al [60] reported a difference in chorionicity by day of embryo transfer with assisted hatching, with an excess of dichorionic placentation with day 3 embryos and more monochorionic placentation with day 5 embryos.

By contrast to the above and in agreement with prior reports [23], there was an increased rate of monozygotic twinning with blastocyst transfer which is generally thought to be correlated with better embryo quality. The cause of increased twinning in this situation could relate to blastocyst stage embryos being more sensitive to the effects of mechanical manipulation in the laboratory or transient changes in temperature or pH during monitoring or embryo transfer. This mechanism, therefore, might be completely different from a mechanism related to poor quality embryos.

Limitations

The SART CORS is a clinical and surveillance database, and its use for research is secondary. Although a sample of the data is validated yearly, it is still possible that some data is entered incorrectly, including number of embryos transferred and the number of fetal heartbeats on early ultrasound. In addition, from this database, it cannot be determined if embryo splitting and embryo loss occurred prior to the early ultrasound, nor is it possible to differentiate the number of gestational sacs on early ultrasound, only number of fetal heartbeats. As a related issue, this database does not contain information on zygosity or chorionicity. Lastly, this database also does not permit evaluation of a spontaneous conception after an ART conception, which would be an alternative explanation for FHB > number of embryos transferred. Spontaneous conception could take place if oocytes that remain in the ovary following retrieval are subsequently ovulated and fertilized. This scenario is theoretically likely, particularly in women with polycystic ovarian syndrome, who often have large numbers of eggs following ovarian stimulation.

Several studies have attempted to determine chorionicity and zygosity prospectively in women treated with ART, albeit with substantial attrition as gestation progressed [26, 28, 33, 34]. The ideal prospective study design to evaluate the risk of monozygosity after ART would be to monitor women after embryo transfer with daily ultrasound examinations through 10–12 weeks gestation, as well as tracking frequency of intercourse (to account for spontaneous conceptions). Among those pregnancies with number of FHB > number of embryos transferred, to subsequently 1) determine chorionicity during the first trimester, 2) perform pathology evaluation of the placenta(s) at birth to confirm placental structure and the absence/presence of fetal losses, and 3) perform HLA typing of the surviving infants for confirmation of zygosity.

Conclusions

The use of fresh embryos, AZH, and transferring at day 5–6 is associated with an increased risk of monozygosity. The effect of AZH on the risk of monozygosity is greater with day 2–3 embryos compared to day 5–6 embryos. The lower rates of splitting with more embryos are transferred may be an artifact of the higher rate of embryo loss; additional embryo splits may be hidden by corresponding embryo losses. Therefore, the rate estimated when one embryo is transferred provides a lower bound for monozygosity with ART.

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References

1. Practice Committee of the American Society for Reproductive Medicine. Multiple gestation associated with infertility therapy: an American Society for Reproductive Medicine practice committee opinion. *Fertility and Sterility*. 2012; 97:825–834. [PubMed: 22192352]

