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Twin fetal facial expressions at 30–33 + 6 weeks of gestation

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Abstract

Objective: To assess the characteristics of twin fetal facial expressions at 30–33+6 weeks of gestation using four-dimensional (4D) ultrasound to clarify twin fetal brain development and maturation.

Methods: Frequencies of seven fetal facial expressions were studied using 4D ultrasound for 15 minutes in 30 singleton pregnancies and 18 twin pregnancies [four mono-chorionic diamniotic (MD) and 14 dichorionic diamniotic (DD) twins] at 30–33+6 weeks of gestation. Comparison of the frequency in each facial expression was performed between singleton and twin fetuses.

Results: Mouthing was the most frequent facial expression at 30–33+6 weeks of gestation, followed by blinking in twin and singleton fetuses. Both facial expressions were significantly more frequent than other expressions ($P < 0.05$). The frequencies of mouthing and scowling in twin fetuses were significantly lower than those in singleton fetuses, but there were no significant differences in the frequencies of the five other facial expressions between the fetal groups.

Conclusion: Our results suggest that restricted twin fetal behavior before 20 weeks of gestation may still affect the frequencies of twin facial expressions early in the third trimester of pregnancy. Moreover, the frequencies of facial expressions in twins are different from those of singleton fetuses.

Keywords: 4D ultrasound; facial expression; singleton fetus; third trimester of pregnancy; twin fetus.

Introduction

Degani et al. [1] studied fetal temperament in twins late in the first trimester of pregnancy and found that differences in activity during this period were followed by temperamental differences postnatally. In our previous studies [2, 3], twin fetuses showed less frequent arm movements compared with singleton fetuses at 12–13 weeks, while the frequencies of eight fetal movements (head antelexion, head retroflexion, body rotation, hand-to-face movement, general movement, arm movement, leg movement, and mouthing) in twins were significantly lower than those in singleton fetuses at 14–19 weeks. The reason for these differences in fetal movements between singleton and twin fetuses is currently unknown. One possible explanation is the restricted space available to twins *in-utero*. Mulder et al. [4] also found that twin fetuses were less active regarding general movement than singletons during gestation. Therefore, restricted space before 20 weeks *in-utero* may affect fetal behavioral movements in twin pregnancies. A question raised here is whether these neurobehavioral restrictions in twin fetuses persist in the third trimester of pregnancy.

Four-dimensional (4D) ultrasound observation of fetal behavior has been used to indirectly assess developmental maturation of the fetal brain and central nervous system (CNS) developmental stages of fetal neurobehavior [5, 6]. Behavior is a product of the functioning CNS; therefore, by investigating fetal behavior, it is possible to make a deduction regarding the functioning of the fetal brain [7]. Fetal facial expressions can mirror the fetal brain function and development in the latter half of pregnancy [8, 9]. These expressions suggest the maturation and development of different brain and CNS, which control these actions [9]. There have been numerous studies on 4D ultrasound assessment of fetal facial expressions [10–15]. To the best of our knowledge, however, there has been no study on 4D ultrasound assessment of twin fetal facial expressions.

In healthy appropriate-for-gestational-age (AGA) fetuses, the frequency of mouthing movement assessed by 4D ultrasound was significantly higher than those of six other facial expressions (yawning, smiling, tongue expulsion, scowling,

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sucking, and blinking) early in the third trimester of pregnancy [12, 15]. However, mouthing was the most frequent facial expression, but there was no significant difference between mouthing and blinking in fetal growth restriction (FGR) [16]. There has been no investigation on 4D ultrasound assessment of the frequency of twin fetal facial expressions in the third trimester of pregnancy.

According to a 4D ultrasound study, the coordination of different parts of the fetal brain controlling facial expressions became apparent after 36 weeks of gestation in healthy AGA fetuses [17]. However, the control of facial expressions by the brain in FGR fetuses may be most evident compared with AGA fetuses at 32–35 weeks of gestation due to the acceleration of neurological maturation and development [18]. A question raised here is whether the control of facial expressions by the brain in twin fetuses is apparent compared with singleton fetuses early in the third trimester of pregnancy, because twin fetal weight is lower than that of singleton fetuses.

In the present study, we evaluated the characteristics of twin fetal facial expressions at 30–33 + 6 weeks of gestation using 4D ultrasound to clarify twin fetal brain development and maturation.

