


## BRIEF REPORT

# Developmental trajectories of general and breathing movements in fetal twins

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## Abstract

This study examined the developmental trajectories of general and breathing movements in fetal twins. Fetal movement patterns were assessed from real-time ultrasound recordings performed at 12-15, 20-23, and 28-32 weeks of gestation in 42 twin pairs. Results indicated that both general movements and breathing movements followed a curvilinear, inverted U-shaped curve. Developmental trajectories were unrelated within pairs of twins and were not associated with gestational age at birth and birth weight. However, sex differences were found for general movements with males displaying more time making general movements at 21 weeks and a steeper decline in time spent making general movements during the second half of pregnancy than females. These age-related changes in fetal movements may reflect CNS development. These findings also suggest that twins' behavioral development is largely independent of co-twin development, gestational age at birth, and birth weight, but not of fetal sex.

## KEYWORDS

breathing movements, fetal behavior, fetal movements, general movements, twins

## 1 | INTRODUCTION

Spontaneous fetal movements are a well-established marker of the maturation of the central nervous system (CNS) during singleton pregnancy (Bots, Broeders, Farman, Haverkorn, & Stolte., 1978; de Vries & Fong, 2006, 2007; Mulder & Visser, 2016). Fetal movements appear between 7.5 and 15 weeks of gestation, and most follow a developmental course (de Vries, Visser, & Prechtl, 1982; Lüchinger, Hadders-Algra, van Kan, & de Vries, 2008). For example, the incidence of general movements (whole-body movements with variable amplitude, speed, and patterning of body parts) increases until the end of the first trimester and has a gradual decline during the second half of pregnancy (D'Elia, Pighetti, Moccia, & Santangelo, 2001;

de Vries et al., 1982; de Vries, Visser, & Prechtl, 1985; Govindan, Wilson, Murphy, Russel, & Lowery, 2007; Kisilevsky, Hains, & Low, 1999; Lüchinger et al., 2008; Roodenburg, Wladimiroff, van Es, & Prechtl, 1991; ten Hof et al., 1999). This decreasing trend during the second half of pregnancy has been attributed to the development of inhibitory neural mechanisms and to the gradual development of different fetal behavioral sleep states (Nijhuis, Prechtl, Martin, & Bots, 1982; ten Hof et al., 1999; van Kan, de Vries, Lüchinger, Mulder, & Taverne, 2009). Breathing movements, characterized by a simultaneous inward movement of the thorax and an outward movement of the abdomen, are another example of a fetal movement pattern that appears to follow a clear developmental course during of gestation. Breathing movements gradually increase with advancing gestational

age (de Vries et al., 1985; Govindan et al., 2007; Kisilevsky et al., 1999), and some studies report a decline near term (Carmichael, Campbell, & Patrick, 1984; Pillai & James, 1990; Roodenburg et al., 1991; van Vliet, Martin, Nijhuis, & Prechtl, 1985). However, there is a wide variation in the percentage of time spent breathing in uncomplicated pregnancies, 0.1%–79.6% at 30–31 weeks of gestation and 0%–86.2% at 38–39 weeks of gestation (Patrick, Campbell, Carmichael, Natale, & Richardson, 1980).

Spontaneous fetal movements are also a marker of the maturation of the CNS in monoamniotic twins (Mayumi et al., 2013). However, very little is known about fetal movements in all types of twins (Nowlan, 2015). The developmental trend of twins' spontaneous activity from 10 to 22 weeks of gestation was described (Piontelli, Bocconi, Boschetto, Kustermann, & Nicolini, 1999). Although the authors provided information on the spontaneous activity for each twin of the 15 pairs included in the sample, no average developmental trend was reported for the 13 specific movement patterns studied. The developmental trends of general movements and breathing movements between 20 and 35 weeks of gestation in 18 twin pairs were also studied (Mulder, Derks, de Laat, & Visser, 2012). General movements decreased with advancing gestation, whereas breathing movements increased over time and then appeared to decline during the last weeks of gestation, as previously found in singletons (Carmichael et al., 1984; Mulder et al., 2012; Pillai & James, 1990; Roodenburg et al., 1991). A significant intra-pair association of spontaneous fetal movements was found, suggesting that twins' fetal behavioral development is non-independent (Mulder et al., 2012; Mulder, Derks, & Visser, 2004). Information on developmental patterns of twins' fetal movements studied by four-dimensional ultrasound (4-D) are lacking. So far, this technology has been used to study inter-twin differences in fetal activity (Degani, Leibovitz, Shapiro, & Ohel, 2009), inter-twin contacts (Castiello et al., 2010; Hata, Sasaki, & Yanagihara, 2012; Kurjak et al., 2013; Sasaki, Yanagihara, Naitoh, & Hata, 2010), reflex movements (Hata, Kanenishi, Sasaki, & Yanagihara, 2011), and to assess differences between singletons and twins (AboEllail et al., 2018; Kurjak et al., 2013; Mori et al., 2018).