2. Center for Disease Control and Prevention. 2010 Assisted Reproductive Technology Success Rates: National Summary and Fertility Clinic Reports. Washington, DC: US Dept. of Health and Human Services; 2012. American Society for Reproductive Medicine, and Society for Assisted Reproductive Technology.
3. Practice Committee of the American Society for Reproductive Medicine. Criteria for number of embryos to transfer: A committee opinion. *Fertility and Sterility*. 2013; 99:44–46. [PubMed: 23095140]
4. Practice Committee of the American Society for Reproductive Medicine. Elective single-embryo transfer. *Fertility and Sterility*. 2012; 97:835–842. [PubMed: 22196716]
5. Templeton A, Morris JK. Reducing the risk of multiple births by transfer of two embryos after in vitro fertilization. *New Engl J Med*. 1998; 339:573–577. [PubMed: 9718375]
6. Jain T, Missmer SA, Hornstein MD. Trends in embryo-transfer practice and in outcomes of the use of assisted reproductive technology in the United States. *New Engl J Med*. 2004; 16:1639–1645. [PubMed: 15084696]
7. Luke B, Brown MB, Grainger DA, Cedars M, Klein N, Stern JE. Practice patterns and outcomes with the use of single embryo transfer in the United States. *Fertil Steril*. 2010; 93:490–498. [PubMed: 19376512]
8. Fieni S, Gramellini D, Piantelli G, Verrotti C, Cavallotti D. Twin-twin transfusion syndrome: A review of treatment options. *Acta Bio-Medica*. 2004; 75:34–39. [PubMed: 15301288]
9. Harkness UF, Crombleholme TM. Twin-transfusion syndrome: Where do we go from here? *Sem Perinatol*. 2005; 29:296–304.
10. Newman, RB. Chapter 8, Obstetrical complications unique to multiple gestations. In: Newman, RB.; Luke, B., editors. *Multifetal Pregnancy: A Handbook for Care of the Pregnant Patient*. Philadelphia: Lippincott Williams & Wilkins; 2000. p. 149-172.
11. Yovich JL, Stanger JD, Grauaug A, Barter RA, Lunay G, Dawkins RL, et al. Monozygotic twins from in vitro fertilization. *Fertility and Sterility*. 1984; 41:833–837. [PubMed: 6539252]
12. Edwards RG, Mettler L, Walters DE. Identical twins and in vitro fertilization. *J in Vitro Fertil Embryo Transfer*. 1986; 3:114–117.
13. Derom C, Vlietinck R, Derom R, Van den Berghe H, Thiery M. Increased monozygotic twinning rate after ovulation induction. *Lancet*. 1987; 1:1236–1238. [PubMed: 2884372]
14. Avrech O, Schoenfeld A, Amit S, Ovadia J, Fisch B. Dizygotic triplet following in-vitro fertilization. *Human Reproduction*. 1993; 8:2240–2242. [PubMed: 8150931]
15. Alikani M, Noyes N, Cohen J, Rosenwaks Z. Monozygotic twinning in the human is associated with the zona pellucida architecture. *Hum Reprod*. 1994; 9:1318–1321. [PubMed: 7962440]
16. Belaisch-Allart J, Elaoufir A, Mayenga JM, Segard L, Bernard JP, Plachot M, Mandelbaum J, Cohen J, Labbé F. Monozygotic triplet pregnancy following transfer of frozen-thawed embryos. *Human Reproduction*. 1995; 10:3064–3066. [PubMed: 8747075]
17. Biljan MM, Hewitt J, Kingsland CR, Taylor CT. Trizygotic quadruplet pregnancy following in-vitro fertilization: An additional factor against replacement of three embryos in young patients? *Human Reproduction*. 1995; 10:2169–2170. [PubMed: 8567861]
18. Hershlag A, Paine T, Cooper GW, Scholl GM, Rawlinson K, Kvapil G. Monozygotic twinning associated with mechanical assisted hatching. *Fertility and Sterility*. 1999; 71:144–146. [PubMed: 9935131]
19. Sills ES, Tucker MJ, Palermo GD. Assisted reproductive technologies and monozygous twins: Implications for future study and clinical practice. *Twin Research*. 2000; 3:217–223. [PubMed: 11463142]
20. Abusheika N, Salha O, Sharma V, Brinsden P. Monozygotic twinning and IVF/ICSI treatment: A report of 11 cases and review of literature. *Hum Reprod Update*. 2000; 6:396–403. [PubMed: 10972526]
21. Sheiner E, Har-Vardi I, Potashnik G. The potential association between blastocyst transfer and monozygotic twinning. *Fertil Steril*. 2001; 75:217–218. [PubMed: 11163843]
22. Schieve LA, Meikle SF, Peterson HB, Jeng G, Burnett NM, Wilcox LS. Does assisted hatching pose a risk for monozygotic twinning in pregnancies conceived through in vitro fertilization? *Fertility and Sterility*. 2000; 74:288–294. [PubMed: 10927046]

