Estimating the Risk of Monochorionic Twins in IVF Pregnancies From the Perspective of a Prenatal Diagnosis Unit

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The aim of the present work was to estimate the risk of monochorionic twin (MCT) pregnancies in in vitro fertilization (IVF) cycles using data from a prenatal diagnosis unit. This was a retrospective cross-sectional study reporting on the frequency of IVF pregnancies among women attending a prenatal diagnosis service specifically dedicated to the management of monochorionic pregnancies. The observed rate was compared with the local regional rate of IVF births (2.2%). A binomial distribution model was used to calculate the 95% CI of proportions. One hundred and forty-five monochorionic pregnancies were selected. Ten of these were achieved with IVF, corresponding to a rate of 6.9% (95% CI: 3.5–11.8), significantly higher than the background rate in the local population of 2.2%. When considering exclusively monochorionic pregnancies achieving delivery of two viable newborns ($n = 132$), the number of IVF pregnancies was nine (6.8%, 95% CI: 3.7–12.5). We did not detect major differences in pregnancy outcome between IVF and natural monochorionic pregnancies, with the exception of the proportion of newborns with a neonatal birth $< 2,500$ g (100% vs. 80%, $p = .03$). In conclusion, data obtained from the perspective of a prenatal diagnosis unit suggest that women undergoing IVF face a 3- to 4-fold increased risk of monochorionic pregnancies.

∎ **Keywords:** IVF, in vitro fertilization, monozygotic, monochorionic, twin

Twin pregnancy is a well-known and threatening complication of IVF cycles (Kulkarni et al., 2013). During the past decade, intensive efforts have been made to reduce the rate of twin pregnancies worldwide by boosting policies of elective single embryo transfer. Even though additional efforts are needed, several countries have now shrunk the rate of twin pregnancies to less than 10% (Bhattacharya & Kamath, 2014).

In line with this current attempt of the scientific and medical communities to minimize the complications of IVF in general and the risk of twins in particular, there is now a growing interest in the association between IVF and the occurrence of monozygotic twins (Delrieu et al., 2012; Franasiak et al., 2015; Gee et al., 2014; Kanter et al., 2015; Knopman et al., 2010, 2014; Luke et al., 2014; Nakasuji et al., 2014; Osianlis et al., 2014; Ren et al., 2013; Tocino et al., 2015; Vitthala et al., 2009). This aspect deserves the utmost consideration because the clinical management of monozygotic twin pregnancies may be demanding (Corsello & Piro, 2010; Sperling et al., 2006). Of note, 75% of these pregnancies are MCT (Shulman and van Vugt, 2006), a condition associated with major obstetrical complications.

Even if monozygotic twin pregnancies are generally deemed to be increased in IVF pregnancies (Delrieu et al., 2012; Hall, 2003; Vitthala et al., 2009), the available evidence is still not fully consistent and estimates of the magnitude of this risk vary widely in the literature. While the rate of monozygotic twins in natural conceptions has been reported to be approximately 0.4% (Gee et al., 2014; Hall, 2003; Imaizumi & Nonaka, 1997), the rates reported in IVF pregnancies vary between 0.7 and 13% (Delrieu et al., 2012; Gee et al., 2014; Knopman et al., 2010, 2014; Nakasuji et al., 2014; Osianlis et al., 2014; Ren et al., 2013; Sobek et al., 2015; Tocino et al., 2015; Vitthala et al., 2009). These discrepancies may be explained by differences in characteristics of the studied populations and in methodological aspects. In fact, study designs used to investigate this issue mainly pro-

vide results that are exposed to significant confounders or diagnostic inaccuracies (Blickstein, 2005; Osianlis et al., 2014). Few studies report DNA testing to confirm the diagnosis and all lack a control group. Of utmost relevance here is that the available studies on the risk of monozygotic twins in IVF pregnancies do not attempt to provide data on the local background rate of monozygosity in natural pregnancies and refer to previous out-of-date evidence (Gee et al., 2014; Hall, 2003; Imaizumi & Nonaka, 1997; Tong et al., 1997). Even if the rate of monozygotic twins is believed to be constant all over the world, regardless of race and age, this assumption is actually speculative and may expose the results to significant inaccuracies.

