

The Risk of Birth Defects in Dichorionic Twins Conceived by Assisted Reproductive Technology

Tomoyuki Kuwata¹, Shigeki Matsubara¹, Akihide Ohkuchi¹, Takashi Watanabe¹, Akio Izumi¹, Yoko Honma², Yukari Yada², Hiroaki Shibahara¹, and Mitsuaki Suzuki¹

¹ Department of Obstetrics, Gynecology, Jichi Medical School, Minamikawachi-machi, Tochigi, Japan

² Department of Pediatrics, Jichi Medical School, Minamikawachi-machi, Tochigi, Japan

The purpose of this study was to examine whether dichorionic twins conceived by assisted reproductive technology (ART; intracytoplasmic sperm injection [ICSI], in vitro fertilization [IVF], gamete-intrafallopian tube transfer [GIFT]) have a higher risk of birth defects compared to dichorionic twins conceived naturally. We reviewed the medical records of 406 mothers with dichorionic twin pregnancies, who received continuous antenatal care from ≤ 20 weeks of gestation and gave birth to infants after ≥ 24 weeks of gestation in our institute. Birth defects were diagnosed at the time of hospital discharge according to the International Classification of Diseases, 10th Revision. Occurrence of birth defects was compared between twins conceived by ART and those conceived naturally using logistic regression analysis. Overall, 51 of 812 infants ($51/812 = 6.2\%$) had birth defects. The incidence of birth defects in ART-conceived twins was significantly higher than that of naturally conceived twins with an odds ratio of 6.9 (95% confidence interval [CI] 2.1, 22.5), 3.7 (95% CI 1.2, 12.0), and 4.3 (95% CI 1.4, 14.3) for ICSI, IVF, and GIFT, respectively. The higher frequency of birth defects in ART-conceived twins was still significant after adjusting for higher maternal age in the ART group, with an adjusted odds ratio of 6.7 (95% CI 2.1, 21.9), 3.6 (95% CI 1.1, 11.5), and 3.7 (95% CI 1.2–11.8) for ICSI, IVF, and GIFT, respectively. Dichorionic twins conceived by ART, compared to dichorionic twins conceived naturally, had a much higher risk for birth defects diagnosed at hospital discharge.

Following the worldwide introduction of assisted reproductive technology (ART; i.e., intracytoplasmic sperm injection [ICSI], in vitro fertilization [IVF], gamete-intrafallopian tube transfer [GIFT], etc.), critical concern about the incidence of fetal/neonatal birth defects prompted studies addressing this issue. Whereas some showed no increased risk of birth defects in infants conceived by ART (Bonduelle et al., 1996; Van Steirteghem, 1998; Wennerholm et al.,

1996), others suggested an increase (Bergh et al., 1999; Kurinczuk & Bower, 1997). Two recent studies on a large population (Hansen et al., 2002; Ludwig & Katalinic, 2002), using appropriate data for comparison, showed that infants conceived by ART had a higher chance of having birth defects than naturally conceived infants. This risk persisted in singletons, after excluding twin pregnancies and thereby eliminating this effect on the incidence of birth defects. Singleton infants conceived by ART have a higher risk to have birth defects compared to naturally conceived singleton infants.

Compared to naturally conceived pregnancies, ART leads to more multiple pregnancies, the majority of which are twin pregnancies. Birth defects are more frequent in twin infants than singleton infants (Bonduelle et al., 2002a; Ericson & Kallen, 2001) and monozygotic twins have a higher risk for birth defects than dichorionic twins (Corney et al., 1983; Cunningham et al., 2001; Wennerholm et al., 2000). Based on available data, we know that (1) birth defects possibly occur more frequently in singleton infants conceived by ART than in naturally conceived singletons, (2) birth defects occur more frequently in twins than in singletons, and (3) chorionicity affects the incidence of birth defects in twin gestation. What is unknown, however, is whether or not twin infants conceived by ART have a higher risk of birth defects than twins conceived naturally. The present study is an effort to address this issue. We performed this study by analyzing 406 mothers (thus 812 twin infants) receiving care from ≤ 20 weeks of gestation and giving birth after ≥ 24 weeks of gestation in our perinatal center. The study population was confined to dichorionic diamniotic twins.

Received 24 September, 2003; accepted 29 January, 2004.