23. Milki AA, Jun SH, Hinckley MD, Behr B, Giudice LC, Westphal LM. Incidence of monozygotic twinning with blastocyst transfer compared to cleavage-stage transfer. *Fertil Steril*. 2003; 79:503–506. [PubMed: 12620430]
24. Jain JK, Boostanfar R, Slater CC, Francis MM, Paulson RJ. Monozygotic twins and triplets in association with blastocyst transfer. *J Assisted Reprod Genet*. 2004; 21:103–107.
25. Wright V, Schieve LA, Vahratian A, Reynolds MA. Monozygotic twinning associated with day 5 embryo transfer in pregnancies conceived after IVF. *Hum Reprod*. 2004; 19:1831–1836. [PubMed: 15192064]
26. Skiadas CC, Missmer SA, Benson CB, Gee RE, Racowsky C. Risk factors associated with pregnancies containing a monochorionic pair following assisted reproductive technologies. *Hum Reprod*. 2008; 23:1366–1371. [PubMed: 18378561]
27. Chang HJ, Lee JR, Jee BC, Suh CS, Kim SH. Impact of blastocyst transfer on offspring sex ratio and the monozygotic twinning rate: A systematic review and meta-analysis. *Fertil Steril*. 2009; 91:2381–2390. [PubMed: 18718582]
28. Knopman J, Krey LC, Lee J, Fino E, Novetsky AP, Noyes N. Monozygotic twinning: An eight-year experience at a large IVF center. *Fertil Steril*. 2010; 94:502–510. [PubMed: 19409556]
29. Aston KI, Peterson CM, Carrell DT. Monozygotic twinning associated with assisted reproductive technologies: A review. *Reproduction*. 2008; 136:377–386. [PubMed: 18577552]
30. Schachter M, Raziel A, Friedler S, Strassburger D, Bern O, Ron-El R. Monozygotic twinning after assisted reproductive techniques: A phenomenon independent of micromanipulation. *Hum Reprod*. 2001; 16:1264–1269. [PubMed: 11387303]
31. Sharara FI, Abdo G. Incidence of monozygotic twins in blastocyst and cleavage stage assisted reproductive technology cycles. *Fertil Steril*. 2010; 93:642–645. [PubMed: 19217097]
32. Papanikolaou EG, Fatemi H, Venetis C, Donoso P, Kolibianakis E, Tournaye H, Tarlatzis B, Devroey P. Monozygotic twinning is not increased after single blastocyst transfer compared with single cleavage-stage embryo transfer. *Fertil Steril*. 2010; 93:592–597. [PubMed: 19243755]
33. Alikani M, Cekleniak NA, Walters E, Cohen J. Monozygotic twinning following assisted conception: An analysis of 81 consecutive cases. *Hum Reprod*. 2003; 18:1937–1943. [PubMed: 12923153]
34. Sills ES, Moomjy M, Zaninovic N, Veeck LL, McGee M, Palermo GD, Rosenwaks Z. Human zona pellucida micromanipulation and monozygotic twinning frequency after IVF. *Hum Reprod*. 2000; 15:890–895. [PubMed: 10739838]
35. Li L, Long X, Du H, Zhang W, Shi Y. Incidence and potential causes affecting monozygotic twin formation following in vitro fertilization and embryo transfer. *J Reprod Contraception*. 2012; 24:93–101.
36. Moayeri SE, Behr B, Lathi RB, Westphal LM, Milki AA. Risk of monozygotic twinning with blastocyst transfer decreases over time: An 8-year experience. *Fertil Steril*. 2007; 87:1028–1032. [PubMed: 17343858]
37. Dhont M, De Sutter P, Ruyssink G, et al. Perinatal outcome of pregnancies after assisted reproduction: A case-control study. *Am J Obstet Gynecol*. 1999; 181:688–695. [PubMed: 10486485]
38. Schieve LA, Meikle SF, Ferre C, Peterson HB, Jeng G, Wilcox LS. Low and very low birth weight in infants conceived with use of assisted reproductive technology. *N Engl J Med*. 2002; 346:731–737. [PubMed: 11882728]
39. Helmerhorst FM, Perquin DAM, Donker D, et al. Perinatal outcome of singletons and twins after assisted conception: A systematic review of controlled studies. *BMJ*. 2004; 328:261–265. [PubMed: 14742347]
40. Wang YA, Sullivan EA, Black D, et al. Preterm birth and low birthweight after assisted reproductive technology-related pregnancy in Australia between 1996 and 2000. *Fertil Steril*. 2005; 83:1650–1658. [PubMed: 15950632]
41. Smith GCS, Smith MFS, McNay FD, et al. First-trimester growth and the risk of low birthweight. *N Engl J Med*. 1998; 339:1817–1822. [PubMed: 9854117]
42. Bukowski R, Smith GCS, Malone FD, et al. Fetal growth in early pregnancy and risk of delivering low birthweight infant: Prospective cohort study. *BMJ*. 2007; 334:836–840. [PubMed: 17355993]

