Presenting Twins Are Exposed to Higher Levels of Inflammatory Mediators than Nonpresenting Twins as Early as the Midtrimester of Pregnancy

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Abstract

Objective

Presenting twins are less likely to develop respiratory complications than non-presenting twins. The precise reason for this difference is not well understood, although it is known that the presence of inflammation reduces the risk of respiratory morbidity at birth. To further investigate this association, we compared the concentrations of inflammatory biomarkers in mid-trimester amniotic fluid (AF) of asymptomatic twin pairs.

Study Design

The study population consisted of women with twin pregnancies who underwent mid-trimester amniocentesis (15–20 weeks) for routine clinical indications and delivered at term. AF was analyzed for pro-inflammatory cytokines (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IFN-γ, TNF-α), matrix metalloproteinases (MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-12), and chemokines (Complement Factor D/Adipsin, Serpin E1/PAI-1, Adiponectin/Acrp30, CRP, CCL2/MCP-1, Leptin, Resistin) using Luminex Performance Assay multiplex kits. Data were analyzed using Wilcoxon signed rank test.

Results

A total of 82 twin pairs were enrolled. Mid-trimester AF concentrations of IL-8, MMP-8, CRP, MCP-1, leptin, and resistin were significantly higher in the presenting twin compared with the non-presenting twin (p<0.05 for each). Differences in AF concentrations of IL-8, MMP-8, and CRP persisted after adjustment for the fetal growth restriction at the time of birth and chorionicity.
Conclusion

These data suggest that, as early as the mid-trimester, the presenting fetus in an otherwise uncomplicated twin pregnancy is exposed to higher levels of pro-inflammatory mediators (especially IL-8, MMP-8, and CRP) than its non-presenting co-twin. Whether this pro-inflammatory milieu reduces the risk of neonatal respiratory morbidity at birth or has other functional implications needs to be further evaluated.

Introduction

The incidence of twin births has increased dramatically over the past few decades primarily because of infertility therapy, making up 3.21% of all births in 2009 in the United States[1]. Twin pregnancies are associated with increased risks of neonatal mortality and morbidity compared with singleton pregnancies,[2–4] and non-presenting twins (second twins) are at higher risk for adverse outcomes than presenting twins (first twins) [5,6]. Mode of delivery has been implicated as one of the major causes of the increased mortality and morbidity in second twins, because adverse outcomes are more common when planned vaginal delivery is attempted[5,7–10].

Second twins are also more likely to develop respiratory complications than first twins, independent of gestational age and mode of delivery[11–13]. The precise reason for this difference is not well understood, although it has been shown that the presence of inflammation reduces the risk of respiratory morbidity in singleton pregnancies[14]. We postulate that first twins are exposed to higher levels of inflammatory mediators than second twins, and that this may be true throughout pregnancy and not only at term when the cervix is effaced and dilated thereby allowing for ascending infection and inflammation. To further investigate this association, we compared the concentrations of inflammatory biomarkers in mid-trimester amniotic fluid (AF) collected from asymptomatic twin pairs.

Materials and Methods

Study design

In this retrospective cohort study, consecutive twin pregnant women who underwent clinically indicated mid-trimester amniocentesis (15–20 weeks of gestation) for fetal karyotyping and delivered at term were enrolled. Cases with subsequent preterm delivery, major fetal structural anomaly or aneuploidy, or fetal death in utero were excluded. Amniocentesis was performed after informed written consent and an aliquot of amniotic fluid from each twin was stored at -70°C until assayed after centrifugation at 2000 rpm. The Institutional Review Board at Seoul National University Hospital approved the study and the patients provided their written consent for the collection and use of clinical information and these samples for research purpose.

