Background: Although the clinical importance of chorionicity in twin pregnancies has been studied widely, the significance of perinatal determination of zygosity using molecular genetic analyses remains controversial. The purpose of this study was to determine the frequency and clinical significance of twin gestations according to zygosity and chorionicity in a Korean population.

Methods: We enrolled 569 women who delivered twin newborns (> 24 weeks) at Seoul National University Hospital between 1999 and 2008. Chorionicity was established by histologic examination of placentae. Zygosity was determined with sex of neonates, chorionicity, and DNA analysis of umbilical cord blood. Results: The frequency of dizygotic (DZ) twins was 71.0% (404/569 pairs) based on the opposite sex (238/404 [58.9%]) and DNA analyses (166/404 [41.1%]); that of monozygotic (MZ) twins was 29.0% (165/569), including monochorionic (MC) (72.1% [119/165]) and dichorionic (DC) twins (27.9% [46/165]), which was confirmed by DNA analyses. Among spontaneously conceived twins, the frequency of MZ twins was more than twice that of DZ twins. The risk of low birth weight was 1.8-fold higher among MZDC twins and 1.9-fold higher among MZMC twins than among DZDC twins (p < .05). Bronchopulmonary dysplasia occurred more frequently among MZMC twins than among DZDC twins (adjusted OR 8.42, 95% CI 1.82–39.08, p < .01). However, there is still controversy over the association between zygosity and perinatal outcome (Carroll et al., 2005; Dube et al., 2002; Potter, 1963; Schinzel et al., 1979; West et al., 1999). Although monozygosity has been suggested to be a risk factor for congenital anomalies, growth restriction, and perinatal death in some studies (Potter, 1963; Schinzel et al., 1979; West et al., 1999), we cannot assert that this increased risk of adverse perinatal outcome is a result of monozygotic (MZ) twinning. Because monochorionicity, which accounts for the majority of cases of monozygosity, has been reported to be associated with a higher risk of perinatal mortality and morbidity compared with

Keywords: twin, zygosity, chorionicity, placenta, DNA analysis
dichorionicity, which mainly originates from the presence of intertwined vascular anastomoses in monochorionic (MC) placentation (Acosta-Rojas et al., 2007; Carroll et al., 2005; Dube et al., 2002; Hack et al., 2008). Therefore, it is essential to differentiate between the effects of zygosity and chorionicity by stratifying twins into the following three subgroups, dizygotic dichorionic (DZDC), monozygotic dichorionic (MZDC) and monozygotic monochorionic (MZMC) twins.

How, then, can we make a precise determination of zygosity? Twins with different genders of fetuses or neonates are DZ; MC twins with a common placenta are MZ. In the case of same-sex twins with two placentae, additional methods to diagnose zygosity should be attempted. Although blood group analysis has been used for the determination of zygosity, the test lacks accuracy because newborns with the same blood type are not necessarily MZ twins (Scardo et al., 1995). A direct comparison of DNA variations using molecular genetic analyses is the most reliable test for determination of zygosity, especially in the case of same-sex twins with DC placentae (Guilherme et al., 2009; Yang et al., 2006). In DNA analysis, the genes are directly used; several unlinked genetic loci are tested at the same time and a pattern unique to each twin is obtained (Yang et al., 2006). The probability of false assignment of zygosity is very low (up to $10^{-4}$), as the number of tested loci is theoretically unlimited (Yang et al., 2006). Von Wurmb-Schwark et al. (von Wurmb-Schwark et al., 2004) demonstrated that only 8 short tandem repeat (STR) loci were reliable to obtain reliable and reproducible results with a high certainty of zygosity in twin studies.

The purpose of this study was to determine zygosity and chorionicity among consecutive twin pregnancies on a relatively large scale using DNA analysis along with histologic examinations of placentae, leading to inform us of the true distribution of zygosity and chorionicity in a Korean population, and to evaluate the clinical significance of the stratification of twin gestations according to zygosity and chorionicity.

