

Birth Defects after Assisted Reproductive Technology in Japan: Comparison between Multiples and Singletons, 2004-2009

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Abstract

More than 2% of all infants in Japan are born after ART (Assisted Reproductive Technology) and this percentage is rapidly increasing. The relationship between ART and birth defects according to plurality is seldom examined. Japanese complete nationwide data of ART from 2004-2009 presented by the Japan Society of Obstetrics and Gynecology, which include information on birth defects, were used for analyses. There were a total of 177,548 pregnancies after ART. The Relative Risk (RR) and 95% Confidence interval (CI) for birth defect categories according to the International Classification of Disease, 10th edition (ICD-10) were calculated with singletons as the reference group. In multiples compared to singletons, the percentage of pregnancies with any birth defects per 10,000 pregnancies was significantly higher (RR=1.68, 95% CI 1.47-1.93), the percentage of birth defects per live births was not significantly higher (RR=0.92, 95% CI 0.80-1.06). The number of birth defects in the same fetus/neonate was significantly greater in multiples compared to singletons ($p < 0.05$). The most common birth defects of major classification were congenital malformations of the circulatory system for both singletons and multiples. RRs per pregnancy were significant for seven out of the 11 main categories. RRs per pregnancy were significant for congenital malformations of the nervous system (RR=3.58, 95% CI 2.49-5.14), other congenital malformations (RR=2.45, 95% CI 1.38-4.34), congenital malformations of genital organs (RR=2.25, 95% CI 1.19-4.24), congenital malformations of the circulatory system (RR=2.22, 95% CI 1.81-2.73), cleft lip and cleft palate (RR=2.01, 95% CI 1.20-3.35), congenital malformations of the eye, ear, face and neck (RR=1.74, 95% CI 1.02-2.98), and congenital malformations and deformations of the musculoskeletal system (RR=1.42, 95% CI 1.00-2.01). Some subcategories or individual diseases were more common in multiples compared to singletons. RRs of any birth defects per pregnancy and live births both decreased when patent ductus arteriosus was excluded.

Keywords: Birth defects; Assisted reproductive technology; Multiple pregnancy; Multiple births; Live births; Epidemiologic study; Prevalence; National data

Introduction

As is well known, multiple births occur far more often in Assisted Reproductive Technology (ART) than spontaneous conception in almost all developed countries [1-6]. The multiple-birth rate (per 1,000 live births) increased twice during the past two decades, mainly due to the increase of iatrogenic multiples, including ART, rather than spontaneous dizygotic twinning of higher maternal age in Japan [7]. According to the Japanese ART and vital statistics, the percentage of ART live births was rapidly increased from 0.22% (2,626/1,208,989) in 1992, 1.64% (18,168/1,110,721) in 2004, to 2.49% (26,680/1,070,035) in 2009. Thus, the use of ART is becoming widespread in Japan.

There are many epidemiologic studies on birth defects. Two important factors of comparison when examining the prevalence of birth defects are method of conception (spontaneous vs. iatrogenic or ART) and plurality (singletons vs. twins or multiple births), and the combination of both factors.

Concerning the comparison of birth defects between spontaneous and ART, larger studies have suggested that children born after ART have an increased risk of birth defects compared with children conceived spontaneously [8,9]. Data from meta-analyses consistently suggest that the overall risk of major birth defects in children born after ART is about 30% higher than in children conceived spontaneously [10,11]. A nationwide survey in Sweden also showed a slightly increased risk for birth defects after IVF (In vitro fertilization), even adjusting for possible confounding factors, such as year of birth, maternal age, and parity [12]. However, the first large-scale report of birth defects in 15,405 offspring conceived by ART in China found that infants born after IVF/ICSI (intracytoplasmic sperm injection) have a birth defect frequency comparable to that in the general Chinese population [13].