Quantification of proteins

Stored AF samples were analyzed for cytokines, matrix metalloproteinases and chemokines. Cytokines including interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IFN-γ, and TNF-α were measured with Bio-Plex Pro assays (BIO-RAD Laboratories, Inc., Hercules, CA, USA). Matrix metalloproteinases (MMPs; MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-12) and chemokines (Complement Factor D/Adipsin, Serpin E1/PAI-1, Adiponectin/Acrp30, C-Reactive Protein, CCL2/MCP-1, Leptin, Resistin) were measured with
Luminex Performance Assay (R&D Systems, Inc., Minneapolis, MN, USA). In these multiplex assays, the antibodies are covalently coupled to microspheres with a unique fluorescent dye, thereby enabling the determination of concentrations of each analyte using a Bio-Plex 200 analyzer (BIO-RAD). For the purpose of analysis, values below the lower limit of detection for each analyte were recorded as the lower limit of quantification (LLOQ).

Statistical methods
The clinical characteristics and analyte concentrations in AF were compared between the first and second twin. Continuous variables were compared using Wilcoxon signed rank test and categorical variables were compared using McNemar test. A generalized estimating equation (GEE) was used to adjust confounding variables in the relationship between birth order and the AF concentrations of analytes. GEE has been used in multivariate analysis for multiple outcomes from the same subject[15,16] and in family-based association studies.[17] Statistical analyses were conducted using the IBM SPSS version 20. $P<0.05$ was considered significant.

Results
During the study period, a total of 152 twin pregnant women underwent genetic amniocentesis at 15–20 weeks of gestation. Cases with major fetal anomalies (n = 7), cases which were lost to follow up (n = 19), and cases in which the presenting and non-presenting twins are not definitely defined (n = 3) were excluded. In the remaining 123 women, 82 women delivered at term and were included in the final analysis.

Table 1 shows the clinical characteristics of the study population. Amniocentesis was performed at a mean of 17.0 weeks of gestation, and the most common indication for genetic amniocentesis was advanced maternal age. The mean gestational age at delivery was 37.8 weeks and cesarean delivery was performed in 66% of cases.

Table 2 compares the concentrations of analytes in the AF collected from first and second twins. A total of 15 analytes were detected in significant quantities (i.e., were measurable in >90% of cases). Among these, the mean AF concentrations of IL-8, MMP-8, CRP, MCP-1, leptin, and resistin were higher in the first twin than in the second twin. (IL-8: 568.53 vs

Table 1. Clinical characteristics of the study population.

| Characteristics                          | Twin pairs (n = 82)       |
|------------------------------------------|---------------------------|
| Maternal age (years)                     | 35 ± 3                    |
| Nulliparity                              | 59 (72%)                  |
| Gestational age at amniocentesis (weeks) | 17.0 ± 0.7                |
| Indication for amniocentesis             |                           |
| Advanced maternal age                    | 68 (83%)                  |
| Abnormal serum screening                 | 8 (10%)                   |
| Abnormal ultrasound                      | 1 (1%)                    |
| Maternal request                         | 5 (6%)                    |
| Gestational age at delivery (weeks)      | 37.8 ± 0.7                |
| Birth weight (grams) *                   | 2723 ± 337 (presenting twin) / 2667 ± 377 (non-presenting twin) |
| Cesarean delivery                        | 54 (66%)                  |

* $p = NS$ between presenting and non-presenting twins (analyzed with Wilcoxon signed rank test).

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Table 2. Concentrations of analytes in mid-trimester amniotic fluid in presenting versus non-presenting twins.