**Materials and Methods**

This was a retrospective cohort study of twin gestations who were delivered in a tertiary referral center (Seoul National University Hospital, Seoul, Korea) from January 1999 and June 2008. A total of a consecutive series of 695 twin pregnancy pairs and 1390 neonates who weighed 500 g or more, and with a gestational period of at least 24 weeks, were delivered during this study period. We excluded 73 twin pairs because of unknown zygosity; all of them were same-sex twins; some had no results of placental examinations; others were DC with no umbilical cord blood stored for DNA analysis. If the procedure of DNA extraction failed for at least one twin, the entire set of twins was excluded from the study. Fifty-three cases were complicated by lethal or major congenital anomalies (identified prenatally and confirmed postnatally) and/or intrauterine fetal deaths or stillbirths (at least one twin). These cases were included only for the assessment of the rate of perinatal mortality and major congenital anomalies (Table 1). Of 622 cases, 569 twin pairs in which both infants were live born without any major congenital anomaly were included for the analysis of neonatal outcome.

The study cohort was stratified into three categories as follows: DZDC, MZDC and MZMC twin pairs. Chorionicity was established by the examination of placental membrane. (After macroscopic examinations, microscopic sections of the membranes were analyzed for the determination of the chorionic and amniotic types.) Zygosity was determined with sex of neonates, chorionicity, and DNA analysis of umbilical cord blood. The opposite sex twins were assumed to be DZ. Among the same-sex twin pairs, cases with MC placentae were considered to be MZ. In the case of same-sex twins with DC placentae, molecular genetic techniques were performed to confirm the zygosity.

Umbilical cord blood was collected in ethylenediamine-tetra-acetic acid (EDTA)-containing tubes by venipuncture of the umbilical vein at birth. Retrieval of umbilical cord blood was performed after written informed consent was obtained. The Institutional Review Board of Seoul National University Hospital approved the collection and use of these samples and information for research purposes. The Seoul National University has a Federal Wide Assurance with the Office for Human Research Protection of the

**Table 1**

<table>
<thead>
<tr>
<th>Major congenital anomaly</th>
<th>No twin</th>
<th>FDIU</th>
<th>Both twins</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No twin</td>
<td>—</td>
<td>8</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>One twin</td>
<td>36</td>
<td>7*</td>
<td>—</td>
<td>43</td>
</tr>
<tr>
<td>Both twins</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>15</td>
<td>1</td>
<td>53*</td>
</tr>
</tbody>
</table>

Note: FDIU, fetal death in utero

* Of 7 cases, 6 cases had one anomalous twin died in utero and the other healthy liveborn twin; 1 case had one liveborn but anomalous twin and the other died in utero without any anomaly. Therefore, the total sets of twins are 53, but not 54.
Department of Health and Human Services of the United States. Each sample was then centrifuged and the interface layer including mostly Buffy coat layer but also small amount of plasma and erythrocytes was stored in polypropylene tubes at −70°C until assayed. DNA was extracted from the Buffy coat using a commercial DNA isolation kit (QIAamp® DNA Blood Mini Kit; QIAGEN, Valencia, CA, USA) and processed according to the manufacturer’s instructions. The AmpFISTR® Profiler Plus® (Applied Biosystems, Foster City, CA, USA) allowed the co-amplification and three-color detection of the short tandem repeat (STR) loci, including D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820, and the gender marker, amelogenin. PCR products were analyzed using an ABI PRISM® 3130xl DNA Analyzer (Applied Biosystems) and GeneMapper® Software v4.0 (Applied Biosystems). Figure 1 displays a schematic diagram of the above process. Whenever all of the STR loci and the gender-determining marker, amelogenin, were identical in the PCR-amplified microsatellite analysis, the compared newborns were determined as MZ twins (Figure 2B); if not, they were DZ (Figure 2A).

The mode of conception is recorded as ‘spontaneous’ or ‘assisted conception’. For the variables ‘hypertensive disorders during pregnancy’ and ‘diabetes during pregnancy’, the data do not allow the distinction between chronic or pregnancy induced hypertension and overt or gestational diabetes. Perinatal mortality included stillbirths and neonatal deaths. Neonatal mortality was defined as death at 28 days or less after birth. Neonatal morbidity included early-onset neonatal proven and suspected sepsis, respiratory distress syndrome (RDS), pneumonia, bronchopulmonary dysplasia (BPD), intraventricular hemorrhage (IVH), and necrotizing enterocolitis (NEC), which were diagnosed according to the definitions described in detail in the previous studies (Yoon et al., 1995). Small for gestational age (SGA) was defined as a birth weight below the 10th percentile, adjusted for gestational age, sex and parity; large for gestational age (LGA) was above the 90th percentile (Kato, 2004). Birthweights below 2500 and 1500 g were defined as low birthweight (LBW) and very low birthweight (VLBW), respectively. Preterm birth was defined as a delivery that occurred before 37 completed weeks of gestation; early preterm birth was defined as delivery that occurred before 32 weeks.