Address for correspondence: Shigeki Matsubara, Department of Obstetrics and Gynecology, Jichi Medical School, Minamikawachi-machi, Tochigi, 329-0498 Japan. Email: matsushi@jichi.ac.jp

Materials and Methods

Between January 1990 and July 2001, 624 mothers gave birth to twin infants after ≥ 24 weeks of gestation in Jichi Medical School Hospital. Among 624 women, there were 469 women with dichorionic twins, 143 with monochorionic twins, and the remaining 12 were of unspecified chorionicity. From 469 women with dichorionic twins, we excluded women who were referred to us after ≥ 20 weeks of gestation ($n = 53$) or because of suspicion of fetal morphological abnormalities ($n = 2$), thus minimizing referral bias. Also excluded were mothers who became pregnant after receiving frozen-thawed embryo transfer ($n = 8$). The remaining 406 mothers (812 infants) comprised the present study population. Among these 406, there were 225 same-sex twin pregnancies (116 male–male and 109 female–female) and 181 different-sex twin pregnancies. Therefore, approximately 90% ($181 \times 2/406 = 89.2\%$) of twin pregnancies were estimated to be dizygotic and the remaining 10% monozygotic. There was no higher order pregnancy that was reduced to a twin pregnancy. All 406 women were arranged to give birth at our institution and were followed regularly at our antenatal clinic beginning before 20 weeks of gestation. The chorionicity of the placenta was confirmed by postpartum histological examination. Gestational ages were calculated by the last menstrual period or the date of the fertilization in ART cases, confirmed by the echographic examination performed at the first trimester. All women were hospitalized at 34 weeks of gestation according to our clinical protocol. We classified 406 women (812 infants) according to the mode of conception: (1) ICSI ($n = 42$ mothers), (2) IVF ($n = 74$), (3) GIFT ($n = 83$), (4) ovulation induction using clomiphene citrate or hMG/hCG (with or without intrauterine insemination) without ART ($n = 113$), and (5) natural pregnancy without infertility treatment ($n = 94$).

Systematic examination for birth defects was performed in the delivery room by at least two experienced neonatologists. Examinations were repeated on

postnatal days 1, 5, and at the time of hospital discharge. Birth defects were diagnosed and classified according to International Classification of Diseases, 10th Revision (World Health Organization, 1992). Chromosomal analyses were performed in clinically suspicious cases.

Data were reported as the median and interquartile range. The Chi-square test or Fisher's exact test was used to compare the categorical data. For multiple group comparisons among continuous variables, we used the Kruskal-Wallis test. Significances of individual differences were evaluated by using the Mann-Whitney tests if the Kruskal-Wallis test was significant. The contribution of age and the mode of conception to the occurrence of birth defects was evaluated using multivariate logistic regression analysis. Logistic regression analysis was also used to calculate both crude and adjusted odds ratio. Ninety-five percent confidence intervals (95% CIs) were determined using the associated standard errors. All analyses were performed with the SPSS software package (Version 9.0J for Windows, SPSS, 1999, Chicago, Illinois, USA). A level of $p < .05$ was accepted as significant.

Results

Maternal age and the percentage of nulliparous women were significantly higher in the ART-twin group than in the naturally conceived twin group (Table 1). Gestational days at delivery and infant weight were not different between the natural conception group and the infertility treatment group (Table 1). There was one case of intrauterine fetal death, which was conceived after ICSI and suffered by holoprosencephaly.

Fifty-one of 812 infants ($51/812 = 6.2\%$) had birth defects. Table 2 indicates the birth defects observed in these 51 infants according to the mode of conception. Overall, the infants conceived by ART were more likely to have birth defects than naturally conceived infants; ICSI especially induced a high incidence of birth defects (Table 3). This remained true

Table 1
Characteristics of 406 Pregnant Women, According to the Mode of Conception

	Natural Conception ($n = 94$) (Group 1)	Infertility treatment			Ovulation Induction ($n = 113$) (Group 5)	<i>p</i> -value	Significant Pair(s)
		ICSI ($n = 42$) (Group 2)	ART IVF ($n = 74$) (Group 3)	GIFT ($n = 83$) (Group 4)			
Maternal age	29.5 (27.3–32.8)	31.5 (30.3–34.5)	32.5 (30.5–34.8)	34.5 (30.5–36.5)	30.5 (28.5–34.5)	< .001	1 vs 2, 1 vs 3, 1 vs 4 2 vs 4, 3 vs 5, 4 vs 5
Nullipara (%)	48 (51%)	37 (88%)	60 (81%)	76 (92%)	88 (78%)	< .001	1 vs 2, 1 vs 3, 1 vs 4 1 vs 5, 4 vs 5
Gestational days at delivery	255 (247–260)	258 (246–259)	257 (251–259)	253 (242–260)	254 (242–260)	.375	
Birth weight (g)	2404 (2176–2644)	2368 (1958–2533)	2353 (2146–2531)	2360 (2026–2550)	2328 (2105–2505)	.227	