In this study, we suggest approaching the issue from a different perspective; that is, using data from obstetric antenatal care units. In other words, we selected women with a diagnosis of MCT from a prenatal diagnosis unit and assessed whether the prevalence of pregnancies that were achieved with IVF differed from the local proportion of IVF pregnancies. This approach is expected to overcome the diagnostic confounders that typically affect case studies of children born from IVF since all included women were ascertained in the same manner by physicians who are experts in prenatal diagnosis. The exclusive inclusion of MCT pregnancies also protects our findings from the diagnostic inaccuracies that can occur when focusing on monozygotic pregnancies in general (i.e., the need for DNA testing for a definitive diagnosis). Moreover, this study design overcomes the above-mentioned limit of the unknown background rate of monozygosity. Finally, it also allows the recruitment of women early in pregnancy, prior to the occurrence of possible pregnancy complications (intrauterine demise) that may affect the birth of two viable children. This confounder may be significant if pregnancy outcome differs between natural and IVF MCT pregnancies.

Materials and Methods

This is a retrospective cross-sectional study reporting on women attending a specific service of the prenatal diagnosis unit of the Fondazione Ca' Granda, Ospedale Maggiore Policlinico of Milan, Italy, which is exclusively dedicated to the monitoring and management of MCT pregnancies. Women were identified through the use of an electronic database. We included twin pregnancies with a sonographic diagnosis of monochorionicity that progressed beyond 16 weeks' gestation. Higher order pregnancies (>2 fetal poles) were excluded. Pregnancies were diagnosed as monochorionic on the basis of the presence of a unique placenta and the absence of the twin peak sign (lambda sign) at the first sonographic assessment performed in our institution (between 8 and 16 weeks' gestation; Sepulveda et al., 1996). All ultrasound assessments were performed by expert gynecologists with extensive and long-standing experience in obstetric sonography. Women who had been referred after 16 weeks' gestation were excluded.

Data were retrospectively obtained from outpatient charts. They were completed using inpatient charts from the same institution. Women could be contacted by phone if relevant data were missing or if inconsistencies emerged. The data collected included baseline clinical characteristics, mode of conception, and pregnancy outcome. The study was accepted by the local Institutional Review Board.

A five-year period from 2007 to 2011 was used to achieve the scheduled sample size of at least 130 women. This sample size was calculated on the basis of an expected rate of IVF pregnancies during the study period of 2.2% (regional data extrapolated from 277 043 births; Parazzini et al., 2015), setting type 1 and 2 errors at 0.05 and 0.20, respectively and stating as clinically relevant a three-fold increase in the risk of MCT in IVF pregnancies. A binomial distribution model was used to calculate the 95% confidence interval (95% CI) of the proportions. The primary aim of the study was to determine the rate of IVF pregnancies among MCT pregnancies. The secondary aim was to compare the pregnancy outcome of IVF and natural pregnancies. Data were analyzed using the software SPSS 18.0 (Chicago, IL., USA). Data were compared using the Student's t test or Fisher's exact test, as appropriate; p values below .05 were considered statistically significant.

Results

One hundred and forty-five MCT pregnancies were selected. Ten of these were achieved with IVF, corresponding to a rate of 6.9% (95% CI: 3.5–11.8%), significantly higher than the natural background rate of 2.2% (Parazzini et al., 2015). The odds ratio (OR) of MCT in IVF pregnancies was 3.3 (95% CI: 1.6–5.9). When considering exclusively MCT pregnancies achieving delivery of two viable newborns ($n = 132$), the number of IVF pregnancies was nine (6.8%, 95% CI: 3.7–12.5%) and the corresponding OR was 3.3 (95% CI: 1.7–6.4).

A comparison of the baseline pre-pregnancy characteristics of women who had IVF pregnancies and those who conceived naturally is illustrated in Table 1. Women achieving pregnancy with IVF were older and the time to pregnancy was longer.