Statistical analysis was performed with the SPSS statistical package 12.0 (SPSS Inc., Chicago, IL, USA). Differences between categorical variables were analyzed using χ² test. Fisher’s exact test was used to assess pairwise concordance rates for MZ and DZ twin pairs (Novak et al., 2009). Differences between continuous variables were tested using independent sample t tests, Mann-Whitney U-tests or Kruskal-Wallis test, where appropriate. The Bonferroni correction method was introduced for an adjustment for multiple comparisons. Possible confounders were adjusted for by logistic regression analyses.

**Results**

Of 569 pairs of live born twins, 404 pairs were DZ (71.0%). Gender examination allowed the classification of male-female or female-male pairs as DZ (238/404 [58.9%]). DNA analyses were performed to confirm the zygosity in the case of the same-sex pairs with DC placentae; 41.1% (166/404) were DZ. The same-sex twin pairs with MC placentae were classified as MZ (119/165 [72.1%]). In the case of the same-sex pairs with DC placentae, genetic studies were conducted and 27.9% (46/165) was MZ. The frequency of assisted conception was 68.2% (388/569), of which 11.6% (45/388) was DZ. Among spontaneously conceived twins, MZ twins occurred twice as often as DZ twins (66.3% [120/181] vs. 33.7% [61/181]).

Maternal characteristics are presented in Table 2. Among DZDC twin pairs, maternal age was more advanced than among MZ twin pairs (p < .01) and the frequency of assisted conception was significantly higher than that of MZ twin pairs (p < .001). Hypertensive disorders during pregnancy were observed more frequently among MZMC twin pairs than DZDC twin pairs, but not statistically significant. Selective fetal reduction was more frequently conducted among DZ than among MZ twins. However, based on a multiple comparison test using
Figure 2

DNA profiles of dizygotic twins (A) and monozygotic twins (B) obtained by electrophoretic STR analyses using the AmpFLSTR® Profiler Plus® (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM® 3130xl DNA Analyzer (Applied Biosystems). Blue, green and yellow peaks are representative of the short tandem repeat (STR) loci, including D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820, and the gender marker, amelogenin. Red peaks are size standards for fragment analyses.
the Bonferroni correction, there was no significant difference between any two of DZDC, MZDC, and MZMC. There was no significant difference among the three groups (DZDC, MZDC, and MZMC) in the frequency of multiparous women, diabetes during pregnancy and cesarean delivery. The mean gestational age at delivery of DZ twins was slightly more advanced than that of MZ twins, but not statistically significant.

As shown in Table 3, the frequencies of early preterm births were significantly lower among MZMC twin pairs than DZDC twins (adjusted odds ratio [aOR] 2.38, 95% confidence interval [CI] 1.07–5.29, p < .05) after adjusting for maternal age, parity, mode of conception, selective fetal reduction, and hypertensive disorders during pregnancy. However, there was no significant difference of preterm births and early preterm births rate among the three groups with respect to the frequencies of spontaneous preterm births.

DZ twins were heavier than MZ (Table 4). Low birth weight (LBW) occurred less frequently among DZ twins than among MZDC and MZMC twins (aOR, 1.77, 95% CI, 1.01–3.12; and aOR, 1.88, 95% CI, 1.22–2.89, respectively; Table 5). The frequencies of very low birth weight had a similar trend of those of LBW, but not statistically significant (Table 5). The frequencies of 1-minute and 5-minute Apgar score less than 7 and admission to the neonatal intensive care unit were significantly higher among MZMC twin pairs than DZDC (p < .05). A low 1-minute Apgar score occurred more frequently among MZMC twins than MZDC (38.7% vs. 17.4%, p < .05). The frequencies of neonatal mortality and morbidity, except BPD, were not significantly different among the three groups. The risk of BPD in MZMC twins was higher than in DCDZ twins (5.5% [13/235] vs. 2.1% [17/797]; aOR, 8.42, 95% CI, 1.82–39.08, p < .01). The frequency of BPD was higher in MZDC (3.3%...
Table 4
Perinatal Outcomes of the Study Population According to the Zygosity and Chorionicity