| Characteristics | unit     | Presenting twin (n = 82) | Non-presenting twin (n = 82) | P value* | Percent <LLOQ |
|-----------------|----------|--------------------------|-----------------------------|----------|---------------|
| IL-1β           | pg/mL    | 1.67 ± 1.23              | 1.66 ± 1.26                 | NS       | 100           |
| IL-2            | pg/mL    | 2.82 ± 2.62              | 3.25 ± 3.33                 | NS       | 84.1          |
| IL-4            | pg/mL    | 0.36 ± 0.24              | 0.36 ± 0.24                 | NS       | 98.2          |
| IL-5            | pg/mL    | 1.23 ± 1.54              | 1.23 ± 1.57                 | NS       | 100           |
| IL-6            | pg/mL    | 350.98 ± 542.31          | 279.52 ± 428.32             | NS       | 1.2           |
| IL-8            | pg/mL    | 568.53 ± 993.94          | 448.16 ± 654.53             | <0.05†   | 0.0           |
| IL-10           | pg/mL    | 3.45 ± 1.82              | 3.51 ± 1.69                 | NS       | 97.6          |
| IL-12           | pg/mL    | 3.43 ± 1.81              | 3.07 ± 1.66                 | 0.091    | 99.4          |
| IL-13           | pg/mL    | 1.68 ± 1.63              | 1.67 ± 1.71                 | NS       | 100           |
| IL-15           | pg/mL    | 6.07 ± 7.12              | 6.17 ± 6.67                 | NS       | 60.4          |
| GM_CSF         | pg/mL    | 25.41 ± 8.36             | 24.61 ± 7.39                | NS       | 0.0           |
| IFN-r          | pg/mL    | 7.61 ± 10.78             | 6.46 ± 8.37                 | NS       | 80.5          |
| TNF-a          | pg/mL    | 6.19 ± 2.94              | 6.26 ± 2.77                 | NS       | 100           |
| MMP-1          | pg/mL    | 760.96 ± 874.26          | 742.51 ± 855.49             | NS       | 0.0           |
| MMP-2          | pg/mL    | 167922.2 ± 57259.1       | 167635.6 ± 63210.0          | NS       | 0.0           |
| MMP-3          | pg/mL    | 1654.33 ± 910.69         | 1598.21 ± 956.81            | NS       | 0.0           |
| MMP-8          | pg/mL    | 2090.55 ± 2341.75        | 1680.63 ± 1991.43           | <0.05†   | 1.2           |
| MMP-9          | pg/mL    | 714.68 ± 584.30          | 673.85 ± 516.87             | NS       | 7.3           |
| MMP-12         | pg/mL    | 21.76 ± 31.86            | 23.68 ± 38.30               | NS       | 57.9          |
| Complement factor D | ng/mL | 1292.9 ± 510.3 | 1215.0 ± 603.9 | NS       | 0.0 |
| Serpin E1      | ng/mL    | 177.7 ± 98.2             | 158.5 ± 100.1               | 0.052    | 0.0           |
| Adiponectin    | ng/mL    | 68.4 ± 35.6              | 61.1 ± 33.8                 | 0.052    | 0.0           |
| CRP            | ng/mL    | 76.6 ± 170.7             | 49.3 ± 79.5                 | <0.05†   | 0.0           |
| CCL2/MCP-1     | pg/mL    | 0.57 ± 0.26              | 0.52 ± 0.26                 | <0.05    | 0.0           |
| Leptin         | ng/mL    | 17.0 ± 11.0              | 15.5 ± 10.7                 | <0.05    | 0.0           |
| Resistin       | ng/mL    | 16.7 ± 11.8              | 14.6 ± 11.5                 | <0.01    | 0.0           |

LLOQ, lower limit of quantification; NS, not significant.
* analyzed with Wilcoxon signed rank test.
† Significant after adjustment for fetal growth restriction and chorionicity.

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448.16 pg/mL, respectively, p<0.05; MMP-8: 2090.55 vs 1680.63 pg/mL, respectively, p<0.05; CRP: 76.6 vs 49.3 ng/mL, respectively, p<0.05; MCP-1: 0.57 vs 0.52 ng/mL, respectively, p<0.05; Leptin: 17.0 vs 15.5 ng/mL, respectively, p<0.05; Resistin: 16.7 vs 14.6 ng/mL, respectively, p<0.01). The differences in AF concentrations of IL-8, MMP-8, and CRP between the first and second twin remained significant after adjustment for the fetal growth restriction at the time of birth and chorionicity (Table 2).