Pregnancy and neonatal outcomes in women who become pregnant by IVF and in those who conceived naturally are shown in Table 2. The rates of delivery of two viable twins (90% and 91%), twin-to-twin transfusion syndrome (10% and 18%), delivery before 34 weeks' gestation (22% and 25%), and small for gestational age (11% and 23%) were similar ($p = 1.00$, $p = 1.00$, $p = 1.00$ and $p = .20$, respectively). A statistically significant difference emerged for the proportion of newborns with a neonatal birth weight $\geq 2{,}500$ g (0% vs. 20%, $p = .03$).

The main cycle characteristics of the 10 women achieving pregnancy by IVF-ICSI are shown in Table 3. All women were transferred at the cleavage stage. No transfer at the

TABLE 1

Baseline Characteristics of the Studied Women According to the Mode of Conception

Characteristics	IVF pregnancies $n = 10$	Natural pregnancies $n = 135$	p
Age (years)	35.2 ± 2.3	32.5 ± 4.3	.007
Ethnic group			.60
Caucasian	10 (100%)	120 (89%)	
Other	0 (0%)	15 (11%)	
Smoking	1 (10%)	9 (7%)	.52
Previous deliveries	0 (0%)	15 (11%)	.60
Pre-pregnancy BMI (kg/m²)	20.2 ± 1.8	21.3 ± 2.5	.15
Time to pregnancy (months)	30 (16–36)	6 (1–12)	<.001

Note: Data is reported as number (%), mean ± SD or median (interquartile range).

TABLE 2

Pregnancy Outcome According to the Mode of Conception

Characteristics	IVF pregnancies $n = 10$	Natural pregnancies $n = 135$	p
Twin-to-twin transfusion syndrome	1 (10%)	24 (18%)	1.00
Intrauterine demise[a]	1 (10%)	12 (9%)	1.00
PIH-Preeclampsia[b]	2 (22%)	15 (11%)	.29
Preterm birth (< 34 weeks' gestation) [b]	2 (22%)	34 (25%)	1.00
Other obstetrical complications [b]	0 (0%)	16 (12%) [c]	.60
Mode of delivery[b]			1.00
Vaginal	0 (0%)	3 (2%)	
Cesarean section	9 (100%)	132 (98%)	
Newborn sex[d]			.08
Male	6 (33%)	147 (57%)	
Female	12 (67%)	111 (43%)	
Neonatal weight <2,500 g[d]	18 (100%)	206 (80%)	.03
Weight < 10th centile[d,e]	2 (11%)	59 (23%)	.20
Neonatal death[d]	0 (0%)	1 (0.4%)	1.00
Admitted to NICU[d]	11 (61%)	133 (52%)	.47
Neonatal morbidity[d]	1 (6%)[f]	18 (6%)[g]	1.00

Note: PIH = pregnancy-induced hypertension. NICU = Neonatal Intensive Care Unit.
[a]Both twins died at 21 weeks' gestation in one IVF-ICSI pregnancy. In the remaining cases (all from natural conception), only one of the two twins died.
[b]Refers to 9 IVF-ICSI pregnancies and 135 natural pregnancies (the woman with the intrauterine demise of both twins is excluded).
[c]Gestational diabetes mellitus ($n = 8$), placenta previa ($n = 2$), cholestasis ($n = 2$), cervical cerclage ($n = 2$), post-partum hemorrhage ($n = 1$), and varicella infection ($n = 1$).
[d]Data refers to the 276 viable newborns (18 IVF-ICSI cases and 258 natural conceptions).
[e]Based on local data (Parazzini et al., 1991).
[f]Intestinal obstruction ($n = 1$).
[g]Respiratory distress syndrome ($n = 6$), cardiovascular malformations ($n = 2$), intestinal obstruction ($n = 2$), clubfoot ($n = 1$), cystic lymphangioma ($n = 1$), sacral teratoma ($n = 1$), ovarian cyst ($n = 1$), aplasia cutis ($n = 1$), and severe hydronephrosis ($n = 1$).

blastocyst stage was recorded. Assisted hatching was never performed. All women transferred two embryos.