Regarding the effect of ART on birth defects according to plurality, many studies and meta-analyses have shown an increased risk for singletons conceived by ART [14-16], but there is controversy over whether the risk is increased or not in twins born after ART [14,16-18]. One contributing factor is that ART usually produces dizygotic twins, who have a better pre- and perinatal outcome than monozygotic twins [19-21]. According to Joy et al. [22], chorionicity accounts for most of the differences between naturally conceived twins and ART twins.

Concerning the comparison of birth defects between twins and singletons, it has been reported that the prevalence of birth defects is higher in multiples than in singletons in total (not stratified by the method of conception), as shown in national studies [19,21,23-29] and in an international study [30]. Some studies, however, found no association between multiple births and birth defects [31-37]. Thus, the findings are still inconsistent.

Data collection of ART, birth defects and births records (vital statistics) are not systematically managed by the Japanese government and record linkage is virtually impossible in Japan. Given the increasing use of ART, however, the outcome of multiple pregnancies in ART needs to be accurately estimated. With this background, the present author performed preliminary analyses of the overall prevalence of birth defects after ART in Japan [38]. The purpose of the present study was to

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		Singletons (N=1,246 mothers)		Multiple births (N=238 mothers with 256 fetuses/neonates)		p
		N	%	N	%	
Method of treatment	IVF	308	24.7	66	27.7	
	ICSI	300	24.1	63	26.5	
	IVF and ICSI	42	3.4	12	5.0	
	Frozen embryo transfer	594	47.7	97	40.8	n.s.
	Unknown/missing values	2	0.2	0	0.0	
Blastocyst transfer	Yes	660	53.0	98	41.2	
	No	580	46.5	137	57.6	<0.01
	Unknown/missing values	6	0.5	3	1.3	
Maternal age	Range		23-46		25-43	
	Mean ± SD		35.2 ± 4.1		33.8 ± 3.8	<0.001
	Median		35.0		34.0	
Gestational weeks	Range		10-42		12-41	
	Mean ± SD		36.5 ± 5.8		34.4 ± 4.9	<0.001
	Median		38.0		36.0	
	Unknown/missing values	153	12.3	5	2.1	
Sex (fetuses/neonates)	Male	595	47.8	131	51.2	
	Female	474	38.0	100	39.1	n.s.
	Unknown/missing values	177	14.2	25	9.8	
Perinatal outcome (fetuses/neonates)	Abortion (< 22 weeks) ^a	73	5.9	13	5.1	n.s.
	Stillbirths (≥ 22 weeks) ^b	24	1.9	6	2.3	
Abortion	Live births	1024	82.2	235	91.8	
	Unknown/missing values	125	10.0	2	0.8	
	Spontaneous	3	4.1	0		
Early neonatal death (neonatal death up to day 6 after birth)	Artificial	51	69.9	11	84.6	
	Unknown/missing values	19	26.0	2	15.4	
	Yes	32	3.1	22	9.4	<0.001
	No	716	69.9	174	74.0	
	Unknown/missing values	276	27.0	39	16.6	

^a: Percentage of singletons and multiples within each year were calculated.

^b: Abortion was defined as occurring under 22 weeks of gestation and stillbirth was defined as occurring at 22 or more weeks of gestation in the original data. Unknown/missing values were excluded in the statistical tests.

Table 1: Demographic and perinatal outcome data of ART pregnancies with birth defects of known plurality.

estimate birth defects after ART according to disease classification, and to calculate the Relative risk (RR) and 95% Confidence interval (CI) with singletons for reference in order to further examine the effect of ART on multiple births.

Materials and Methods

Outline of Japanese Birth Defects Data after ART

The method for collecting data is described in detail elsewhere [38]. Almost all medical institutions performing ART are registered with the Japan Society of Obstetrics and Gynecology (JSOG). The JSOG administers questionnaire surveys for these medical institutions. Some of the survey data are presented in simple annual reports of aggregate, not individual, data. From 2004 to 2009 (the latest), the individual list of all ART pregnancies resulting in birth defects was presented every year in the JSOG annual reports on ART (in Japanese). The presented items are method of treatment (In-vitro fertilization (IVF), Intracytoplasmic sperm injection (ICSI), frozen embryo transfer and others (duplicative methods), and do not include simple ovulation stimulation/enhancement), blastocyst transfer (yes, no, unknown), maternal age, perinatal outcome (spontaneous/artificial abortion (<22 weeks), stillbirths (≥22 weeks), and live births) and their gestational week, plurality (singleton, twins, triplets/+, and unknown), sex (male, female, unknown), early neonatal infant death up to day 6 (yes, no, unknown), and name of disease. The response rate for ART surveillance between 2004-2009 was 97.7-99.5%, and the mean response rate

