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REGULAR ARTICLE

Foetal mouth movements: Effects of nicotine

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Abstract

Aim: To assess whether foetal mouth movement frequency changes across gestation and whether there are differences between cigarette and e-cigarette exposure conditions in comparison to a non-exposed group of foetuses.

Method: Pregnant women underwent 4-dimensional (4D) foetal ultrasound scans at 32 weeks (106 scans) and 36 weeks of gestational age (87 scans) at James Cook University Hospital, UK. The 4D scans were coded using the Fetal Observable Movement System (FOMS). Measures of maternal smoking status, stress, depression, anxiety, attachment and time of scan were also collected. There were four exposure groups: non-smokers, light smokers (<10 per day), heavy smokers (11–20 per day) and e-cigarette users.

Results: No significant differences in relative frequency of mouth movements between the exposure groups at 32- and 36 weeks of gestational age were found. Foetal mouth movements declined from 32 to 36 weeks of gestation for non-exposed and e-cigarette-exposed foetuses.

Conclusion: Due to variability in foetal behaviour, examining mouth movements alone may not be the most appropriate method for assessing group differences. However, in line with other research, mouth movement frequency declined between 32- and 36 weeks of gestational age. A combination of foetal behavioural assessments is needed to assess the effects of cigarette and e-cigarette exposure on foetal neurobehavioural development.

KEYWORDS
cigarette smoking, e-cigarettes, Fetal behaviour, pregnancy

1 | INTRODUCTION

Mouth movements can provide an indication of the central nervous system (CNS) development in the foetus, with the potential to identify normal and abnormal development in utero.[1] In attempts to begin to examine this, research using 4D ultrasound scans has identified that when the mother experiences extreme sickness and lack of nutrition in her pregnancy, these foetuses had significantly higher rates of mouth movements as identified by the Fetal Observable Movement System (FOMS)[2] at 32 weeks of gestation in comparison to non-affected foetuses.[3] Similarly for genetic disorders such as Prader–Willi Syndrome, a postnatally diagnosed foetus

Abbreviations: 2D/4D, 2-/4-dimensional; CNS, Central nervous system; CO, Carbon monoxide; ECG, Electrocardiogram; FENS, Fetal Neurobehavioural Assessment System; FOMS, Fetal Observable Movement System; KANET, Kurjak's Antenatal Neurodevelopmental Scoring Test; nAChRs, Nicotine acetylcholine receptors; NBAS, Neonatal Behavioural Assessment Scale; OSF, Open Science Framework.

displayed significantly fewer mouth movements in comparison to a control group of healthy foetuses.[4] Given that these foetuses displayed different patterns of behaviour in comparison to healthy controls, it could be argued that maternal health status and foetal genetic disorders can affect the development and function of the CNS differently. Additionally, maternal smoking during pregnancy has been shown to lead to different foetal mouth movement profiles.[5]

One small study indicated that foetuses exposed to maternal smoking ($N = 4$) had an overall higher rate of mouth movements, as identified by the FOMS, in comparison to non-exposed foetuses ($N = 16$).[5] The authors suggested that the foetal CNS was affected as a consequence of maternal smoking during pregnancy resulting in differences in mouth movements between the exposure groups.[5]

Similar results have been shown when assessing gross foetal body movements via 2D ultrasound scans.[6] In contrast, when assessing quality and quantity of global foetal movements, comparing non-exposed, light exposed (<10 per day) and heavy exposed (11–20 cigarettes per day), the only significantly different group was the heavy exposed foetuses. These foetuses demonstrated a decrease in movements that were sluggish in comparison to the other two groups where the movement was brisk.[7] The evidence is contradictory for the effects of maternal smoking on foetal movements, possibly owing to the differences in methodology (i.e., number of cigarettes smoked, 2D and 4D ultrasound scans, gross body movements and facial movements).

Similarly, frequency of foetal mouth movements declines from 24 to 36 weeks of gestational age, at the rate 1.5% for smoke-exposed and 3% for non-smoke-exposed foetuses, per additional gestational week.[5] Similar results have been shown in studies assessing gross foetal body movements and complex body movements.[8]

The present study will use the same methodology as Reissland et al.[5] to examine foetal facial movements in relation to nicotine exposure but with a larger sample of foetuses including two cigarette (light and heavy) and one e-cigarette-exposed group compared to a control group. New to this study is the effects of e-cigarettes on foetal behaviour, specifically mouth movements. The effects could be very different from smoking cigarettes especially in light of previous research attributing the effects of smoking on foetal activity to carbon monoxide (CO) exposure due to placenta insufficiency as a result of reduced oxygenation.[7,9]

Firstly, we expect differences in foetal mouth movement profiles across the four exposure groups. Secondly, as the CNS development becomes more coordinated and precise movements can be observed, we anticipate that mouth movement frequencies will differ at 32- and 36 weeks of gestational age.