Fetal movements appear to be associated with a number of factors including fetal sex, gestational age at birth, and birth weight. Regarding fetal sex, the results are inconsistent, with some studies showing that male fetuses are more active than female fetuses (Almli, Ball, & Wheeler, 2001; DiPietro, Hodgson, Costigan, Hilton, & Johnson, 1996), while most studies failed to find any sex differences in fetal movements incidence (Conde et al., 2010; de Vries, Visser, & Prechtl, 1988; Hata et al., 2016; Mulder et al., 2012; Reissland, Francis, Aydin, Mason, & Exley, 2014; Robles de Medina, Visser, Huizink, Buitelaar, & Mulder, 2003). The absence of breathing movements has been identified as the best predictor of preterm birth (Boots et al., 2014). Reduced body and breathing movements (with ruptured membranes) have been found in fetuses born prematurely (Kisilevsky et al., 1999). Similarly, reduced fetal movements at term have also been associated with the birth of small for gestational age (SGA) infants (Pagani et al., 2014).

This study examined the developmental trajectories of general and breathing movements in fetal twins. The effect of co-twin, fetal sex, gestational age at birth, and birth weight was also examined. Our study attempts to address some of the limitations of prior research on twins' spontaneous fetal movements by employing a prospective longitudinal design in a larger population covering the three trimesters of pregnancy and the use of a dyadic statistical approach.

## 2 | METHODS

### 2.1 | Participants

Forty-five twin pairs monitored in antenatal clinics of three public hospitals in northern Portugal (Hospital São Sebastião, Hospital São João and Hospital Pedro Hispano) were enrolled in this longitudinal study at 12–15 weeks of pregnancy. The duration of pregnancy was calculated from the first day of the last menstrual cycle and confirmed by early ultrasound. Three twin pairs were subsequently excluded due to in utero death of one or both twins. Thus, the final sample comprised 42 twin pairs ( $N = 84$  individuals).

The sample included 17 female-female, 13 male-male, and 12 male-female twin pairs. Most pregnancies were bichorionic bi-amniotic (90.5%). More than half of the women had no complications during pregnancy (61.9%) and delivered by cesarean section (64.3%). The mean gestational age at birth was 36.1 weeks ( $SD = 2.7$ , range = 27.7–38.7) and half of the twin pairs were born preterm (<37 weeks of gestation). More than a quarter (26.2%) was born small for gestational age (weight <10th percentile). Most twins had normal 1-min and 5-min Apgar scores (89.3% and 98.8%, respectively). In about one third of twin pairs (31.0%), at least one was admitted to an intensive care unit.

### 2.2 | Procedure

After approval from the Ethics Committee of each participating hospital, parents of twins were recruited from those referred by their physicians. Inclusion criteria included carrying a twin pregnancy, having less than 15 weeks of gestation, and knowing how to read and write in Portuguese to complete self-administered questionnaires. Written informed consent was obtained from both parents. Fetal movements were recorded after the routine ultrasound assessment at 12–15, 20–23, and 28–32 weeks of gestation for 20 min using real-time ultrasound with a multifrequency transabdominal probe. These observations were recorded on DVD for later off-line fetal movement analysis. A longitudinal view of the fetuses was preferred with the head, trunk, and upper limbs visible, as well as the potential contact area of the twins. The evaluation of fetal movements was done without magnification of the imaging, trying to obtain the maximum scan area. Slight movements of the probe were done during the evaluation trying to identify movements of the extremities. Whenever possible, both fetuses were monitored simultaneously ( $n = 81$  recordings).

to distinguish between active (spontaneous and evoked by the co-twin movements) and passive fetal movements. However, when an adequate view of both twins was not attained they were monitored in two consecutive periods ( $n = 40$  recordings).