throughout the 6 years was 99.0% (3,646/3,683), meaning that almost a complete database reflecting the current situation of ART and birth defects in Japan could be constructed.

The author used these case report data as initial information for the present secondary data analyses. All methods of fertility treatment were treated as ART in the present study, because the classification of these methods is not necessarily consistent and mutually exclusive.

The types of defects were reclassified according to the International Classification of Diseases, tenth edition (i.e., ICD-10, 2003 version). Diseases that were classified in the category of ICD-10 code Q00-Q99 (i.e., congenital malformations, deformations and chromosomal abnormalities) were selected and analyzed in the present study. Other congenital diseases that were not classified in Q00-Q99, such as congenital hypothyroidism, were excluded. In this reclassification, 114 out of 1,360 (8.4%) singletons and 26 out of 282 multiples (9.2%) were excluded, with no significant difference in the exclusion percentage between singletons and multiples. In total, 1,502 abortions, stillbirths or live births with birth defects (number of fetuses or neonates), consisting of 1,246 (83.0%) singletons, 247 (16.4%) twins, and 9 (0.6%) triplets were included. Twins and triplets/+ were treated in one category as multiples in the present study.

The number of stillbirths according to plurality was not being reported in the JSOG data. The number of total ART live births, singletons and multiples, were available from 2007-2009. For the

Number of birth defects		Singletons (N=1,246)		Multiples (N=256)		P
Total	Number of diseases in the same category	N	%	N	%	
1		1,070	85.9	215	84.0	<0.05
2		135	10.8	23	9.0	
	1	61		12		
	2	74		11		
3-		41	3.3	18	7.0	
	1	8		7		
	2	18		4		
	3-	15		7		

χ^2 test was performed for the 3x2 contingency table.

Table 2: Number of birth defects in the same fetus/neonate.

multiple births, the births were counted as live only when all neonates were born alive. For example, if both members of certain twin pairs are alive, then they are counted as two live births (neonates). On the other hand, when one member of certain twin pairs are alive (the other a stillbirth), then they are counted as no live births (neonates), or as one live singleton. The author estimated the number of ART singletons and multiples between 2004-2006 using approximation formulae [7].

Statistical Analyses

First, demographic and perinatal outcome data was analyzed. Then, the crude percentage of birth defects after ART per pregnancy (number of mothers), and live births according to the disease classification were calculated according to plurality and their RR with the corresponding 95% CI. For multiple pregnancies, pregnancies with at least one fetus/neonate with birth defects were counted as one pregnancy with birth defects. In other words, each twin pair concordant with respect to any birth defects was regarded as one pregnancy with birth defects. Statistical analysis was conducted using SAS for Windows ver 9.2.

Results

Demographic and perinatal outcome data of ART pregnancies with birth defects are summarized according to plurality in Table 1. Blastocyst transfer, maternal age and gestational was significantly different between singletons and multiples. Males were more frequent in both singletons and multiples. Early neonatal death was more frequent in multiples, although unknown/missing values of early neonatal death in singletons were very high (27.0%).

The number of birth defects in the same fetus/neonate is shown in Table 2. Of fetuses/neonates with any birth defects, about 15% had two or more birth defects. The proportion of fetuses/neonates who had three or more birth defects was greater in multiples ($p < 0.05$).