<table>
<thead>
<tr>
<th></th>
<th>DZDC</th>
<th>P*</th>
<th>MZDC</th>
<th>P†</th>
<th>MZMC</th>
<th>P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 808</td>
<td></td>
<td>N = 92</td>
<td></td>
<td>N = 238</td>
<td></td>
</tr>
<tr>
<td>Birthweight (g)&lt;sup&gt;11&lt;/sup&gt;</td>
<td>2393.0 ± 572.6</td>
<td>NS</td>
<td>2313.5 ± 603.0</td>
<td>NS</td>
<td>2298.2 ± 576.1</td>
<td>.015 &lt; .05</td>
</tr>
<tr>
<td>Discordancy ≥ 20%&lt;sup&gt;5&lt;/sup&gt;</td>
<td>70 (17.9%)</td>
<td>NS</td>
<td>6 (13.6%)</td>
<td>NS</td>
<td>26 (22.8%)</td>
<td>NS NS</td>
</tr>
<tr>
<td>LBW</td>
<td>417 (51.6%)</td>
<td>NS</td>
<td>54 (58.7%)</td>
<td>NS</td>
<td>145 (60.9%)</td>
<td>.012 &lt; .05</td>
</tr>
<tr>
<td>VLBW</td>
<td>58 (7.2%)</td>
<td>NS</td>
<td>10 (10.9%)</td>
<td>NS</td>
<td>25 (10.5%)</td>
<td>NS NS</td>
</tr>
<tr>
<td>SGA</td>
<td>65 (8.0%)</td>
<td>NS</td>
<td>7 (7.6%)</td>
<td>NS</td>
<td>25 (10.5%)</td>
<td>NS NS</td>
</tr>
<tr>
<td>LGA</td>
<td>114 (14.1%)</td>
<td>NS</td>
<td>12 (13.0%)</td>
<td>NS</td>
<td>21 (8.8%)</td>
<td>0.036 NS</td>
</tr>
<tr>
<td>HCA &amp;/or funisitis&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>54 (14.0%)</td>
<td>NS</td>
<td>7 (15.2%)</td>
<td>NS</td>
<td>22 (18.8%)</td>
<td>NS NS</td>
</tr>
<tr>
<td>1-minute Apgar score &lt; 7</td>
<td>141 (17.5%)</td>
<td>NS</td>
<td>13 (14.1%)</td>
<td>&lt; .01</td>
<td>66 (27.7%)</td>
<td>&lt; .01 &lt; .01</td>
</tr>
<tr>
<td>5-minute Apgar score &lt; 7</td>
<td>42 (5.2%)</td>
<td>NS</td>
<td>7 (15.2%)</td>
<td>NS</td>
<td>31 (25.0%)</td>
<td>&lt; .001 &lt; .001</td>
</tr>
<tr>
<td>Umbilical cord blood pH&lt;sup&gt;∥&lt;/sup&gt;</td>
<td>7.27 ± 0.07</td>
<td>NS</td>
<td>7.27 ± 0.06</td>
<td>.048</td>
<td>7.26 ± 0.08</td>
<td>&lt; .01 &lt; .05</td>
</tr>
<tr>
<td>NICU admission rate</td>
<td>161 (20.0%)</td>
<td>NS</td>
<td>19 (20.7%)</td>
<td>NS</td>
<td>72 (30.3%)</td>
<td>.001 &lt; .01</td>
</tr>
<tr>
<td>NICU admission (days)</td>
<td>7.1 ± 21.0</td>
<td>NS</td>
<td>9.7 ± 26.4</td>
<td>NS</td>
<td>8.2 ± 18.9</td>
<td>&lt; .01 &lt; .05</td>
</tr>
<tr>
<td>Neonatal sepsis</td>
<td>10 (1.3%)</td>
<td>NS</td>
<td>2 (2.2%)</td>
<td>NS</td>
<td>1 (0.4%)</td>
<td>NS NS</td>
</tr>
<tr>
<td>RDS</td>
<td>41 (5.1%)</td>
<td>NS</td>
<td>7 (7.7%)</td>
<td>NS</td>
<td>10 (4.3%)</td>
<td>NS NS</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>3 (0.4%)</td>
<td>NS</td>
<td>0 (0.0%)</td>
<td>NS</td>
<td>0 (0.0%)</td>
<td>NS NS</td>
</tr>
<tr>
<td>BPD</td>
<td>17 (2.1%)</td>
<td>NS</td>
<td>3 (3.3%)</td>
<td>NS</td>
<td>13 (5.5%)</td>
<td>&lt; .01 &lt; .05</td>
</tr>
<tr>
<td>IVH</td>
<td>8 (1.0%)</td>
<td>NS</td>
<td>0 (0.0%)</td>
<td>NS</td>
<td>1 (0.4%)</td>
<td>NS NS</td>
</tr>
<tr>
<td>NEC</td>
<td>9 (1.1%)</td>
<td>NS</td>
<td>1 (1.1%)</td>
<td>NS</td>
<td>4 (1.7%)</td>
<td>NS NS</td>
</tr>
<tr>
<td>Neonatal morbidity&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>55 (6.8%)</td>
<td>NS</td>
<td>9 (9.8%)</td>
<td>NS</td>
<td>21 (8.8%)</td>
<td>NS NS</td>
</tr>
<tr>
<td>Neonatal mortality</td>
<td>8 (1.0%)</td>
<td>NS</td>
<td>1 (1.1%)</td>
<td>NS</td>
<td>1 (0.4%)</td>
<td>NS NS</td>
</tr>
</tbody>
</table>