Except for those who delivered before the scheduled last prenatal assessment ( $n = 3$ ), the other participants completed all prenatal assessments. Nevertheless, two recordings were not available for analysis due to technical problems.

Recordings of simultaneously monitored twins were scored by two researchers (I.T. and E.M.), each scoring the movements of one fetus. The scoring of the fetus positioned on the left or right side of the monitor screen was randomized at the first recording and was maintained at subsequent recordings whenever the identification of each twin was possible (e.g. by sex difference). Recordings of twins separately monitored were all scored by one researcher (I.T.). Hand-held pushbuttons were used to score two prenatal movement patterns – general and breathing movements – and the data were fed into a computer. General movements (spontaneous or evoked) were scored by pressing one button as long as the movement was performed. Passive movements due to co-twin movement were overlooked. As fetal breathing movements last  $<1$  s, they were marked as discrete events.

Smoothing procedures previously applied to data from singletons (Mulder et al., 2004; ten Hof et al., 1999) and twins (Mulder et al., 2012) were performed with an in-house software package (UMC Utrecht). Accordingly, a single burst was considered when consecutive general movements occurred within 1 s of each other, as suggested in previous research (ten Hof et al., 1999). Breathing movements occurring within 6 s apart were regarded as bouts of continuous breathing activity, consistent with previously developed criteria (Mulder et al., 2012; Patrick et al., 1980).

The duration of general movement bursts and breathing movement bouts (in seconds) was determined, summed, and expressed as percentage of observation time (also commonly known as % incidence) for each fetus and fetal assessment. The following fetal movement parameters were also calculated: *mean number*; *mean total duration of bursts/bouts*; *mean and median burst/bout duration*; *median onset-onset interval*: median interval between the onset of a burst/bout and the onset of the next successive burst/bout (ten Hof et al., 1999).

## 2.3 | Measures

Twins' fetal behavior was assessed through the observation of real-time ultrasound recordings. General movements involve the whole body (head, limbs, and trunk) and have variable amplitude, speed, and patterning of body parts. Breathing movements are characterized by a simultaneous inward movement of the thorax and an outward movement of the abdomen. After extensive training, interrater reliability on the identification of these fetal movement patterns was calculated using 6 hr of recordings of fetal data. There was good to excellent agreement between the two raters (I.T. and E.M.) for general movements and breathing movements (intraclass correlation coefficients: 0.65 and 0.94, respectively).

## 2.4 | Data analysis

Dyadic growth curve models were estimated using multilevel modeling to examine the developmental trends of fetal movement patterns and to account for non-independence of scores within twin pairs and across time. This procedure is highly innovative for this kind of research and has not been used for similar purpose in fetal twin studies. Twins were treated as indistinguishable dyads because no meaningful criterion could be used to systematically distinguish each twin member (Kenny, Kashy, & Cook, 2006). Based on previous research, fetal sex was potential meaningful criterion to distinguish each twin member. However, as our sample included both different-sex and same-sex twins this criterion could not be used. Accordingly, each twin member was classified as Twin 1 or Twin 2, even though this classification was arbitrary (Kashy, Donnellan, Burt, & McGue, 2008). Although twins are treated as indistinguishable for the statistical analysis, the assignment of each twin as either 1 or 2 was maintained in all time points. If the assignment to Twin 1 or Twin 2 was switched for some dyads, the estimates would change (Kashy et al., 2008). All parameters of the statistical models used were constrained to be the same across twins in the same pair (for a detailed explanation of dyadic growth curve models see Kashy et al., 2008; Olsen & Kenny, 2006). Time was centered at 21 weeks of gestation so that the intercept represents the average incidence of fetal movements at mid-pregnancy. Slope coefficients represent the average change in fetal movements for each 1-week increase in time. Natural logarithmic transformation of the fetal breathing movement variable was performed based on a positively skewed distribution.