Key Notes

- Relative frequency of mouth movement does not differ between exposure groups.
- Rate of mouth movements declines across gestation for non-exposed and e-cigarette-exposed foetuses.
- More specific assessments of foetal behaviour may be necessary to identify neurobehavioural differences across nicotine exposure groups.

2 | METHOD

2.1 | Participants

The foetal scans for this research were undertaken at James Cook University Hospital, Middlesbrough and the Friarage Hospital, Northallerton, UK. A total of 123 pregnant women were recruited to participate in the study assessing the impact of smoking status on foetal mouth movements. Potential participants who met the inclusion criteria were identified by the hospital sonographers at their 20-week anomaly scan. The inclusion criteria consisted of currently not taking any medication or recreational drugs for a medical or mental health condition, not diagnosed with a medical problem that may affect the foetus, low-risk pregnancy, BMI between 18–25 and aged between 18–40 years old.

Pregnant women provided informed consent prior to participating in the research. Ethical approval was granted by Durham University and the NHS ethics committee (REC reference, 11/NE/0361).

The number of women recruited in each smoking status group and scans coded at 32 and 36 weeks is shown in Table 1. Although we were able to recruit 123 women into the study, not all scans could be coded and analysed due to a variety of reasons. At 32 weeks, some scans were not analysed due to the foetal mouth areas not visible ($N = 16$) or because of technical difficulties with the recording of the scan ($N = 1$). At 36 weeks, additional to the factors mentioned above ($N = 25$) some women dropped out of the research ($N = 9$) or had already given birth ($N = 2$). The number of scans analysed at 32 weeks differs by 1 participant between this paper and the pre-registration report, as further examination identified one of the scans was not of good enough quality. Based on the data at 32 weeks, the smallest effect size the achieved sample was powered to detect (80%) was $d = 0.64$, and $d = 0.72$ at 36 weeks.

Mothers attended a 30-min 4D ultrasound appointment with an NHS qualified sonographer at James Cook University Hospital or the Friarage Hospital. The scan lasted approximately 15–20 min, and time of day the scan took place was recorded. During this appointment, all mothers regardless of exposure group were asked to do a Smokerlyzer breath test using the Bedfont Smokerlyzer piCObaby™ to obtain a CO reading for both mother and foetus. This was used to assess level of CO at the time of the scan. Associations between maternal psychological state and foetal movement[5,10] have been well documented, and therefore, we collected measures of stress (Perceived Stress Scale (PSS)),[11] anxiety, depression (Hospital Anxiety and Depression Scale (HADS))[12] and attachment (Antenatal Attachment Scale).[13] Additionally, mothers completed a smoking questionnaire indicating the number of cigarettes smoked per day, whether they had

quit smoking and whether they use nicotine replacement therapy or e-cigarettes. If using an e-cigarette, milligrams of nicotine were identified via maternal self-report, ranging from 3–16 mg (M = 7.76 mg, S. D. = 4.76). None of the mothers reported dual use of an e-cigarette and cigarettes. The scans took place at 32- and 36 weeks of gestational age.

There is limited observational research assessing the effects of time of day on foetal behaviour and activity, with most research focussing on maternal perceptions of movements. According to such research, mothers have an increased awareness of foetal movements from afternoon (12–6 PM) to evening (6–8 PM) and night-time (8-midnight).[14] However, there are a number of factors that may influence the perception of increased awareness of foetal movements in the evening such as maternal positioning and relaxation,[15] hence the importance of including an objective measure of foetal movement in relation to time of day in the present study.

The 4D ultrasound scans were coded frame by frame offline using the Observer XT. The Fetal Observable Movement System (FOMS) that assesses foetal facial muscles was used to code a number of different mouth movements (see Figure 1).[2] The only facial movements coded were mouth movements, as was the case in Reissland et al.[5] Reliability of coding was assessed on approximately 10% of the scans by an independent coder, blind to the study conditions. Based on 20 scans, mean Cohen's Kappa the mean was 0.86 and ranged between 0.75–0.98. Mean re-test reliability was 0.97 and ranged between 0.92–1, indicating high reliability.