The number, rate (per 10,000 pregnancies), RR and 95% CI of birth defects in different organ systems are shown in Table 3 Included as supplementary data. The rate of birth defects was significantly higher in multiple pregnancy when assessed per 10,000 pregnancies (78.1 for singletons, 131.5 for multiples, RR=1.68, 95% CI 1.47-1.93). The RRs per pregnancy were significant with regard to seven main categories, 14 subcategories, and four diseases, i.e. ventricular septal defect, tetralogy of Fallot, Patent ductus arteriosus (PDA), and exomphalos. The RRs per pregnancy were the highest for congenital malformations of the nervous system (RR=3.58, 95% CI 2.49-5.14).

The number, rate (per 10,000 live births), RR and 95% CI of birth defects in different organ systems are shown in Table 4 Included as supplementary data. The rate of birth defects was not significantly higher in multiple pregnancy when assessed per 10,000 live births (102.4 for singletons, 94.6 for multiples, RR=0.92, 95% CI 0.80-1.06).

Rrs per live birth were significant regarding two main categories, six subcategories, and PDA. RRs were significant regarding congenital malformations of the nervous system (RR=3.62, 95% CI 2.31-5.68) and chromosomal abnormalities not classified elsewhere (RR=0.56, 95% CI 0.35-0.88).

The RRs of any birth defects per pregnancy and of any birth defects per live births both decreased when PDA was excluded.

Discussion

According to Mayor [39], the risk of congenital malformation in children born after ART is higher than previously thought, and is a public health issue. The present study for the first time showed the nationwide prevalence of birth defects after ART according to disease classification and plurality in Japan.

Few studies compared birth defects between singletons and multiples after ART. Of them, Pinborg et al. [40] compared neonatal outcome, including birth defects, between twins and singletons after ART using a Danish national cohort, and concluded that neonatal outcome in IVF/ICSI twins is considerably poorer than in singletons. For birth defects, the rate of major malformation was not significantly different between twins and multiples, whereas the total malformation rate (major plus minor) was higher in twins than in singletons.

One important cautionary note is that multiple births after ART usually produce dizygotic twins, and their pre- and perinatal outcome, including the prevalence of birth defects [32], is better than that of monozygotic twins [20]. Therefore, the higher proportion of birth defects in multiples compared to singletons frequently seen in spontaneous pregnancies or total pregnancies, which was not stratified by the method of conception, i.e. spontaneous or iatrogenic [27-30], may be diminished in ART pregnancies.

The present percentage of total birth defects after ART may be lower overall compared with other studies seen in many reviews [10,12,18]. Nevertheless, the main objective of this study was to evaluate the birth defect rate in multiple births compared to singletons, and not to compare the birth defect rates across different populations. Therefore, the comparison of birth defects in multiple births and singletons may be biased only if there is differential reporting according to plurality, which is not likely to occur [30].

The number of birth defects that occurred in the same fetus/neonate was greater in multiples than in singletons, as shown in Table 2. This result is in accordance with that of Doyle et al. [34], which in contrast did not agree with that of Zhang et al. [29]. But the latter study simply used a dichotomous classification for the number of diseases (1 or ≥ 2). If this dichotomous classification was adopted, the present data would show no significant difference in the numbers of diseases between singletons and multiples.

The percentage of birth defects after ART per pregnancy is clearly higher in multiple than in singleton pregnancies, whereas the percentage of birth defects per live birth in multiple pregnancies was equal in the two groups. This seemed reasonable given the fact that birth defects per pregnancy were counted based on the number of pregnant woman, not on the number of fetuses/neonates. The possibility of having at least one fetus/neonate with birth defects, which is counted as one pregnancy with birth defects, is higher in a multiple pregnancy. The rate of birth defects per live birth is important for estimating the risk of birth defects of multiple birth babies or for analyzing the etiology of birth defects themselves, while knowing the proportion of birth defects per pregnancy is important for counseling couples who are thinking

of undertaking ART. For example, if a couple becomes pregnant with twins, they hope to give birth to two healthy babies, not to at least one healthy baby.

According to the recent vital statistics in Japan, the mean number of children under 6 years old in Japanese families is currently about 1.3. This, combined with present results, suggested that the risk of having birth defects in at least one baby in one family after ART may become higher in families with multiples than in families without multiples.