Note: MZMC, monozygotic monochorionic; MZDC, monozygotic dichorionic; DZDC, dizygotic dichorionic; LBW, low birthweight; VLBW, very low birthweight; NICU, neonatal intensive care unit; NS, not significant

* Comparison between DZDC and MZDC
† Comparison between MZDC and MZMC
‡ Comparison between DZDC and MZMC
§ Comparison among DZDC, MZDC and MZMC
∥ Values are shown as the mean ± standard deviation.
¶ Denominators are 404 for DZDC, 46 for MZDC and 119 for MZMC, respectively.
# Neonatal morbidity was defined as a composite variable including one or more of the following: congenital neonatal proven or suspected sepsis, respiratory distress syndrome, congenital pneumonia, bronchopulmonary dysplasia, intraventricular hemorrhage and necrotizing enterocolitis

[3/90]) than in DCDZ, but not significant. To assess genetic influence on development of BPD, we calculated concordance rates in MZ and DZ twins (0.78 vs. 0.21, p < .01, Table 6).

For analysis of the rate of perinatal mortality and major congenital anomalies, an additional 53 twin pairs were included (Table 1). Cases complicated with intrauterine fetal death or stillbirth were 16 twin pairs (one twin, n = 15; both twins, n = 1) and those complicated with major congenital anomaly were 45 twin pairs (one twin, n = 43; both twins, n = 2). Of 53 twin pairs, 7 pairs had one twin with major congenital anomaly and one twin died in utero; 6 pairs had one anomalous twin died in utero and the other healthy liveborn twin; 1 pair had one liveborn but anomalous twin and the other died in utero without any anomaly. Four infants died at 28 days or less after birth; one was complicated with Potter’s syndrome, one prune belly syndrome, one Edwards syndrome and one esophageal atresia and double outlet right ventricle. Among 1138 liveborn infants with no congenital anomaly, 10 twin infants died during the neonatal period; 9 twins were delivered at 32 or less weeks of gestation and died of prematurity; one twin was complicated by respiratory distress syndrome, who was delivered at 34–2 weeks of gestation. Figure 3 describes the perinatal mortality rate according to zygosity and chorionicity. The perinatal mortality rate was 15 per 1000 total births in DZDC twins, 20 per 1000 total births in MZDC and 56 per 1000 total births in MZMC (p < .001 by linear-by-linear association). The perinatal mortality rate in MZMC was significantly higher than in DCDZ even after adjusting for gestational age at delivery and major congenital anomalies (p < .01 by logistic regression analysis). As shown in Figure 4, the rate of major congenital anomalies was 2.9% (25/862) in DZDC twins, 3.1% (3/98) in MZDC and 6.7% (19/284) in MZMC (p < .01 by linear-by-linear association).

Of 142 MC twin pairs, 8 cases were complicated by twin-to-twin transfusion syndrome (TTTS); 4 twin pairs in which both twins were liveborn with no major
CI 0.48–4.07, respectively), but the differences were not significant. Not surprisingly, gestational age at delivery had the most important role in reducing the risk of neonatal morbidity (aOR, 0.45, 95% CI, 0.39–0.52).