**Discussion**

**Main findings**

The principal findings of this study were that AF concentrations of IL-8, MMP-8, CRP, MCP-1, leptin, and resistin were significantly higher in the presenting compared with the non-presenting twin already in the mid-trimester (p<0.05 for each). The differences in AF concentrations of IL-8, MMP-8, and CRP between the first and second twin remained significant after adjustment for the fetal growth restriction at the time of birth and chorionicity.
Inflammation and respiratory morbidity in singleton pregnancy

Previous studies have shown that inflammation can promote fetal lung maturation in both animals and humans. In animal studies, intra-amniotic administration of endotoxin or inflammatory cytokine accelerated lung maturation by increasing the production of airway surfactant and improving lung gas exchange, which has been suggested as the mechanism by which antenatal corticosteroids act to promote functional maturation of the fetal lung [18–24]. Recent studies suggest that chorioamnionitis is associated with the decreased risk of neonatal respiratory distress syndrome, thereby supporting the theory that pulmonary maturation is accelerated in the presence of antenatal inflammation [14, 25–30]. Increased endogenous cortisol levels have been suggested as the possible mechanism for this relationship, since cortisol can increase surfactant synthesis in the presence of inflammation as well as increase pulmonary fluid clearance and subsequent gas exchange in the presence of MMPs and neutrophils [25].

Different respiratory morbidity in twin pairs

In twin pairs, the second twin is at increased risk of respiratory morbidity as compared with the first twin [11–13]. Some have suggested that immediate postnatal depression may be attributed to the increased incidence of respiratory complication, but the development of respiratory distress syndrome due to surfactant deficiency is independent of birth asphyxia, gestational age, and mode of delivery [13, 31]. Other mechanisms for the observed higher incidence of respiratory morbidity in the second twin have been suggested, including decreased exposure to the protective effects of labor, the development of acute uteroplacental insufficiency after delivery of the first fetus, and lower production of surfactant [9, 11].

In this study, we postulated that a relatively lack of exposure to inflammatory mediators may provide the underlying mechanism to explain the differences in lung maturation between twin pairs. To this end, we compared the concentrations of inflammatory cytokines in mid-trimester amniotic fluid between twin pairs. The AF concentrations of IL-8, MMP-8, and CRP were higher in the first twin, and this difference remained significant after adjustment for the fetal growth restriction at the time of birth and chorionicity. We included only twin pregnant women who delivered at term, because intra-amniotic infection and/or inflammation in mid-trimester amniotic fluid itself is associated with an increased risk of preterm delivery [32–36].

Strengths and Limitations

To our knowledge, this is the first study comparing levels of inflammatory cytokines in asymptomatic mid-trimester AF between twin pairs. To avoid the confounder of preterm birth, we limited our analysis only to twin pregnancies who delivered at term. Even in these twin pregnancies, the concentrations of inflammatory markers in mid-trimester AF were higher in the first twin within twin pairs. This could indeed provide a possible explanation for the decreased incidence of respiratory morbidity in the first twin, even in the late preterm or term period, and even after cesarean delivery in the absence of labor [11].

There are several limitations of our study. First, because of a relatively small number of cases and inclusion of only those twin pregnancies that delivered at term, the association between mid-trimester AF concentrations of inflammatory markers and the subsequent development of respiratory morbidity could not be examined in further detail. In the current study population, respiratory morbidity occurred in only one case. In this case, the mid-trimester AF concentrations of IL-8 was 238.8 pg/mL (very low), MMP-8 was 1724.1 pg/mL (low normal), and CRP was 252.2 ng/mL (high) (see Table 2). Further studies are needed to examine the association between mid-trimester exposure to intra-amniotic inflammation and subsequent respiratory morbidity. Second, it is possible that the presenting twin identified in mid-pregnancy
may not be the presenting twin at birth. According to one study, a discrepancy between antenatal labeling and the anticipated birth order was observed in about 30% of cases [37]. Third, the study included a measurement of AF cytokines at only a single point in time. Serial AF concentrations of these inflammatory biomarkers would be more useful in helping to understand the relationship between antenatal inflammation and fetal lung development, but this does not seem to be plausible in human studies. Lastly, IL-6 is well known marker for inflammatory condition, and the elevated AF concentrations of IL-6 has been reported to be associated with intra-amniotic infection and adverse pregnancy outcomes [32,38,39]. However in the current study, the mid-trimester AF concentrations of IL-6 were higher in the presenting twin compared with the non-presenting twin, but did not reach statistical significance. Further studies with a larger study population are needed to demonstrate the role of mid-trimester AF IL-6 in the pathogenesis of preterm birth.