The most common birth defects of major classification were congenital malformations of the circulatory system. This result was in accordance with previous Japanese studies of birth defects in general [25] or other studies [21,30,40].

The percentage of some birth defects according to organ system, especially malformations of the nervous system is significantly higher in multiples than in singletons. Very few studies have compared each type of birth defect after ART between singletons and multiples. Therefore, the birth defect findings in spontaneous pregnancy or pregnancy, neither of which were stratified by ART status, are described below. However, caution is again needed given that ART usually produces dizygotic twins, who tend to have a better pre⁹ and perinatal outcome than monozygotic twins. The difference between singletons and multiples may be diminished in ART subjects.

Many studies [21,28,30,34,35,41] have reported a higher proportion of birth defects of the nervous system in twins or multiples compared to singletons. Live births with anencephaly were all multiples in the present study. Anencephaly is frequently reported to be higher in twins/multiples than in singletons [27,28,34].

A higher proportion of PDA in multiples are frequently observed [24,27,28], and is attributed to the prematurity or shorter gestational age of multiples [27,40]. According to Pinborg et al. [40], after exclusion of PDA, which is strongly associated with preterm birth, no significant differences in any malformation rates were observed between twins and singletons. PDA was also frequently seen among multiples in the present study, and the RRs of any birth defects per pregnancy and of any birth defects per live births both decreased when this disease was excluded.

Chromosomal abnormalities not classified elsewhere were significantly lower in multiple live births. One possible reason was the lower mean maternal age of women with multiples, because maternal age is well known to be strongly related to chromosomal abnormalities [37]. However, the reason why the mean maternal age of women with multiples was lower than women with singletons was unclear. One explanation may be that the implantation rates are higher for younger patients and therefore they tend to have higher multiple pregnancy rates if the same numbers of embryos are transferred.

This study has the following limitations, most of which could be attributed to the dataset; namely, the fact that individual information was obtained only from the subjects with birth defects after ART, not the total ART pregnancies. The first and greatest limitation is that the author could not check the reliability of the data directly. Several misspellings or misclassifications of diseases were found in the annual report. This is the essential limitation of secondary data analyses. Second, although the present dataset was from a multi-year nationwide survey, it still did not have sufficiently high statistical power to detect the statistical significance of several diseases with high RR. Third, the prevalences of birth defects per live birth were underestimated in singletons, and, on the other hand, they were overestimated in multiples according to the present definition of live births. Thus, the RRs per live birth

were logically overestimated. Fourth, the author could not control for confounding factors that can affect ART and/or birth defects [12,42], such as maternal age, parity, smoking, and socioeconomic status, medical history, and prenatal care, since these data on the general ART populations were not available. However, to date, many studies have not necessarily controlled for confounding factors [11]. Fifth, follow-up after birth was limited to the early neonatal period, and was incomplete, especially for singletons. Some birth defects are not obvious within a few days after birth. Sixth, all methods of ART, i.e. IVF, ICSI and so on, were treated as ART. Regarding this point, a recent meta-analysis [43] and national study [44] reported that the ICSI procedure represents no significant additional risks of major birth defects in addition to the risks involved in standard IVF. The other limitations are the same as those pointed out by many studies related to birth defects [16,21,29,34,45]; namely, ascertainment bias (both over-ascertainment and under-ascertainment in multiples), the classification or diagnosis, no data on zygosity or the chorionicity of multiples.

Even with all these limitations, the present results overviewed the current situation of births defects after ART according to plurality. The risk of birth defects in ART live births are not significantly different between multiples and singletons, while the risk per pregnancy is clearly higher in multiple births. In conclusion, the overall impact of birth defects after ART would be larger in families with multiples, since the mean number of children would be larger in these families compared to in families without multiples. ART might contribute to the risk of birth defects both directly, by increasing the risk of defects among singletons, and indirectly by increasing the occurrence of twinning [16]. Proper follow-up for all families after ART, especially for families with multiple pregnancies/births, is needed.

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