**Discussion**

Principal findings of this study: In this cohort, 71.0% of twin live births were DZ and 29.0% were MZ. DNA analyses were performed in 37.3% of live born twins because of the same-sex twin pairs with DC placenta; 41.1% of DZ and 27.9% of MZ needed the confirmative analyses. The frequency of assisted conception was 68.2%, of which 11.6% was MZ. Among spontaneously conceived twins, the frequency of MZ was twice that of DZ (66.3% vs. 33.7%). After adjustment for maternal age, parity, mode of conception, SFR and hypertensive disorders in pregnancy, preterm delivery before 32 weeks of gestation occurred 2.4-fold more often among MZMC than among DZDC, though the causal relationship between spontaneous preterm births and zygosity and chorionicity was lacking. The risk of LBW was 1.8-fold higher among MZDC and 1.9-fold higher among MZMC than among DZDC. However, in terms of the risk for overall neonatal morbidity associated with preterm deliveries, there were no differences among MZMC, MZDC and DZDC. The perinatal mortality rate was 15 per 1000 total births in DZDC twins, 20 per 1000 total births in MZDC and 56 per 1000 total births in MZMC. The rate of congenital anomalies was 3.8% of total twin births; 2.9% in DZDC twins, 3.1% in MZDC and 6.7% in MZMC.

Twin distribution according to zygosity: To date, there is a paucity of information regarding twin distribution according to zygosity considering a method of conception. Thorough review of a mode of conception in every twin pregnancy showed that, among spontaneously conceived twins, the frequency of MZ pairs was 66.3%, which was twice the frequency of DZ pairs. Although the causes of twinning are incompletely understood, DZ results from relatively elevated serum gonadotropin levels, leading to double ovulation and the frequency of DZ varies widely among different populations. In 1986, when assisted reproductive technologies were not prevalent, MacGillivray (MacGillivray, 1986) demonstrated that the frequency of twin births varies significantly among different races and ethnic groups. For example, twinning rates per 1000 births were 3.0 in monozygotes and 1.3 in dizygotes in Japan. This is consistent with our results and the evidence in support of levels of follicle-stimulating hormone (FSH) varying among different races (Nylander, 1973; Soma et al., 1975).

Preterm births and zygosity and chorionicity: In this study, among MZMC twins, the frequency of early preterm birth was higher than among DZDC twins. There was a suggestion of increased frequency of preterm birth among MZMC twins, but not significant. However, an increase in the frequency of preterm or...
Table 7
Risk Factors for Neonatal Morbidity

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted OR (95% CI)*</th>
<th>P</th>
<th>Adjusted OR (95% CI)*</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old age (≥35 years)</td>
<td>0.63 (0.38–1.04)</td>
<td>NS</td>
<td>0.92 (0.37–2.25)</td>
<td>NS</td>
</tr>
<tr>
<td>Multiparity</td>
<td>1.24 (0.74–2.05)</td>
<td>NS</td>
<td>1.05 (0.43–2.55)</td>
<td>NS</td>
</tr>
<tr>
<td>Assisted conception</td>
<td>1.73 (1.02–2.92)</td>
<td>&lt; .05</td>
<td>1.82 (0.64–5.20)</td>
<td>NS</td>
</tr>
<tr>
<td>Selective fetal reduction</td>
<td>1.73 (0.88–3.38)</td>
<td>NS</td>
<td>1.53 (0.50–4.74)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertensive disorders during pregnancy</td>
<td>0.79 (0.42–1.49)</td>
<td>NS</td>
<td>1.08 (0.42–2.79)</td>
<td>NS</td>
</tr>
<tr>
<td>DZDC</td>
<td>1.00</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>MZDC</td>
<td>1.48 (0.70–3.09)</td>
<td>NS</td>
<td>1.93 (0.48–7.80)</td>
<td>NS</td>
</tr>
<tr>
<td>MZMC</td>
<td>1.32 (0.78–2.23)</td>
<td>NS</td>
<td>1.39 (0.48–4.07)</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age at delivery (per 1-week increase)</td>
<td>0.45 (0.39–0.51)</td>
<td>&lt; .001</td>
<td>0.45 (0.39–0.52)</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

Note: MZMC, monozygotic monochorionic; MZDC, monozygotic dichorionic; DZDC, dizygotic dichorionic; NS, not significant

*Adjusted for maternal age, parity, mode of conception, selective fetal reduction, hypertensive disorders during pregnancy and gestational age at delivery

early preterm births might not be attributed to increased risks of spontaneous preterm births. These findings corroborate that the prevalence of prematurity, especially that due to spontaneous preterm labor, among twin pregnancies might have little causal relationship with zygosity and chorionicity, as suggested in the previous studies (Carroll et al., 2005; Dube et al., 2002).