43. Dickey RP, Taylor SN, Lu PY, et al. Spontaneous reduction of multiple pregnancy: Incidence and effect on outcome. *Am J Obstet Gynecol.* 2002; 186:77–83. [PubMed: 11810089]
44. Pinborg A, Lidegaard Ø, la Cour Freiesleben N, et al. Vanishing twins: A predictor of small-for-gestational age in IVF singletons. *Hum Reprod.* 2007; 22:2707–2714. [PubMed: 17728356]
45. Shebl O, Ebner T, Sommergruber M, et al. Birthweight is lower for survivors of the vanishing twin syndrome: A case-control study. *Fertil Steril.* 2008; 90:310–314. [PubMed: 17931633]
46. Luke B, Brown MB, Grainger DA, et al. The effect of early fetal losses on singleton assisted-conception pregnancy outcomes. *Fertil Steril.* 2009; 91:2578–2585. [PubMed: 18565521]
47. Luke B, Brown MB, Grainger DA, et al. The effect of early fetal losses on twin assisted-conception pregnancy outcomes. *Fertil Steril.* 2009; 91:2586–2592. [PubMed: 18804206]
48. Luke B, Brown MB, Nugent C, Gonzalez-Quintero VH, Witter FR, Newman RB. Risk factors for adverse outcomes in spontaneous versus assisted conception twin pregnancies. *Fertil Steril.* 2004; 81:315–319. [PubMed: 14967366]
49. Kalish RB, Gupta M, Perni SC, Berman S, Chasen ST. Clinical significance of first trimester crown-rump length disparity in dichorionic twin gestations. *Am J Obstet Gynecol.* 2004; 191:1437–1440. [PubMed: 15507979]
50. Tai J, Grobman WA. The association of crown-rump length discordance in twin gestations with adverse perinatal outcomes. *Am J Obstet Gynecol.* 2007; 197:369.e1–369.e4. [PubMed: 17904963]
51. Hediger ML, Luke B, Grainger D, Misiunas R, Anderson E, Witter F, Mauldin J, Newman R, Nugent C, O'Sullivan MJ, Hankins G, D'Alton M. Suboptimal first-trimester growth and very preterm delivery of twins. *Am J Obstet Gynecol.* 2004; 191:S65.
52. Hediger ML, Luke B, Gonzalez-Quintero VH, Martin D, Nugent C, Witter FR, Mauldin JG, Newman RB. Fetal growth rates and the very preterm delivery of twins. *Am J Obstet Gynecol.* 2005; 193:1498–1507. [PubMed: 16202746]
53. Hediger ML, Luke B, Grainger DA, Witter F, Newman R, Gonzalez-Quintero VH. Twin intrapair crown-rump length discordancy and risk of very preterm birth. *Am J Obstet Gynecol.* 2006; 195:S57.
54. Luke B, Brown MB, Stern JE, Grainger DA, Klein N, Cedars M. Effect of embryo transfer number on singleton and twin implantation pregnancy outcomes after assisted reproductive technology. *J Reprod Med.* 2010; 55:387–394. [PubMed: 21043364]
55. Delbaere I, Goetgheuk S, Derom C, Bacquer D, De Sutter P, Temmerman M. Umbilical cord anomalies are more frequent in twins after assisted reproduction. *Hum Reprod.* 2007; 22:2763–2767. [PubMed: 17720701]
56. Danon D, Sekar R, Hack KEA, Fisk NM. Increased stillbirth in uncomplicated monochorionic twin pregnancies. *Obstet Gynecol.* 2013; 121:1318–1326. [PubMed: 23812469]
57. Qiao J, Feng HL. Extra- and intra-ovarian factors in polycystic ovary syndrome: impact on oocyte maturation and embryo developmental competence. *Human Reproduction Update.* 2010; 17:17–33. [PubMed: 20639519]
58. Wells D, Bermúdez MG, Steuerwald N, Malter HE, Thornhill AR, Cohen J. Association of abnormal morphology and altered gene expression in human preimplantation embryos. *Fertil Steril.* 2005; 84:343–355. [PubMed: 16084875]
59. Hammadeh ME, Fischer-Hammadeh C, Ali KR. Assisted hatching in assisted reproduction: a state of the art. *J Assist Reprod Genet.* 2011; 28:119–128. [PubMed: 21042844]
60. Knopman JM, Krey LC, Oh C, Lee J, McCaffrey C, Noyes N. What makes them split? Identifying risk factors that lead to monozygotic twins after in vitro fertilization. *Fertil Steril.* 2013