Model selection was based on a sequential process of comparing nested models using likelihood ratio tests (Singer & Willett, 2003). Maximum likelihood estimation was used for significance assessment, whereas restricted maximum likelihood was used in reporting estimates of best fitting models. Fetal sex, gestational age at birth (term vs. preterm), and birth weight (appropriate vs. small for gestational age) were tested as dichotomous predictors of mean levels and trajectories of fetal movements. Interdependence of fetal movements was assessed by time-specific correlation between the residuals at each time point with the significant predictors controlled for in the model. No significant differences in inter-pair trajectories of change were identified on any of the outcome variables as tested by the inclusion of random slopes, suggesting a similar rate of change among twins. Thus, only intercepts were allowed to covary within and between twin dyads. These analyses were conducted with MLwiN 2.22 (Centre for Multilevel Modelling, University of Bristol).

## 3 | RESULTS

### 3.1 | Descriptive analysis

Fetal movement analyses were based on 161 observation periods that lasted on average for 20-min (range 13-27 min) with a total time

of 4,070 min. There were three observations per twin pair available for analysis (Mean; range 2–3). Descriptive statistics of fetal movement parameters are displayed in Table 1.

### 3.2 | General movements and breathing movements' developmental trajectories

The estimates of the best fitting dyadic growth curve models for both fetal movement patterns are presented in Table 2. Figure 1 illustrates the mean developmental course of each fetal movement pattern.

#### 3.2.1 | General movements

The average percentage of time spent making general movements was 18.7% at 21 weeks of gestation. Significant linear and quadratic fixed effects were found for gestational age. The percentage of time spent making general movements increased from 14.1% at 12 weeks to 17.8% at 19 weeks and, thereafter, decreased progressively reaching the lowest value (5.4%) at 32 weeks of pregnancy. No significant intercept variance was found at 21 weeks of pregnancy.

The median onset-onset intervals between bursts per twin at each time point are presented in Figure S1.

#### 3.2.2 | Breathing movements

The average percentage of time spent making breathing movements was 9.6% (converted value of log transformed variable) at 21 weeks of gestation. Significant linear and quadratic fixed effects were found for gestational age. The predicted percentage of time spent making breathing movements increased steeply from 1.6% at 12 weeks to 11.2% at 26 weeks of pregnancy and, thereafter, decreased

**TABLE 1** Mean number, bursts and bouts duration, and onset-onset intervals (in seconds) of fetal movements obtained in 20 min observation periods at each gestational age (in weeks)

	12–15	20–23	28–32
General movements			
Mean number	88.01	88.91	51.74
Burst duration			
Mean total	176.46	196.01	104.29
Mean	2.22	2.24	1.91
Median	1.13	1.06	1.07
Median duration of onset-onset interval	5.96	7.22	16.86
Breathing movements			
Bout duration			
Mean total	45.72	150.11	189.55
Mean	8.15	7.59	10.23
Median	3.59	3.29	4.33
Median duration of onset-onset interval	44.85	46.03	41.18

progressively reaching 9.2% at 32 weeks of pregnancy. No significant intercept variance was found at 21 weeks of pregnancy.

### 3.3 | Factors associated with general movements and breathing movements' developmental trajectories

Dyadic growth curve models showed no significant intra-pair correlation with the intercepts and with the residuals for the overall percentage of time spent making general movements and breathing movements at the three time points of pregnancy, indicating that over gestation the percentage of time spent moving is independent of the co-twin (Table 2).

Fetal sex was a significant predictor of general movements at mid-pregnancy, such that male fetuses exhibited a higher percentage of time spent making general movements than female fetuses. We found a sex difference in the quadratic effect of gestational age. Males' general movements decrease steeply after mid-pregnancy such that by 32 weeks no differences in the percentage of time spent making general movements can be found between males and females. No significant sex differences were found for breathing movements at mid-pregnancy or throughout the gestation.

There were no significant effects of gestational age at birth and birth weight on mean levels and developmental course of general movements and breathing movements.