Discussion

Our results, based on the perspective of a prenatal diagnosis unit, indicate that the risk of MCT is three to four times higher in IVF pregnancies. To our knowledge, our study design has not been previously employed. Four studies reported the rate of MCT conceived by IVF but none related this finding to the local proportion of IVF pregnancies, thus impeding inferences with respect to the association (Chow et al., 2001; Ghalili et al., 2013; Ortibus et al., 2009; Sperling et al., 2006). Results from these contributions are summarized in Table 4. Surprisingly, the proportions of IVF pregnancies in these studies were higher than the rate observed in our study and varied widely, from 8.1% (Ortibus et al., 2009) to 32.0% (Chow et al., 2001). The reasons for these discrepancies are obscure. Differences in the local rate of IVF pregnancies may play a role, but it is unlikely that they fully explain these findings. Three possible additional reasons may be proposed as follows:

First, diagnostic accuracy may differ among studies. In our setting, the diagnosis of zygosity was made by highly expert physicians who are exclusively dedicated to prenatal diagnosis and we considered MCT pregnancies exclusively. However, it has to be acknowledged that we lack confirmation by DNA analyses and we cannot rule out some misdiagnoses. Noteworthy, the zygosity test was performed in only one out of the four previous studies (Sperling et al., 2006), leading to the exclusion of four cases initially classified as MCT (4 out of 78, corresponding to 5%). On the other hand, it has to be pointed out that the sonographic diagnosis of MCT is nowadays considered highly reliable

TABLE 3

Treatment Cycle Characteristics of the 10 Women Conceiving With IVF

Characteristics	Number (%)
Indication	
Male factor	5 (50%)
Endometriosis/tubal factor	2 (20%)
Unexplained	3 (30%)
Protocol of hyper-stimulation	
Long protocol	5 (50%)
Flare-up protocol	3 (30%)
GnRH antagonists	2 (20%)
Number of oocytes retrieved	
1–3	1 (10%)
4–8	7 (70%)
≥ 9	2 (20%)
Technique used	
IVF	2 (20%)
ICSI	8 (80%)
Embryo stage at transfer	
48 hours	2 (20%)
72 hours	8 (80%)
Blastocyst	0 (0%)
Assisted hatching	0 (0%)
Number of embryos transferred	
1	0 (0%)
2	10 (100%)

(Carroll et al., 2002; Sepulveda et al., 1996; Sperling et al., 2006), such that the skill level of the physicians involved is unlikely to differ markedly among the five contributions (all contributions were published by researchers engaged in prenatal diagnosis) and that there is no rational reason to speculate that diagnostic inaccuracy may selectively affect IVF pregnancies. Therefore, it is implausible that this limitation may explain the extreme variability in the reported rates of IVF pregnancies among MCT pregnancies.

Second, one may speculate on a role for some confounders in the referral fluxes to the involved prenatal diagnosis units. In other words, in some settings, access to the prenatal diagnostic service may have been facilitated for women who became pregnant by IVF. We cannot speculate on the potential role of this confounder in the four previous studies because we do not know the local situation. Even if we cannot fully exclude a similar bias in our study, it has to be pointed out that, in our setting, the IVF and prenatal diagnosis units are two distinct and independent services

that are run by different physicians and that refer to separate departments. Moreover, the prenatal diagnosis unit of our academic institution is a regional referral center for MCT pregnancies that converges cases from the whole region and this may further protect our data from confounders related to referral fluxes.

Third, the characteristics of the studied populations or the IVF protocols may differ among studies. For instance, the risk of MCT in IVF pregnancies has been related to a younger maternal age (Knopman et al., 2010; 2014). In this regard, it is interesting to note that a recent study also suggested that the higher incidence of monozygotic twins after assisted reproduction techniques may be related to genetic information rather than to the IVF technology itself (Sobek et al., 2015). Moreover, there is some evidence, albeit debated, suggesting a role for ovarian hyperstimulation, prolonged culture up to the blastocysts stage, culture medium, number of embryos transferred, and breach of the zona pellucida with ICSI or assisted hatching (Chang et al., 2009; Delrieu et al., 2012; Franasiak et al., 2015; Kanter et al., 2015; Knopman et al., 2010; 2014; Luke et al., 2014; Nakasuji et al., 2014; Ren et al., 2013; Vitthala et al., 2009). In other words, differences may be consequent to local differences in clinical and laboratory protocols (Vitthala et al., 2009). Noteworthy, in the 10 IVF pregnancies included in our study, none of the embryos were transferred at the blastocyst stage and none underwent assisted hatching. Of the utmost interest here is that some authors reported a decrease in the rate of MCT in IVF pregnancies over time, and this has been related to improved expertise (Knopman et al., 2014; Moayeri et al., 2007).