Table 1

Distribution of Study Population by Number of Embryos Transferred and Number of Fetal Heartbeats

Row Numbers		Total	Number of Fetal Heartbeats				Overall
			1	2	3	4	% Split
Number of Embryos Transferred		1	25,697	541	11	1	2.15
		2	171,630	61,448	2,198	73	1.32
Row Percents		Total	1	2	3	4	% Split
Number of Embryos Transferred		1	100.00	2.10	0.04	0.004	2.15
		2	100.00	35.8	1.28	0.04	1.32

Cells with dark outlines represent pregnancies with evidence of monozygosity (number of fetal heartbeats > number of embryos transferred)

Table 2

Description of the Study Population

	Embryo State Number of Embryos Transferred	Fresh		Frozen	
		One Embryo	Two Embryos	One Embryo	Two Embryos
	(N, pregnancies)	(17,784)	(141,088)	(7,913)	(30,542)
	Evidence of monozygosity (%)	2.4	1.4	1.7	0.9
Year of Cycles (%)	Prior to 2007	24.1	35.4	21.3	29.0
Woman's Age (years, %)	<30	16.5	17.9	9.9	13.7
	30–34	37.8	39.1	33.1	36.0
	35–39	28.3	27.9	35.8	30.8
	40–44	13.1	10.9	15.1	13.2
	45	4.3	4.1	6.0	6.4
Woman's Race/Ethnicity (%)	White	47.4	49.3	44.1	46.4
	Asian	3.1	3.4	2.7	3.8
	Black	6.9	5.5	9.3	6.2
	Hispanic	3.0	4.0	3.1	3.7
	Unknown	39.6	37.9	40.8	39.8
Gravidity (%)	Nulligravida	44.6	49.0	21.5	28.5
Oocyte Source (%)	Donor	15.3	18.5	16.9	20.3
Diagnosis (%)	Ovulation Disorder	16.9	16.7	17.2	19.3
ICSI (%)	All or Some	66.5	69.2	8.3	15.5
AZH (%)	All or Some	24.4	23.4	43.5	44.2
FSH dose (%)	750–1999	28.3	26.4	---	---
	2000–2999	21.4	24.5	---	---
	3000–8550	29.3	26.5	---	---
	No FSH Dose or Unrecorded	21.0	22.5	---	---
Day of Embryo Transfer (%)	Day 2–3	33.0	44.4	---	---
	Day 5–6	67.0	55.6	---	---
Autologous Cycles	(N)	(15,061)	(115,023)	(6,574)	(24,327)

	Embryo State	Fresh		Frozen	
		One Embryo	Two Embryos	One Embryo	Two Embryos
	Number of Embryos Transferred				
	Stimulation Protocol (%)				
	Unstimulated	7.8	8.8	54.7	50.2
	GnRHA Flare	9.0	6.5	0.6	1.0
	GnRHA Suppression	55.4	59.7	23.2	29.7
Donor Cycles	(N)	(2,723)	(26,065)	(1,339)	(6,215)
	Stimulation Protocol (%)				
	Unstimulated	0.5	0.6	0.3	0.9
	GnRHA Flare	1.7	1.7	---	---
	GnRHA Suppression	61.3	61.8	---	---

Table 3

Percent Distribution of Fresh Day 2–3 and Day 5–6 Embryos by Morphologic Stage and Grade

			Number of Embryos Transferred	
			One Embryo	Two Embryos
		(N)	(13,493)	(91,112)
Stage	Day 2–3	1–6 cells	6.2	3.3
		7–8+ cells	17.3	28.2
		Morula or Blastocyst	0.4	0.6
		Missing	4.7	7.9
	Day 5–6	1–8+ cells	12.0	7.2
		Morula	1.1	0.7
		Early Blastocyst	5.7	5.2
		Expanded Blastocyst	30.4	26.4
		Hatched Blastocyst	12.2	7.8
		Missing	10.0	12.7
		Total	100.0	100.0
Grade	Day 2–3	Good	14.6	24.3
		Fair	6.1	4.2
		Poor	0.6	0.4
		Missing	7.3	11.2
	Day 5–6	Good	43.1	34.3
		Fair	6.5	5.9
		Poor	0.6	0.4
		Missing	21.3	19.4
		Total	100.0	100.0