## 4 | DISCUSSION

This study showed that general movements and breathing movements of twins followed a clear developmental trend. These age-related changes in fetal movements may reflect CNS development. Sex differences were found for general movements mean levels and developmental trajectories, but not for breathing movements. General movements and breathing movements' developmental trajectories were independent of the co-twin, gestational age at birth, and birth weight.

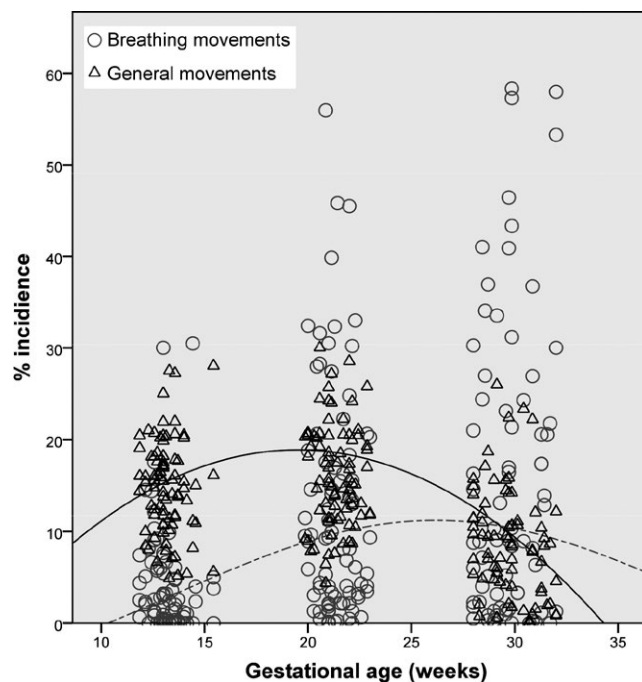
Both fetal movement patterns followed a curvilinear developmental trend. General movements increased up to 18 weeks of gestation and then showed a decline over the second half of gestation, whereas breathing movements increased up to 26 weeks of gestation and declined thereafter. These overall trends are generally in line with the developmental characteristics previously found in singletons (Carmichael et al., 1984; D'Elia et al., 2001; de Vries et al., 1985, 1988; Luchinger et al., 2008; Pillai & James, 1990; Roodenburg et al., 1991; ten Hof et al., 2002, 1999). However, some specific differences were noted. We found an earlier decrease in general movements and breathing movements incidence in twins compared with singletons as reported in the literature (ten Hof et al., 2002). These results might be explained by accelerated maturation, lack of intrauterine space, or anticipation of premature birth (Mulder et al., 2012). It is also possible that the observed differences could be explained by the reduced observation period and the lack of statistical control of prandial effects and circadian rhythms.

**TABLE 2** Estimates (mean; SE) of dyadic growth models for fetal movement patterns

Parameter	General movements		Breathing movements (Ln)	
	<i>b</i>	SE	<i>b</i>	SE
<b>Fixed effects</b>				
Intercept	18.69***	0.98	2.98***	0.06
Female	-2.39***	0.71	—	—
GA	-0.26**	0.08	0.03***	0.01
GA squared	-0.09***	0.02	-0.003**	0.001
GA x Female	-0.06	0.06	—	—
GA squared x Female	-0.03*	0.01	—	—
<b>Random effects</b>				
	General movements		Breathing movements (Ln)	
	Estimate	SE	Estimate	SE
<b>Variances</b>				
Intercept	2.52	2.02	0.02	0.02
Residual	25.45***	2.86	0.22***	0.03
<b>Correlations</b>				
Intercept-intercept	0.56		-0.21	
Between residual	-0.01		0.20	

Note. GA: gestational age; Ln: natural logarithm.

\* $p < 0.05$ . \*\* $p < 0.01$ . \*\*\* $p < 0.001$ .