As a corollary of our study, we investigated pregnancy outcome according to the mode of conception. We observed a higher rate of newborns with a weight at birth below 2,500 g among IVF pregnancies. Conversely, the frequency of other pregnancy complications did not differ between the two groups. Our study is nonetheless underpowered for reliable conclusions. In this regard, it has to be noted that our data are mainly in agreement with the findings of Ghalili et al. (2013), who also evaluated pregnancy outcome according to the mode of conception and who failed to document major differences. Prematurity,

TABLE 4

Case Series on the Proportion of Women Achieving Pregnancy With IVF Among MZTs

Study	Country	Study period	N of IVF	Total N of MZT	% (95% CI)
Chow et al. (2001)	Massachusett, USA	1998–2000	16	50	32.0% (20.3–45.4)
Sperling et al. (2006)	Denmark, Sweden	1999–2003	10	74	13.5% (7.0–22.4)
Ortibus et al. (2009)	Belgium, the Netherlands, Germany	2002–2005	11	136	8.1% (4.3–13.5)
Ghalili et al. (2013)	Australia	2006–2010	76	294	25.9% (21.1–31.0)
Present study	Italy	2007–2011	10	145	6.9% (3.5–11.8)

Note: Studies were identified by searching in PubMed for articles published in the English language between January 2000 and February 2015 and using the following MeSH search terms: 'mzt', 'monozygotic', 'monochorionic' combined with 'ivf', 'in vitro fertilization', 'icsi', 'assisted reproductive', and 'ART' with restriction to the human species. Data was extracted independently by two investigators (V.S. and E.S.) who performed an initial screening of the title and abstract of all articles to exclude citations deemed irrelevant. Manual search of cross references completed the search.

intrauterine growth restriction, and neonatal survival did not indeed differ in their study (Ghalili et al., 2013).

Some limitations of our study should be acknowledged. First, IVF cases may be more likely to be referred to the antenatal care unit compared with natural pregnancies. However, this bias is unlikely to play a critical role here because MCT is one of the most challenging clinical conditions in pregnancy, and affected women are systematically referred to highly specialized services. The decision to refer is thus unlikely to be influenced by the mode of conception. Second, it is plausible that women conceiving by IVF and those conceiving naturally may differ in some characteristics that can be related to the risk of monozygosity. In other words, we cannot exclude that women requiring IVF may be at increased risk per se. Noteworthy, women conceiving naturally were younger in our study. Because a younger maternal age has been associated with the risk of monozygosity in IVF (Knopman et al., 2010; 2014), we cannot exclude the possibility that we have underestimated the risk. Overall, this is a limitation of all observational studies in any area of medicine. In this regard, however, it has to be pointed out that, even though some confounders cannot be excluded, the magnitude of the risk (OR >3) tends to support a causal effect. In a recent luminous editorial on the interpretation of observational studies, Grimes and Schulz (2012) emphasized that associations with an OR below three are more likely to be attributable to biases than to causal association. Third, we could not provide data on risk factors for MCT among the group of IVF pregnancies. In particular, we could not explore the independent effects of transfer at the blastocyst stage (all included women transferred at the cleavage stage), assisted hatching (this technique was never used), and ICSI (only two women underwent classical IVF). As alluded to above, there is currently a burning debate on this point that, to date, has not yet lead to consistent conclusions. This is an important point because the practice of IVF is rapidly changing and transfer at the blastocyst stage is gaining consent. In other words, if blastocyst transfer, for example, proves to be a risk factor for MZT, the figure emerging from our analysis may change in the near future (data here refer to the period 2007–2011 when the blastocyst strategy was uncommon in our region). Fourth, as controls we referred to a population-based rate as estimated in a recent study of our group in the same geographical area (Parazzini et al., 2015). This choice presents some limitations. The study periods of referral are similar but not identical (2010–2012 in the referral study and 2007–2011 in the index study) and data were extracted from birth registers on the basis of subjects' declarations (and thus they are exposed to some inaccuracies). In conclusion, according to data obtained from a prenatal diagnosis perspective, women undergoing IVF face an increased risk of MCT pregnancy, thus confirming previous evidence obtained from IVF case studies. Evidence from other geographical areas using the same study design is needed to confirm our estimation of the risk. Moreover, large multi-center studies are required to assess whether the prognoses of IVF and natural MCT pregnancies differ.