In conclusion, as early as the mid-trimester, the presenting fetus in an otherwise uncomplicated twin pregnancy is exposed to higher levels of pro-inflammatory mediators (IL-8, MMP-8, and CRP) than its non-presenting co-twin. Whether this pro-inflammatory milieu reduces the risk of neonatal respiratory morbidity at birth or has other functional implications needs to be further evaluated.

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Author Contributions
Conceived and designed the experiments: SML JSP. Performed the experiments: SML SMK JHL CWP BJK JKJ. Analyzed the data: SML JSP ERN. Contributed reagents/materials/analysis tools: SML SMK JHL CWP BJK JKJ. Wrote the paper: SML JSP ERN.

References
1. Martin JA, Hamilton BE, Ventura SJ, Osterman MJ, Wilson EC, Mathews TJ. Births: final data for 2010. Natl Vital Stat Rep. 2012; 61: 1–72. PMID: 24964584
2. Wyshak G. Birth Hazard of the Second Twin. JAMA. 1963; 186: 869–870. PMID: 14061080
3. Prins RP. The second-born twin: can we improve outcomes? Am J Obstet Gynecol. 1994; 170: 1649–1656. PMID: 8203422
4. Sibony O, Touitou S, Luton D, Oury JF, Blot PH. A comparison of the neonatal morbidity of second twins to that of a low-risk population. Eur J Obstet Gynecol Reprod Biol. 2003; 108: 157–163. PMID: 12781404
5. Smith GC, Pell JP, Dobbie R. Birth order, gestational age, and risk of delivery related perinatal death in twins: retrospective cohort study. BMJ. 2002; 325: 1004. PMID: 12411358
6. Smith GC, Fleming KM, White IR. Birth order of twins and risk of perinatal death related to delivery in England, Northern Ireland, and Wales, 1994–2003: retrospective cohort study. BMJ. 2007; 334: 576. PMID: 17337456
7. Wen SW, Fung Kee Fung K, Oppenheimer L, Demissie K, Yang Q, Walker M. Neonatal morbidity in second twin according to gestational age at birth and mode of delivery. Am J Obstet Gynecol. 2004; 191: 773–777. PMID: 15467539
8. Wen SW, Fung Kee Fung K, Oppenheimer L, Demissie K, Yang Q, Walker M. Neonatal mortality in second twin according to gestational age, and mode of delivery. Am J Obstet Gynecol. 2004; 191: 778–783. PMID: 15467540
9. Hartley RS, Hitti J. Birth order and delivery interval: analysis of twin pair perinatal outcomes. J Matern Fetal Neonatal Med. 2005; 17: 375–380. PMID: 16009639
10. Herbst A, Kallen K. Influence of mode of delivery on neonatal mortality in the second twin, at and before term. BJOG. 2008; 115: 1512–1517. doi: 10.1111/j.1471-0528.2008.01899.x PMID: 19035987

11. Armson BA, O'Connell C, Persad V, Joseph KS, Young DC, Baskett TF. Determinants of perinatal mortality and serious neonatal morbidity in the second twin. Obstet Gynecol. 2006; 108: 556–564. PMID: 16946215