2.2 | Data analysis

A pre-registration plan was submitted to the Open Science Framework (OSF) (https://mfr.osf.io/render?url=https://osf.io/xn768/?direct%26mode=render%26action=download%26mode=render) outlining our hypotheses, a priori predictions and data analysis plan. We hypothesised that there will be differences in the frequency of foetal mouth movements across the four exposure groups. We also expected that there will be a difference in frequency of foetal mouth movements between the 32- and 36 weeks of gestational data.

The total relative frequency of combined foetal mouth movements per minute was the outcome measured. There are 11 different mouth movements that were coded using the FOMS including lip corner depressor, lip pressor, lip pucker, lip pull, lip stretch, lip suck, lower lip depressor, upper lip raiser, lips parting, mouth stretch and tongue show.

As stated in the OSF plan, we planned to run a correlation between the 32- and 36 weeks of gestational age data, and if the data were correlated, only one ANOVA would be conducted on the 32-week data due to the larger sample. If there was not a significant correlation, two separate ANOVAs tests would be conducted, one referring to movement at

TABLE 1 Number of scans analysed per smoking condition

Smoking status	Recruited	Scans coded at 32 weeks	Scans coded at 36 weeks
Non-smokers	54	46	34
Light cigarette smokers (<10 per day)	38	32	27
Heavy cigarette smokers (11–20 per day)	15	13	12
E-cigarette users	16	15	14
Total	123	106	87

FIGURE 1 Examples of foetal mouth movements including a neutral mouth, tongue show and lower lip depressor

TABLE 2 Means and standard deviation of total relative frequency of mouth movement per minute, stress, depression, anxiety, attachment and maternal CO

	32-week relative frequency M (S.D.)	32-week stress M (S.D.)	32-week depression M (S.D.)	32-week anxiety M (S.D.)	32-week attachment M (S.D.)	32-week maternal CO M (S.D.)	36-week relative frequency M (S.D.)	36-week stress M (S.D.)	36-week depression M (S.D.)	36-week anxiety M (S.D.)	36-week attachment M (S.D.)	36-week maternal CO M (S.D.)
Not exposed	4.66 (4.14)	9.37 (6.09)	2.83 (2.34)	4.49 (2.84)	83.02 (6.14)	0.98 (0.14)	2.67 (2.08)	8.76 (5.83)	3.35 (2.80)	4.41 (3.27)	85.41 (5.08)	0.94 (0.19)
	N = 46						*N* = 34					
Light exposure (<10 per day)	3.78 (4.22)	13.06 (6.81)	5.06 (3.11)	5.59 (3.27)	81.16 (6.42)	2.40 (0.93)	2.83 (2.58)	12.19 (5.96)	4.92 (2.75)	5.46 (3.36)	83.58 (6.94)	2.33 (1.13)
	N = 32						*N* = 27					
Heavy exposure (11–20 per day)	1.97 (0.88)	14.92 (8.98)	5.85 (4.35)	7.31 (4.38)	82.80 (7.85)	3.43 (1.05)	4.29 (4.76)	13.75 (8.63)	5.42 (4.50)	7.50 (4.60)	84.63 (9.38)	3.01 (1.02)
	N = 13						*N* = 12					
E-cigarette-exposed (3–16 mg)	8.58 (10.07)	16.60 (6.82)	4.07 (3.30)	6.33 (3.26)	83.67 (3.55)	0.96 (0.17)	3.32 (2.33)	12.64 (6.29)	3.00 (1.95)	4.62 (2.90)	89.42 (2.81)	0.76 (0.30)
	N = 15						*N* = 14					

32 weeks and one at 36 weeks of gestational age to assess the first hypothesis. Should any potential confounding factors (stress, depression, anxiety, attachment and time of scan) be significantly associated with the outcome measure, then an ANCOVA would be carried out. Time of day the scan took place was not reported as a potential covariate in the OSF plan; however, due to these data being collected and the literature indicating a possible association, it was added at the analysis stage of conducting this research. We outlined that a mixed model ANOVA would be conducted to assess our second hypothesis.