Subjects and methods

This study was conducted from April 2016 to December 2018. Pregnant Japanese women, who were at 30–33 + 6 weeks' gestation and scheduled to undergo routine ultrasound examinations at Kagawa University Hospital, were asked to participate in a 15-min examination of fetal facial expressions. Only healthy non-smokers with singleton, dichorionic diamniotic (DD) or monochorionic diamniotic (MD) twin pregnancies were included in this study. Pregnancies with a high maternal or fetal risk (hypertensive disorders of pregnancy, gestational diabetes, thyroid disease, FGR, polyhydramnios, oligohydramnios, and chromosomal abnormalities) were excluded from the study. The gestational age was calculated from the first day of the last menstrual period, and confirmed by the first- or early second-trimester two-dimensional (2D) sonographic examinations.

A sonographic mid-trimester anomaly scan according to the guidelines of the International Society of Obstetrics and Gynecology was used to exclude the presence of fetal abnormalities, while growth was evaluated using Japanese growth charts [19]. All pregnancies were only examined once. If the fetal face was not clearly displayed because it was covered by body parts or due to inappropriate fetal position, the trial was repeated on another day. The growth of all fetuses was normal, and there were no abnormalities on conducting 4D ultrasound examination. There was no neonate with congenital anomalies or genetic disorders.

All examinations were performed using Voluson E8 (GE Healthcare Japan, Tokyo, Japan) with a curved array trans-abdominal transducer (1–4 MHz). Examinations and data analysis were performed by two experienced examiners (E.N. and M.A.M.A.). The study was conducted following approval by the Ethics Committee of the Kagawa

Table 1: Clinical characteristics of subjects.

Subjects	n	Maternal age (y.o.) mean (SD)	Para median (range)	Examination week (weeks) mean (SD)	Birth weight (g) mean (SD)	Sex (male/female)	Apgar score		UAPH mean (SD)	NICU admission, %	Neonatal abnormality, %
							1 min median (range)	5 min median (range)			
Singleton pregnancy	30	31.6 (5.6)	1 (0–4)	31.8 (1.3)	3133.5 (281.9)	17/13	8 (7–9)	9 (7–10)	7.300 (0.072)	3.3	0
Twin pregnancy	18	30.4 (5.1)	0 (0–3)	31.9 (1.3)	2490.4 (344.7)	9/21	8 (5–9)	9 (7–10)	7.322 (0.062)	33.3	0
Significance		NS	NS	NS	P < 0.0005	P = 0.039	NS	NS	NS	P = 0.003	NS

NICU, neonatal intensive care unit; NS, not significant; SD, standard deviation; UAPH, umbilical artery blood pH; y.o., years old.

University Graduate School of Medicine. All participants provided informed consent after a full explanation of the aim of the study.

Sixty fetuses of 48 pregnant women (30 with a singleton pregnancy and 18 with twin pregnancies) were eligible to be enrolled in the study. Twin pregnancies included 18 cases (14 DD pregnancies and four MD twin fetuses). Out of the 14 DD cases, 10 cases (20 fetuses) showed clear visualization of both fetuses, while the remaining four cases involved only one fetus that could be clearly visualized (four fetuses). MD cases involved two twin cases (four fetuses) that could be clearly visualized, while the two remaining MD cases involved one fetus only each that could be clearly observed. Therefore, twin fetuses included 24 DD and six MD fetuses. Table 1 shows the clinical characteristics of subjects included in the study.

Seven fetal facial expressions (mouthing, yawning, smiling, tongue expulsion, scowling, sucking, and blinking) were assessed using 4D ultrasound. The descriptions of these expressions were precisely explained in our previous study [15].

The frequencies of total movement are expressed as the median and range values. The unpaired *t*-test was used to assess differences in maternal age, gestational age at examination, birth weight, and umbilical artery pH (UApH). A comparison of the sex ratio, incidence of neonatal intensive care unit (NICU) admission, and incidence of neonatal abnormalities between the two groups was made using the chi-square (χ^2) test. The Mann-Whitney *U* test was used to examine differences in parity, Apgar scores, and differences in the frequencies of facial expressions. Facial expression frequencies in singleton or twin fetuses were compared using the Kruskal-Wallis one-way analysis of variance by ranks test. Statistical software SPSS, version 23, for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis, with significance set at a *P*-value <0.05.

Results

When clinical characteristics were compared between singleton and twin cases, there were no significant differences between singleton and twin pregnancies regarding maternal age, parity, examination week, Apgar score, UApH, or incidence of neonatal abnormalities. The birth weight was significantly higher in singleton cases than in twins ($P < 0.0005$). There was a significant difference in the male/female ratio between singleton and twin pregnancies. NICU admission was significantly more frequent in twin cases than in singleton fetuses ($P = 0.003$) (Table 1).