**FIGURE 1** Percentage of time spent making general movements and breathing by each twin ( $n = 161$  observations) and regression lines (solid line - general movements; dashed line - breathing movements) with the expected percentage of time moving over the study period

Our results are also partially consistent with those reported in a previous study on the spontaneous activity of twins during the second half of pregnancy (Mulder et al., 2012). These authors found a linear decrease in general movements and a linear increase in breathing movements from 20 to 35 weeks' gestation. We also found a linear decreasing trend for general movements from 18 weeks' gestation onwards. However, our results show that breathing movements follow a non-linear developmental trend. Several methodological differences may explain the different results, even though the same smoothing procedures have been used (ten Hof et al., 1999). We were not able to control for prandial effects as Mulder et al. (2012) did and previous research indicates that breathing movements increase two to three hours after meals (Patrick et al., 1980). Second, Mulder et al. (2012) monitored both twins simultaneously using two real-time ultrasound machines for 60 min, while in the current study the observation period was limited to 20 min and only one real-time ultrasound machine was used to monitor both twins simultaneously or one after the other when an adequate view of both fetuses was not accomplished. Given that breathing movements usually occur in bursts and not as single events (Roodenburg et al., 1991), it is likely that observation periods of different duration influence the obtained results. In addition, there is a wide variation in the percentage of time that fetuses spend on breathing movements (de Vries et al., 1985; Patrick et al., 1980). Third, the Mulder et al. (2012) study included only normal



dichorionic twin pregnancies, whereas in the current study complicated twin pregnancies were also included.

In line with most previous research (Conde et al., 2010; de Vries et al., 1988; Hata et al., 2016; Mulder et al., 2012; Reissland et al., 2014), no differences were found between males and females in mean levels and trajectories of breathing movements. However, sex differences were found for general movements, such that male were more active than female fetuses at 21 weeks of gestation and males had a steeper decline in general movements incidence during the second half of pregnancy. Other studies have also found that male fetuses are more active than female fetuses (Almli et al., 2001; DiPietro et al., 1996). Sex differences in fetal activity have been found in small samples studies (Almli et al., 2001; DiPietro et al., 1996) and, therefore, it is also possible that they are due to Type I error (DiPietro et al., 2004). Other factors including differences in the rest-activity cycles that appear to emerge around 19–20 weeks (de Vries et al., 1985; Swartjes, van Geijn, Mantel, van Woerden, & Schoemaker, 1990) and differences in the amniotic volume could have also influenced the results reported here. In fact, one study found a higher incidence of general movements in male fetuses, but this difference was not significant when fetal wakefulness (and sleep states in general) was taken into account (Robles de Medina et al., 2003). This study finds lack of intra-pair association in fetal movement patterns during pregnancy and contrasts with previous studies (Mulder et al., 2012, 2004). Methodological differences such as simultaneous versus non-simultaneous observation periods of twins within pairs and short (20 min) versus long (60 min) observation periods may explain differences in results between studies. Similarity in twins' fetal movements are probably noticeable with simultaneous monitoring and for extended periods of observation.

Fetal movement incidence and developmental trajectories were unrelated to gestational age at birth and birth weight. These results are consistent with those reported by Kisilevsky et al. (1999) who found similar maturation patterns for body and breathing movements between the low- and high-risk fetuses suggesting normal/typical functional development in the high-risk groups. The authors argue that this indicates that premature birth is most likely precipitated by a recent insult or unfavorable environment rather than an event commencing early in gestation.

The longitudinal assessment of fetal movements is a major strength of this study. However, several limitations need to be recognized, namely the relatively small sample size, but still larger than in most other studies (Tendais, Visser, Figueiredo, Montenegro, & Mulder, 2013). Other limitations include lack of simultaneous observation of both twins' fetal movements in a third of the recordings, the inability to control for possible confounders like diurnal rhythms and prandial effects.

This study provides new insights into gestational age-dependent changes in twins' fetal general movements and breathing movements using short ultrasound observations after routine ultrasound assessments. Our results suggest that twins may have specific developmental trajectories in fetal movement patterns. Normal references for fetal movements might differ for twins and singletons, especially

during the second half of gestation. The earlier decrease in the time spent making these fetal movement patterns may be considered as an adaptation or anticipation to crowding, diminished uteroplacental supply, and birth before term (Mulder et al., 2012). Future studies are needed to confirm the obtained results in normal pregnancies and those complicated by maternal and fetal conditions.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## SUPPORTING INFORMATION

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