12. Arnold C, McLean FH, Kramer MS, Usher RH. Respiratory distress syndrome in second-born versus first-born twins. A matched case-control analysis. N Engl J Med. 1987; 317: 1121–1125. PMID: 3657879

13. Hacking D, Watkins A, Fraser S, Wolfe R, Nolan T. Respiratory distress syndrome and birth order in premature twins. Arch Dis Child Fetal Neonatal Ed. 2001; 84: F117–121. PMID: 11207228

14. Lee J, Seong HS, Kim BJ, Jun JK, Romero R, Yoon BH. Evidence to support that spontaneous preterm labor is adaptive in nature: neonatal RDS is more common in "indicated" than in "spontaneous" preterm birth. J Perinat Med. 2009; 37: 53–58. doi: 10.1515/JPM.2009.036 PMID: 19099368

15. Khairy P, Ouyang DW, Fernandes SM, Lee-Parritz A, Economy KE, Landzberg MJ. Pregnancy outcomes in women with congenital heart disease. Circulation. 2006; 113: 517–524. PMID: 16449731

16. van Nimwegen FA, Penders J, Stobberingh EE, Postma DS, Koppelman GH, Kerkhof M, et al. Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. J Allergy Clin Immunol. 2011; 128: 948–955 e941–943. doi: 10.1016/j.jaci.2011.07.027 PMID: 21872915

17. Xu WL, Atti AR, Gatz M, Pedersen NL, Johansson B, Fratiglioni L. Midlife overweight and obesity increase late-life dementia risk: a population-based twin study. Neurology. 2011; 76: 1568–1574. doi: 10.1212/WNL.0b013e3182190d09 PMID: 21536637

18. Bry K, Lappalainen U, Hallman M. Intra-amniotic interleukin-1 accelerates surfactant protein synthesis in fetal rabbits and improves lung stability after premature birth. J Clin Invest. 1997; 99: 2992–2999. PMID: 9185523

19. Bry K, Lappalainen U. Intra-amniotic endotoxin accelerates lung maturation in fetal rabbits. Acta Paediatr. 2001; 90: 74–80. PMID: 11227339

20. Lambermont VA, Kuypers E, Collins JJ, Pillow JJ, Newharnam JP, Polglase GR, et al. Effects of intra-amniotic lipopolysaccharide exposure on the fetal lamb lung as gestation advances. Pediatr Res. 2014; 75: 500–506. doi: 10.1038/pr.2014.3 PMID: 24441106

21. Kuypers E, Collins JJ, Kramer BW, Olman G, Nitsos I, Pillow JJ, et al. Intra-amniotic LPS and antenatal betamethasone: inflammation and maturation in preterm lamb lungs. Am J Physiol Lung Cell Mol Physiol. 2012; 302: L380–389. doi: 10.1152/ajplung.00338.2011 PMID: 22610306

22. Collins JJ, Kuypers E, Nitsos I, Jane Pillow J, Polglase GR, Kemp MW, et al. LPS-induced chorioamnionitis and antenatal corticosteroids modulate Shh signaling in the ovine fetal lung. Am J Physiol Lung Cell Mol Physiol. 2012; 303: L778–787. doi: 10.1152/ajplung.00280.2011 PMID: 22962010

23. Jobe AH. Antenatal associations with lung maturation and infection. J Perinatol. 2005; 25 Suppl 2: S31–35. PMID: 15861169

24. Kramer BW. Antenatal inflammation and lung injury: prenatal origin of neonatal disease. J Perinatol. 2008; 28 Suppl 1: S21–27. doi: 10.1038/jp.2008.46 PMID: 18446173

25. Been JV, Zimmermann LJ. Histological chorioamnionitis and respiratory outcome in preterm infants. Arch Dis Child Fetal Neonatal Ed. 2009; 94: F218–225. doi: 10.1136/adc.2008.150458 PMID: 19131431