Note: Data are presented as median (interquartile range)

Table 2
Identified Birth Defect Diagnoses According to the Mode of Conception

	ICSI	IVF	GIFT	Ovulation Induction	Natural conception
Cardiac					
Atrial Septal Defect (ASD)	2	2	7	7	
Ventricular Septal Defect (VSD)		1	1	1	
Endocardial Cushion Defect		1			
Hypoplastic left heart syndrome			1		
ASD+VSD	1				
VSD+Coarctation of the aorta		1			
Facial					
Cleft palate with cleft lip	1				2
Accessory auricle		1	1		1
Gastrointestinal					
Stenosis of duodenum	1				
Atresia of jejunum		1			
Malrotation of intestine		1			
Stenosis of ileum				1	
imperforate anus				1	
Musculoskeletal					
Polydactyly	1		1		
Polysyndactyly	1				
Deformities of feet		1			
Deformities of spine			1		
Brain					
Agenesis of corpus callosum	1				
Holoprosencephaly	1				
Hydrocephalus					1
Urogenital					
Hypospadias	1	2			
Combined					
Spina bifida+VSD+Polydactyly	1				
Spina bifida+Gastroschisis				1	
Gastroschisis+Limb malformations			1		
ASD+Macroglossia (Trisomy 21)			1		
Total	11	11	14	11	4

after adjusting for the effect of maternal age by multivariate analysis (Table 3). The infants conceived after ovulation induction without ART had more birth defects than those conceived naturally but without statistical significance (Table 3). Table 4 indicates the comparison of the incidence of birth defects between ART group versus ovulation induction without ART group. The incidence of birth defects in ART-conceived twins was higher than that of twins conceived by ovulation induction without ART, with only the ICSI group reaching statistical significance. There was one infant with Down syndrome confirmed by chromosomal analysis, who was born after GIFT and suffered from ASD and macroglossia.

Discussion

Recent observation by Hansen et al. (2002) indicated that major birth defects occurred more frequently in infants conceived by ART compared to natural conception with an odds ratio of 2.0 in IVF and 2.0 in ICSI. A prospective, control cohort study by Ludwig and Katalinic (2002) also showed that ICSI-conceived

infants had a higher risk of having major birth defects compared to the naturally conceived infants. Both studies indicated that the risk for birth defects in ART remained when analysis was confined to singletons. Data, however, are scant regarding whether or not twins conceived by ART also have a higher chance for birth defects compared to those conceived naturally. In this study, we found that dichorionic twin infants conceived by ART, compared to dichorionic twin infants conceived naturally, were more likely to have birth defects diagnosed at the time of hospital discharge. This tendency remained after adjusting for maternal age. Furthermore, twin infants conceived by ICSI had a higher chance to have birth defects compared to twins conceived by ovulation induction without ART treatment.

Some previous studies focused on the relationship between the occurrence of birth defects in twins born after ART versus those after natural conception. Lambalk and Hooff (2001) analyzed 1093 twin pregnancies registered to the Dutch National Birth Registry, and found a nonsignificant, slightly higher

Table 3

Risk for Having Birth Defects in Dichorionic Twins who Were Conceived by ART (ICSI, IVF, and GIFT) and Ovulation Induction Without ART, Compared With those who Were Conceived Naturally

	Number of Birth Defects (%)	OR (95%CI)	Adjusted OR (95%CI)
ICSI (<i>n</i> = 84)	11 (13.1)	6.9* (2.1–22.5)	6.7* (2.1–21.9)
IVF (<i>n</i> = 148)	11 (7.4)	3.7** (1.2–12.0)	3.6** (1.1–11.5)
GIFT (<i>n</i> = 166)	14 (8.4)	4.3** (1.4–14.3)	3.7** (1.2–11.8)
Ovulation Induction (<i>n</i> = 226)	11 (4.9)	2.4 (0.7–7.5)	2.3 (0.7–7.3)
Natural conception (<i>n</i> = 188)	4 (2.1)	1.0	1.0

Note: OR = odds ratio, CI = confidence interval
Adjusted OR = OR adjusted for the maternal ages.
p* < .01 *p* < .05

rate of birth defects in the induced twins (IVF + ovulation induction) with a risk ratio of 1.54 which disappeared after correction for maternal age. Their population consisted of dizygotic twins (opposite-sex twins; dichorionic only), and was therefore different from ours (dichorionic twins; either dizygotic or monozygotic). Wennerholm et al. (2000) described an incidence of birth defects even lower in twin infants conceived by ICSI than in those conceived naturally (odds ratio 0.86). However, the population studied by Wennerholm et al. (2000) consisted of both dichorionic and monochorionic twins. The lower rate of birth defects in ART-conceived twins may be attributed to the lower fraction of monochorionic twins in this group, twins that have higher risk of birth defects (Corney et al., 1983; Cunningham et al., 2001; Wennerholm et al., 2000). We do not know the reason for the discrepancy in results between these two previous studies and the present research. Difference in study population, especially in chorionicity, may account for the different results.