As the data did not meet the assumptions of ANOVA, including non-normal data and homoscedasticity, non-parametric tests were used. As the data did not meet the assumptions for an ANCOVA, the correlations will be reported. Significantly correlated variables were included into a regression analysis, with a subsequent Kruskal–Wallis test using the residuals. To correct for multiple comparisons, the Benjamini–Hochberg false discovery rate procedure was applied.[16]

Although not reported in the OSF plan, it was later decided to include a pooled cigarette exposure group analysis to examine whether once light and heavy smokers were combined, as is the case for Reissland et al.[5] whether findings would be similar to those reported in the pilot study.

3 | RESULTS

Table 2 and Figure 2 visually display the means across the exposure groups for both 32- and 36 weeks of gestational age. Table 3 displays the correlations between the relative frequency of foetal mouth movements and stress, depression, anxiety, maternal CO and attachment.

There was no significant correlation between 32- and 36-week data ($r = -0.09$, $p = 0.42$), and due to data not meeting the assumptions of an ANOVA, separate Kruskal–Wallis tests were conducted.

3.1 | 32-weeks of gestation

Based on 106 4D ultrasound scans, there is a significant overall effect of exposure group when assessing frequency of mouth movements, $X^2(3) = 0.812$, $p = 0.04$, d = 0.29. Adjusted pairwise comparisons indicate significant differences between heavy exposed foetuses (11–20 cigarettes per day) and e-cigarette-exposed foetuses, $p = 0.04$, d = 0.89 (see Table 4).

For relative frequency of mouth movements, when time of day was considered, there were no significant differences between the four groups $X^2(3) = 7.38$, $p = 0.06$, d = 0.43.

Pooling together results from both cigarette exposure groups, there is a significant effect when assessing frequency of mouth movement, $X^2(2) = 6.94$, $p = 0.03$, d = 0.40. There are no significant pairwise comparisons for non-exposed compared to both cigarette-exposed ($p = 0.16$) or e-cigarette-exposed ($p = 0.25$) or between cigarette and e-cigarette-exposed foetuses ($p = 0.08$). Accounting for time of day the scan took place, there is no significant difference between the three groups ($X^2(2) = 4.46$, $p = 0.10$, d = 0.31).

3.2 | 36-weeks of gestation

Based on 86 4D ultrasound scans, there was no significant difference between the exposure groups, $X^2(3) = 2.40$, $p = 0.49$, d = 0.15. Taking into account the significant correlation between relative frequency of mouth movements and depression, there are no significant differences between the four smoking groups, $X^2(3) = 2.06$, $p = 0.55$, d = 0.21.

When pooling results from both cigarette exposure groups, there is no significant difference when assessing frequency of mouth movement, $X^2(2) = 1.02$, $p = 0.60$, d = 0.31. There is no significant differences between the three groups when accounting for depression, $X^2(2) = 1.64$, $p = 0.44$, d = 0.55.

3.3 | Mouth movement frequency changes across gestational age

There are 79 sets of paired 32- and 36-week data. Foetuses displayed a greater number of mouth movements per minute at 32 weeks of gestation (M = 4.85, S.D. = 5.89) compared to 36 weeks of gestation (M = 3.08, S.D. = 2.87), Z = −2.36, $p = 0.01$, $r = -0.26$.

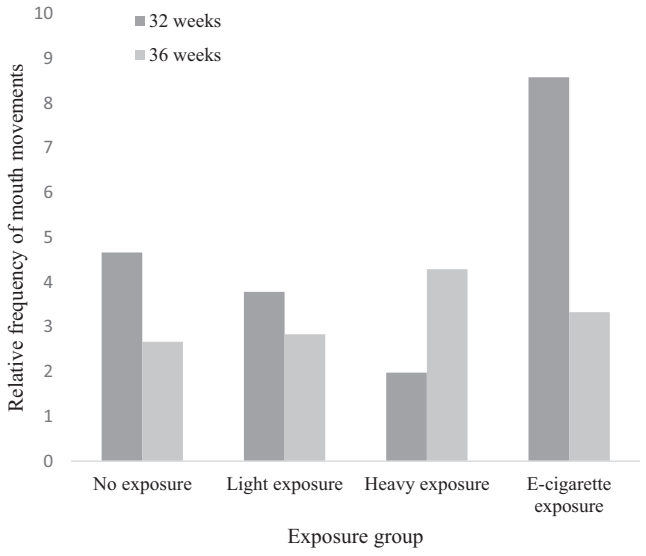

FIGURE 2 Relative frequency of mouth movements at 32 and 36 weeks' gestation split by the exposure group

Results indicate significant differences for the non-exposed group with foetuses displaying a great number of mouth movements at 32 weeks of gestation (Z = −2.22, $p = 0.02$, $r = -0.25$, $N = 32$, M = 5.06, S.D. = 4.56) compared to 36 weeks of gestation (M = 2.79, S.D. = 2.08). Borderline differences were observed for the e-cigarette-exposed foetuses, with a higher number of mouth movements at 32 weeks (Z = −1.85, $p = 0.06$, $r = -0.20$, $N = 13$, M = 9.03, S.D. = 10.67) in comparison to 36 weeks of gestation (M = 3.41, S.D. = 2.41). No differences were observed for the two cigarette-exposed groups.