References

Bhattacharya, S., & Kamath, M. S. (2014). Reducing multiple births in assisted reproduction technology. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *28*, 191–199.

Blickstein, I. (2005). Estimation of iatrogenic monozygotic twinning rate following assisted reproduction: Pitfalls and caveats. *American Journal of Obstetrics and Gynecology*, *192*, 365–368.

Carroll, S. G., Soothill, P. W., Abdel-Fattah, S. A., Porter, H., Montague, I., & Kyle, P. M. (2002). Prediction of chorionicity in twin pregnancies at 10–14 weeks of gestation. *BJOG*, *109*, 182–186.

Chang, H. J., Lee, J. R., Jee, B. C., Suh, C. S., & Kim, S. H. (2009). Impact of blastocyst transfer on offspring sex ratio and the monozygotic twinning rate: A systematic review and meta-analysis. *Fertility and Sterility*, *91*, 2381–2390.

Chow, J. S., Benson, C. B., Racowsky, C., Doubilet, P. M., & Ginsburg, E. (2001). Frequency of a monochorionic pair in multiple gestations: Relationship to mode of conception. *Journal of Ultrasound in Medicine*, *20*, 757–760.

Corsello, G., & Piro, E. (2010). The world of twins: An update. *The Journal of Maternal-Fetal and Neonatal Medicine*, *2* (Suppl. 3), 59–62.

Delrieu, D., Himaya, E., Phillips, S., & Kadoch, I. J. (2012). Monozygotic multiple pregnancies following IVF: A case report series of rare experience. *Reproductive BioMedicine Online*, *25*, 460–465.

Franasiak, J. M., Dondik, Y., Molinaro, T. A., Hong, K. H., Forman, E. J., Werner, M. D., . . . Scott, R. T. Jr. (2015). Blastocyst transfer is not associated with increased rates of monozygotic twins when controlling for embryo cohort quality. *Fertility and Sterility*, *103*, 95–100.

Gee, R. E., Dickey, R. P., Xiong, X., Clark, L. S., & Pridjian, G. (2014). Impact of monozygotic twinning on multiple births resulting from in vitro fertilization in the United States, 2006–2010. *American Journal of Obstetrics and Gynecology*, *210*, 468.e1–e6.

Ghalili, A., McLennan, A., Pedersen, L., Kesby, G., & Hyett, J. (2013). Outcomes of monochorionic diamniotic twin pregnancies: A comparison of assisted and spontaneous conceptions. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, *53*, 437–442.

Grimes, D. A., & Schulz, K. F. (2012). False alarms and pseudo-epidemics: The limitations of observational epidemiology. *Obstetrics & Gynecology*, *120*, 920–927.

Hall, J. G. (2003). Twinning. *Lancet*, *362*, 735–743.

Imaizumi, Y., & Nonaka, K., 1997. The twinning rates by zygosity in Japan, 1975–1994. *Acta Geneticae Medicae et Gemellologiae (Roma)*, *46*, 9–22.

Kanter, J. R., Boulet, S. L., Kawwass, J. F., Jamieson, D. J., & Kissin, D. M. (2015). Trends and correlates of monozygotic twinning after single embryo transfer. *Obstetrics & Gynecology*, *125*, 111–117.

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.

Knopman, J. M., Krey, L. C., Oh, C., Lee, J., McCaffrey, C., & Noyes, N. (2014). What makes them split? Identifying risk factors that lead to monozygotic twins after in vitro fertilization. *Fertility and Sterility*, *102*, 82–89.