Selection bias should be taken into account in evaluating the risk of birth defects between the two population groups. The occurrence of birth defects has a close association with various factors other than the mode of conception (ART versus natural), and therefore when comparing this risk, caution should be taken that the two groups, that is, ART versus natural conception, consist of populations possessing nearly equal backgrounds except for the mode of conception. Among these factors are racial, socioeconomic, and geographical factors (Cunningham et al., 2001). Furthermore, chorionicity also affects the frequency of birth defects in that monochorionicity enhances the risk of birth defects (Corney et al., 1983; Cunningham et al., 2001; Wennerholm et al., 2000). In this study, we dealt with a homogeneous population. Almost all twin pregnancies in this area,

regardless of whether they were conceived naturally or by ART, were referred to our hospital and almost all women were Japanese, and lived in the half urban/half rural area within approximately 30 km of the hospital. Both groups of pregnant women, ART versus natural conception, consisted of a racially, socioeconomically, and geographically homogeneous population, which confirmed the appropriateness of data for comparison. We further confined our study populations to dichorionic twins, and thus excluded the effect of chorionicity on the occurrence of birth defects.

Detection bias is another factor that should be taken into account. Some researchers (Ericson & Kallen, 2001; Hansen et al., 2002) have argued that there may have been closer monitoring of the infants conceived by ART than those conceived naturally, a circumstance which may lead to a higher detection rate of birth defects in ART groups. This detection bias may be particularly heavy considering the detailed follow-up for infants born with identified birth defects after ART, which were then compared with population data from various types of registers. The present study overcame this obstacle by confining our attention to dichorionic twin infants born in our institute. Physicians considered and parents were counseled that twin pregnancies by themselves were at high risk irrespective of whether they were conceived by ART or naturally. All pregnant women in this study, both those who conceived by ART and those who conceived naturally, were admitted to the hospital at 34 weeks' gestation according to our clinical protocol. Therefore, both physicians and parents may have paid approximately the same degree of attention to detecting birth defects in both groups. Furthermore, examination for birth defects was performed repeatedly by the same experienced pediatricians according to our protocol. It is unlikely that there was closer attention to the ART group, thus precluding the possibility of detection bias, and underscoring the appropriateness of the present conclusion.

The twin infants conceived by ovulation induction without ART treatment more frequently had birth defects than those conceived naturally, which,

Table 4

Risk for Having Birth Defects in Dichorionic Twins Conceived after ART Versus those Conceived after Ovulation Induction Without ART

	Number of Birth Defects	OR (95%CI)	Adjusted OR (95%CI)
ICSI (<i>n</i> = 84)	11	2.9* (1.2–7.1)	3.0* (1.2–7.1)
IVF (<i>n</i> = 148)	11	1.6 (0.7–3.7)	1.5 (0.7–3.7)
GIFT (<i>n</i> = 166)	14	1.8 (0.8–4.1)	1.6 (0.7–3.8)
Ovulation Induction (<i>n</i> = 226)	11	1.0	1.0

Note: **p* < .05 versus ovulation induction without ART

however, did not reach statistical significance. Data are controversial whether or not ovulation induction increases the risk of birth defects (Kallen et al., 2002; Van Loon et al., 1992). Some researchers suggested “infertility itself”, expressed by the length of involuntary childlessness, as one possible cause of birth defects (Kallen et al., 2002). In the present study we did not evaluate the infertility status of each woman, and therefore the link between subfertility and birth defects in twin infants remains to be proven.

We do not know the reason for the higher rate of birth defects detected in the ART-twin group, especially the ICSI-twin group. Previous studies indicated that some chromosomal anomalies, including de-novo structural anomalies or sex chromosomal anomalies, were observed more frequently in infants conceived by ICSI (Bonduelle et al., 2002b; Van Steirteghem, 1998). Although we have not analyzed chromosomes except in clinically suspicious cases, such increase in chromosomal abnormalities, if present in this series of ICSI infants, may partly account for the higher incidence of birth defects observed in the ICSI group. It was also reported that some specific defects may occur more frequently in infants after ART, especially those after ICSI; among them, urogenital (Hansen et al., 2002; Kurinczuk & Bower, 1997; Wennerholm et al., 2000), cardiovascular (Hansen et al., 2002; Kurinczuk & Bower, 1997), neural (Bergh et al., 1999), gastrointestinal (Bergh et al., 1999; Kurinczuk & Bower, 1997), and musculoskeletal (Hansen et al., 2002) abnormalities were listed. Although we found a high incidence of cardiovascular abnormalities in twin infants born after GIFT and ovulation induction without ART, the limited incidence of each abnormality made further analysis difficult. Regardless of these problems, by minimizing various study biases (referral, selection, and detection biases), this study indicated a higher risk for birth defects in dichorionic twins born after ART. Data presented here may be useful to care providers in counseling patients about the birth defect risk of twin pregnancies conceived by ART, although further studies are necessary to elucidate the mechanism of this phenomenon.