26. Watterberg KL, Demers LM, Scott SM, Murphy S. Chorioamnionitis and early lung inflammation in infants in whom bronchopulmonary dysplasia develops. Pediatrics. 1996; 98: 210–215. PMID: 8584379

27. Dempsey E, Chen MF, Kokottis T, Vallerand D, Usher R. Outcome of neonates less than 30 weeks gestation with histologic chorioamnionitis. Am J Perinatol. 2005; 22: 155–159. PMID: 15838750

28. Andrews WW, Goldenberg RL, Faye-Petersen O, Cliver S, Goepfert AR, Hauth JC. The Alabama Preterm Birth study: polymorphonuclear and mononuclear cell placental infiltrations, other markers of inflammation, and outcomes in 23- to 32-week preterm newborn infants. Am J Obstet Gynecol. 2006; 195: 803–808. PMID: 16949415

29. Lahra MM, Beeby PJ, Jeffery HE. Maternal versus fetal inflammation and respiratory distress syndrome: a 10-year hospital cohort study. Arch Dis Child Fetal Neonatal Ed. 2009; 94: F13–16. doi: 10.1136/adc.2007.135883 PMID: 18446119

30. Kaukolaa T, Tuimala J, Herva R, Kingsmore S, Hallman M. Cord immunoproteins as predictors of respiratory outcome in preterm infants. Am J Obstet Gynecol. 2009; 200: 100 e101–108. doi: 10.1016/j.amjog.2008.07.070 PMID: 19026401

31. Shinwell ES, Blickstein I, Lusky A, Reichman B. Effect of birth order on neonatal morbidity and mortality among very low birthweight twins: a population based study. Arch Dis Child Fetal Neonatal Ed. 2004; 89: F145–148. PMID: 14977899
32. Yoon BH, Oh SY, Romero R, Shim SS, Han SY, Park JS, et al. An elevated amniotic fluid matrix metalloproteinase-8 level at the time of mid-trimester genetic amniocentesis is a risk factor for spontaneous preterm delivery. Am J Obstet Gynecol. 2001; 185: 1162–1167. PMID: 11717651

33. Cassell GH, Davis RO, Waites KB, Brown MB, Marriott PA, Stagno S, et al. Isolation of Mycoplasma hominis and Ureaplasma urealyticum from amniotic fluid at 16–20 weeks of gestation: potential effect on outcome of pregnancy. Sex Transm Dis. 1983; 10: 294–302. PMID: 6665671

34. Gray DJ, Robinson HB, Malone J, Thomson RB Jr. Adverse outcome in pregnancy following amniotic fluid isolation of Ureaplasma urealyticum. Prenat Diagn. 1992; 12: 111–117. PMID: 1553356

35. Horowitz S, Mazor M, Romero R, Horowitz J, Glezerman M. Infection of the amniotic cavity with Ureaplasma urealyticum in the midtrimester of pregnancy. J Reprod Med. 1995; 40: 375–379. PMID: 7608879

36. Gerber S, Vial Y, Hohlfeld P, Witkin SS. Detection of Ureaplasma urealyticum in second-trimester amniotic fluid by polymerase chain reaction correlates with subsequent preterm labor and delivery. J Infect Dis. 2003; 187: 518–521. PMID: 12552439

37. D’Antonio F, Dias T, Collaborative BTotobSTOR. Does antenatal ultrasound labeling predict birth order in twin pregnancies? Ultrasound Obstet Gynecol. 2013; 41: 274–277. doi: 10.1002/uog.12310 PMID: 23019097

38. Yoon BH, Romero R, Moon JB, Shim SS, Kim M, Kim G, et al. Clinical significance of intra-amniotic inflammation in patients with preterm labor and intact membranes. Am J Obstet Gynecol. 2001; 185: 1130–1136. PMID: 11717646

39. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel L, Hassan S. The role of inflammation and infection in preterm birth. Semin Reprod Med. 2007; 25: 21–39. PMID: 17205421