Mouthing and blinking were the most frequent facial expressions at 30–33+6 weeks of gestation in twin (Figure 1) and singleton fetuses (Figure 2). Both mouthing and blinking were significantly more frequent than the other facial expressions. When the frequency of each facial expression was compared between singleton and twin fetuses, we found that mouthing was more frequent in singleton (median, 4; range, 0–23) than in twin (median, 2; range, 0–8) fetuses ($P = 0.038$) (Figure 3). Scowling was more frequent in singleton (median, 0; range, 0–3) than in twin (median, 0; range, 0–2) fetuses ($P = 0.01$) (Figure 4).

Figure 1: Pattern of facial expressions in twin fetuses at 30–33+6 weeks.

Figure 2: Pattern of facial expressions in singleton fetuses at 30–33+6 weeks.

The frequencies of the other five facial expressions did not show any significant differences between the two groups (Table 2). There were also no significant differences between DD and MD twin fetuses.

Figure 3: Comparison of the frequencies of mouthing between twin and singleton fetuses.

Figure 4: Comparison of the frequencies of scowling between twin and singleton fetuses.

Discussion

In the current study, facial expression frequencies showed that mouthing and blinking are the most frequent facial expressions in both singleton as well as twin fetal groups. These findings are consistent with those reported in our previous study [15]. When facial expressions were compared between singleton and twin fetuses, mouthing was

less frequent in twins than in singletons. The reason for this difference is unknown. One possible cause might be accelerated brain maturation found in twin fetuses. It is known from previous reports that mouthing movements decrease with the advancement of gestation as a sign of brain maturation and development [10]. Reissland et al. [20] found that fetuses of smoker mothers displayed less mouthing movements than those of non-smokers probably due to the effect of stress. Smoking represented the stress factor that contributed to accelerated brain maturation in that study [20]. In the case of twin fetuses, the limitation of the available space *in-utero* represents a stressful situation, which might be responsible for the accelerated brain maturation as indicated by the less frequent mouthing movement compared with those in singleton fetuses at the same gestational age group. Amiel-Tison et al. [21] also reported that neurologic and physical maturity in twins after birth may be accelerated compared with those of singletons.

Regarding scowling, our results demonstrated its lower frequency in twin fetuses than in singletons. Scowling represents a state of fetal expression of pain or discomfort *in-utero* [22]. Twin pregnancy is a special experience where both fetuses experience early twin-to-twin contact [23]. Moreover, researchers found that fetuses developed habituation to stimuli as functional development of the brain reach advanced stages [24]. Therefore, the adaptations to touch stimulation by the co-twin and stressful situation of the crowded uterus might explain the lower frequency of scowling movements in twins in the third trimester compared with singleton fetuses. Such adaptation might reflect accelerated brain development in twin fetuses.

Although mouthing and scowling in twin fetuses are significantly different from those in singleton fetuses, the other facial expressions did not show any difference. This may be due to partial acceleration of the fetal brain development affecting some parts of the brain in twin fetuses while the others are not affected, resulting in selective acceleration of some brain areas as reflected by the changes in frequencies of two out of the seven studied facial expressions. This might be a result of maternal

Table 2: Comparison of the frequencies of facial expressions between singleton and twin fetuses.

Subjects	n	Mouthing median (range)	Yawning median (range)	Smiling median (range)	Tongue expulsion median (range)	Scowling median (range)	Sucking median (range)	Blinking median (range)
Singleton	30	4 (0–23)	1 (0–5)	1 (0–6)	0 (0–4)	0 (0–3)	0 (0–2)	4.5 (0–17)
Twin	30	2.5 (0–8)	1 (0–3)	1 (0–5)	0 (0–3)	0 (0–2)	0 (0–1)	2.5 (0–17)
Significance		P = 0.038	NS	NS	NS	P = 0.01	NS	NS

NS, not significant.

immune activation which happens when environmental factors *in-utero* are not favorable due to crowding of the uterus by the co-twin [25].

A limitation of this study is the small number of subjects, weakening the ability to draw a strong conclusion from the obtained results. This was due to the inability to obtain clear views of fetal faces in the case of a crowded uterus with twins of a relatively large size at such a late gestational age. A second limitation is that although our team has shown good inter- and intra-observer reproducibility in fetal behavior studies [26], fetal facial expressions still lack a universally accepted coding system. Therefore, studies with a larger sample size or multicentric studies using a coding system are needed to determine the real possible impacts of restricted spaces *in-utero* on functional development of the fetal brain and CNS in twin compared with singleton fetuses. The third limitation is that there is a significant difference in fetal sex between the two groups. Regarding the sex difference in fetal behavior and facial expressions between male and female fetuses, our previous study using 4D ultrasound showed that there is no sex difference in fetal behavior and facial expressions in the third trimester of pregnancy [27]. Therefore, a 4D ultrasound study examining fetal facial expressions may not need to consider the factor of fetal sex.

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