References

- Bergh, T., Ericson, A., Hillensjo, T., Nygren, K. G., & Wennerholm, U. B. (1999). Deliveries and children born after in-vitro fertilisation in Sweden 1982–95: A retrospective cohort study. *Lancet*, 354, 1579–1585.
- Bonduelle, M., Legein, J., Buysse, A., Van Assche, E., Wisanto, A., Devroey, P., et al. (1996). Prospective follow-up study of 423 children born after intracytoplasmic sperm injection. *Human Reproduction*, 11, 1558–1564.
- Bonduelle, M., Liebaers, I., Deketelaere, V., Derde, M. P., Camus, M., Devroey, P., et al. (2002a). Neonatal data on a cohort of 2889 infants born after ICSI (1991–1999) and of 2995 infants born after IVF (1983–1999). *Human Reproduction*, 17, 671–694.
- Bonduelle, M., Van Assche, E., Joris, H., Keymolen, K., Devroey, P., Van Steirteghem, A., et al. (2002b). Prenatal testing in ICSI pregnancies: Incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters. *Human Reproduction*, 17, 2600–2614.
- Corney, G., MacGillivray, I., Campbell, D. M., Thompson, B., & Little, J. (1983). Congenital anomalies in twins in Aberdeen and Northeast Scotland. *Acta Geneticae Medicae et Gemellologiae*, 32, 31–35.
- Cunningham, F. G., Gant, N. F., Leveno, K. J., Gilstrap, L. C., III, Hauth, J. C., & Wenstrom, K. D. (2001). Multifetal pregnancy. In F. G. Cunningham, N. F. Gant, K. J. Leveno, L. C. Gilstrap III, J. C. Hauth, & K. D. Wenstrom (Eds.), *Williams Obstetrics* (21st ed., pp. 765–810). McGraw-Hill: New York.
- Ericson, A., & Kallen, B. (2001). Congenital malformations in infants born after IVF: A population-based study. *Human Reproduction*, 16, 504–509.
- Hansen, M., Kurinczuk, J. J., Bower, C., & Webb, S. (2002). The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. *New England Journal of Medicine*, 346, 725–730.
- Kallen, B., Otterblad, P. O., & Nygren, K. G. (2002). Neonatal outcome in pregnancies from ovarian stimulation. *Obstetrics Gynecology*, 100, 414–419.
- Kurinczuk, J. J., & Bower, C. (1997). Birth defects in infants conceived by intracytoplasmic sperm injection: An alternative interpretation. *British Medical Journal*, 315, 1260–1266.
- Lambalk, C. B., & Van Hooff, M. (2001). Natural versus induced twinning and pregnancy outcome: A Dutch nationwide survey of primiparous dizygotic twin deliveries. *Fertility Sterility*, 75, 731–736.
- Ludwig, M., & Katalinic, A. (2002). Malformation rate in fetuses and children conceived after ICSI: Results of a prospective cohort study. *Reproductive Bio-Medicine Online*, 5, 171–178.
- Van Loon, K., Besseghir, K., & Eshkol, A. (1992). Neural tube defects after infertility treatment: A review. *Fertility Sterility*, 58, 875–884.
- Van Steirteghem, A. (1998). Outcome of assisted reproductive technology. *New England Journal of Medicine*, 338, 194–195.
- Wennerholm, U. B., Bergh, C., Hamberger, L., Lundin, K., Nilsson, L., Wikland, M., et al. (2000). Incidence of congenital malformations in children born after ICSI. *Human Reproduction*, 15, 944–948.
- Wennerholm, U. B., Bergh, C., Hamberger, L., Nilsson, L., Reismer, E., Wennergren, M., et al. (1996). Obstetric and perinatal outcome of pregnancies following intracytoplasmic sperm injection. *Human Reproduction*, 11, 1113–1119.
- World Health Organization. (1992). *International statistical classification of diseases and related health problems: Vol 1* (10th ed.). Geneva: WHO Publication.