Kulkarni, A. D., Jamieson, D. J., Jones, H. W. Jr, Kissin, D. M., Gallo, M. F., Macaluso, M., & Adashi, E. Y. (2013). Fertility treatments and multiple births in the United States. *The New England Journal of Medicine*, *369*, 2218–2225.

Luke, B., Brown, M. B., Wantman, E., & Stern, J. E. (2014). Factors associated with monozygosity in assisted reproductive technology pregnancies and the risk of recurrence using linked cycles. *Fertility and Sterility*, *101*, 683–689.

Moayeri, S. E., Behr, B., Lathi, R. B., Westphal, L. M., & Milki, A. A. (2007). Risk of monozygotic twinning with blastocyst transfer decreases over time: An 8-year experience. *Fertility and Sterility*, *87*, 1028–1032.

Nakasuji, T., Saito, H., Araki, R., Nakaza, A., Nakashima, A., Kuwahara, A., & Sakumoto, T. (2014). The incidence of monozygotic twinning in assisted reproductive technology: Analysis based on results from the 2010 Japanese ART national registry. *Journal of Assisted Reproduction and Genetics*, *31*, 803–807.

Ortibus, E., Lopriore, E., Deprest, J., Vandenbussche, F. P., Walther, F. J., Diemert, A., . . . Lewi, L. (2009). The pregnancy and long-term neurodevelopmental outcome of monochorionic diamniotic twin gestations: A multicenter prospective cohort study from the first trimester onward. *American Journal of Obstetrics and Gynecology*, *200*, 494.e1–e8.

Osianlis, T., Rombauts, L., Gabbe, M., Motteram, C., & Vollenhoven, V. (2014). Incidence and zygosity of twin births following transfers using a single fresh or frozen embryo. *Human Reproduction*, *29*, 1438–1443.

Parazzini, F., Cipriani, S., Bulfoni, G., Bulfoni, C., Frigerio, A., Somigliana, E., & Mosca, F. (2015). The risk of birth defects after assisted reproduction. *Journal of Assisted Reproduction and Genetics*, *32*, 379–385.

Parazzini, F., Cortinovis, I., Bortolus, R., & Fedele, L. (1991). Standards of birth weight in Italy. *Annali di Ostetricia, Ginecologia, Medicina Perinatale*, *112*, 203–246.

Ren, X., Liu, Q., Chen, W., Zhu, G., & Zhang, H. (2013). Effect of the site of assisted hatching on vitrified-warmed blastocyst transfer cycles: A prospective randomized study. *Journal of Assisted Reproduction and Genetics*, *30*, 691–697.

Sepulveda, W., Sebire, N. J., Hughes, K., Odibo, A., & Nicolaides, K. H. (1996). The lambda sign at 10–14 weeks of gestation as a predictor of chorionicity in twin pregnancies. *Ultrasound in Obstetrics and Gynecology*, *7*, 421–423.

Shulman, L. S., & van Vugt, J. M. G. (2006). *Prenatal medicine*. Washington, DC: Taylor & Francis.

Sobek, A. Jr., Zbořilová, B., Procházka, M., Šilhánová, E., Koutná, O., Klásková, E., . . . Sobek, A. (2015). High incidence of monozygotic twinning after assisted reproduction is related to genetic information, but not to assisted reproduction technology itself. *Fertility and Sterility*, *103*, 756–760.

Sperling, L., Kiil, C., Larsen, L. U., Qvist, I., Schwartz, M., Jørgensen, C., . . . Tabor, A. (2006). Naturally conceived twins with monochorionic placentation have the highest risk of fetal loss. *Ultrasound in Obstetrics and Gynecology*, *28*, 644–652.

Tocino, A., Blasco, V., Prados, N., Vargas, M. J., Requena, A., Pellicer, A., & Fernández-Sánchez, M. (2015). Monozygotic twinning after assisted reproductive technologies: A case report of asymmetric development and incidence during 19 years in an international group of in vitro fertilization clinics. *Fertility and Sterility*, *103*, 1185–1189.

Tong, S., Caddy, D., & Short, R. V. (1997). Use of dizygotic to monozygotic twinning ratio as a measure of fertility. *Lancet*, *349*, 843–845.

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.