Birthweight and zygosity and chorionicity: Even after adjusting for maternal age, parity, mode of conception, selective fetal reduction, hypertensive disorders during pregnancy and gestational age at delivery, MZ twins had a higher risk of LBW, which can be attributed to intrauterine growth restriction. However, the risk of small babies less than 10th percentile for gestational age failed to reach any statistical significance but suggested only a trend to increase the rate of small babies among MZMC twins. These findings were consistent with the findings of previous studies (Carroll et al., 2005; Dube et al., 2002).

Neonatal morbidity and zygosity and chorionicity: We attempted to determine risk factors for neonatal morbidity. The risk of most neonatal morbidities, including neonatal sepsis, RDS, IVH, and NEC, did not demonstrate any significant difference among the three groups, DZDC, MZDC, and MZMC. However, MZ twins, primarily MZMC, had a greater risk of BPD than DZ twins. Immaturity, barotraumas and oxygen toxicity have been thought to play major roles in the pathogenesis of BPD (Yoon et al., 1997). When
considering the higher rate of admission to NICU and twin-to-twin transfusion syndrome (TTTS), a MG-specific disease, it is not surprising that the risk of development of BPD was higher among MCMZ than DCDZ. On the other hand, evidences supporting the hypothesis that there is a genetic predisposition in developing BPD independent of environmental factors has been suggested in recent studies (Bhandari et al., 2006; Lavoie et al., 2008). Bhandari et al. demonstrated that pair-wise concordance rate of BPD was significantly higher in MZ twins than in DZ (Bhandari et al., 2006). In the current study, the pair-wise concordance of BPD among MZ was significantly higher than among DZ (p < .01). Lavoie et al. proposed that heritability contribute the development of BPD and target therapy with the aid of advances in human genome mapping prevent BPD (Lavoie et al., 2008).

Despite individual differences in the frequency of each disease entity, such as neonatal sepsis, RDS, BPD, IVH, and NEC, among the DZDC, MZDC and MZMC twins, there were no significant differences in overall neonatal morbidity among the three groups. Two major possibilities must be considered: (1) every neonatal morbidity is closely related with preterm delivery, of which the frequencies failed to show any significant difference among the three groups in our study and (2) more importantly, among MZ twins, survivors might be healthier and have fewer postnatal problems than those complicated by stillbirths and early neonatal deaths.

Strengths and weaknesses of this study: The findings of this retrospective study must be interpreted with caution. The limitations of the cohort size, particularly within the stratified categories, had the potential for selection bias among practitioners, and changes in obstetric and neonatal practice over a 10-year time span must be acknowledged. Nevertheless, in this study, the cross-distribution of zygosity and chorionicity in twin pregnancies was demonstrated with the introduction of the established technique for zygosity determination, DNA analysis, which is widely used in forensic medicine and paternity testing for human identification, on a relatively large scale. Moreover, the use of a well-controlled perinatal database with the bank of umbilical cord blood samples and the agreement of our findings with other recent publications lends support to our findings.

In conclusion, our results show the cross-distribution of twin pregnancies based on zygosity and chorionicity with DNA analyses and histologic examinations of placentae. Although accurate determination of zygosity along with chorionicity plays a major role in future consideration of organ transplantation and twin studies, the value of zygosity along with chorionicity in relation to overall neonatal morbidity may be limited. Prenatal diagnosis of zygosity with chorionic villi or amniocytes must be considered in selective fetal reduction or termination when chorionicity is indeterminate, of which a randomized controlled study in the
future would be of use for maternal-fetal-medicine specialists.

**Author’s Role**

JK Jun initiated the idea of this study. Jun JK and Kim A contributed to the study design and data analysis. Lee KA and Oh KJ prepared the data files for analysis. Lee KA conducted the analyses and collated the results. Lee KA and Jun JK drafted the paper. Oh KJ and Lee SM provided statistical advice. All authors contributed to the interpretation of the data and all were involved in the critical revision of the paper.

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