Table 4

Factors Associated with Monozygosity (Adjusted Odds Ratios and 95% CIs)

Model 1 *	Fresh, One Embryo			Fresh, Two Embryos			Frozen, One Embryo			Frozen, Two Embryos		
	AOR	95% CI	P Value	AOR	95% CI	P Value	AOR	95% CI	P Value	AOR	95% CI	P Value
Year of Cycle 2007	1.00	Reference	0.09	1.00	Reference	<0.0001	1.00	Reference	0.88	1.00	Reference	0.28
2006	0.81	0.64 1.03		0.83	0.75 0.91		0.97	0.63 1.49		0.86	0.65 1.13	
Ovulation Disorders	1.30	1.02 1.67	0.03	1.22	1.09 1.36	0.0006	1.01	0.64 1.59	0.97	1.15	0.86 1.55	0.36
Donor Oocytes	1.25	0.88 1.78	0.22	1.32	1.13 1.54	0.0004	0.91	0.47 1.75	0.77	1.32	0.88 1.97	0.18
Suppression Protocol (Autologous)	1.21	0.99 1.48	0.07	1.09	0.99 1.20	0.07	0.77	0.49 1.19	0.23	1.08	0.83 1.41	0.57
AZH (None)	1.00	Reference	0.41	1.00	Reference	0.47	1.00	Reference	0.49	1.00	Reference	0.001
AZH (All/Some)	0.91	0.72 1.15		1.04	0.94 1.15		1.13	0.79 1.62		1.50	1.17 1.91	
ICSI (None)	1.00	Reference	0.07	1.00	Reference	0.38						
ICSI (All/Some)	0.83	0.68 1.02		0.96	0.87 1.06							
Day of Transfer Day 2-3	1.00	Reference	<0.0001	1.00	Reference	<0.0001						
Day of Transfer Day 5-6	1.59	1.27 2.00		2.33	2.08 2.56							
FSH Dose <2000	1.00	Reference		1.00	Reference							
FSH Dose 2000-2999	0.90	0.69 1.17	0.02	0.84	0.74 0.95	<0.0001						
FSH Dose 3000	0.64	0.49 0.85		0.68	0.60 0.78							
No FSH Dose or Unrecorded	0.81	0.58 1.13		1.07	0.92 1.23							
Model 2 **												
Year of Cycle 2007				1.00	Reference	0.01						
2006				0.88	0.80 0.97							
Suppression Protocol (Autologous)				1.10	1.00 1.22	0.04						
AZH (None)										1.00	Reference	0.001
AZH (All/Some)										1.50	1.17 1.91	
AZH (None) on Day 2-3	1.00	Reference		1.00	Reference							
AZH (All/Some) on Day 2-3	2.05	1.37 3.08	<0.0001	2.47	2.07 2.94							
AZH (None) on Day 5-6	2.43	1.73 3.41		3.36	2.92 3.87							
AZH (All/Some) on Day 5-6	1.77	1.11 2.85		3.29	2.71 3.99							

	Fresh, One Embryo			Fresh, Two Embryos			Frozen, One Embryo			Frozen, Two Embryos		
	AOR	95% CI	P Value	AOR	95% CI	P Value	AOR	95% CI	P Value	AOR	95% CI	P Value
Model 1 *												
ICSI (None)	1.00	Reference		1.00	Reference							
ICSI (All/Some)	0.81	0.66 0.99	0.04	0.89	0.81 0.98	0.02						
FSH Dose <2000 IU				1.00	Reference							
FSH Dose 2000–2999 IU				0.83	0.74 0.94	<0.0001						
FSH Dose 3000 IU				0.70	0.62 0.80							
No FSH Dose or Unrecorded				1.07	0.92 1.24							

* Model 1: univariate tests, adjusted for woman's age, race and ethnicity, and gravidity

** Model 2: multivariate model after backwards stepping, adjusted for woman's age, race and ethnicity, gravidity, and all other factors in the model