Time of day the scans took place did not significantly differ between the four exposure groups at either 32 weeks ($X^2(3) = 1.28$, $p = 0.73$, d = 0.26) or 36 weeks of gestational age ($X^2(3) = 3.34$, $p = 0.34$, d = 0.13). Time of day the scan took place was not significantly different between 32- and 36 weeks of gestational age, Z = −0.14, $p = 0.88$, d = 0.03. Nor for the individual exposure groups; non-exposed Z = −0.14, $p = 0.88$, d = 0.03, light exposed Z = −0.59, $p = 0.55$, d = 0.00, heavy exposed Z = −0.77, $p = 0.44$, d = 0.00 and e-cigarette-exposed Z = −0.83, $p = 0.40$, d = 0.00.

Assessing maternal mental health scores across the two time points, there were no significant differences for stress (Z = −1.79, $p = 0.07$, $r = -0.20$, $N = 79$), depression (Z = −0.62, $p = 0.53$, $r = -0.06$, $N = 79$) or anxiety (Z = −0.93, $p = 0.34$, $r = -0.10$, $N = 79$). However, there were significant differences for attachment between the two time points (Z = −5.40, $p < 0.001$, $r = 0.60$, $N = 79$), with attachment increasing over time (32 weeks M = 81.99, S.D. = 6.21; 36 weeks M = 85.73, S.D. = 5.92.

4 | DISCUSSION

We expected different foetal mouth movement profiles across the four exposure groups, with movements overall declining from 32 to 36 weeks of gestational age. Initially, the findings of this study suggest that there are overall differences in foetal mouth movements at 32 weeks of gestation, as indicated by a significant difference in the pairwise comparison between heavy smoke-exposed foetuses displaying significantly reduced movements compared to e-cigarette-exposed foetuses. However, when accounting for the time of day the scan took place, the overall result is borderline, with a medium effect size, and thus, no further group differences were explored. No significant differences were found at 36 weeks of gestational age,

TABLE 3 Correlations between relative frequency and potential covariates

		Time of day	Stress	Anxiety	Depression	Attachment	Maternal CO
Relative frequency 32 weeks	Correlation	**−0.21**	−0.02	−0.10	−0.03	0.06	−0.18
	Significance	**0.03**[a]	0.78	0.30	0.73	0.51	0.06
Relative frequency 36 weeks	Correlation	−0.02	0.09	0.17	**0.25**	−0.10	0.07
	Significance	0.83	0.41	0.11	**0.01**[a]	0.36	0.53

[a]Significant correlation.

Bold indicates significant correlations less than 0.05.

TABLE 4 Pairwise comparisons

Group	Significance	Adjusted sig (Benjamini-Hochberg)	Effect size and variance d (CI) *V*
Non versus <10	0.202	0.166	0.21 (−0.66, 0.24) *0.05*
Non versus 11–20	**0.038**[a]	0.083	0.72 (−1.35, −0.09) *0.10*
Non versus e-cigarettes	0.289	0.250	0.64 (0.04, 1.23) *0.09*
<10 versus 11–20	0.278	0.208	0.49 (−0.15, 1.15) *0.11*
<10 versus e-cigarettes	0.052	0.125	0.72 (−1.35, −0.09) *0.10*
11–20 versus e-cigarettes	**0.011**[a]	**0.041**[a]	0.89 (−1.66, −0.11) *0.15*

[a]Significant correlation.

Bold indicates pairwise comparisons at the 0.05 level of significance.

in line with previous research.[17] In contrast to previously published research including Stroud et al.[6] and Habek,[7] our research does not support the hypothesis that foetal mouth movement frequency differs between the exposure groups. The findings support the hypothesis that total relative frequency of foetal mouth movements per minute differs between 32- and 36 weeks of gestational age, with the overall rate declining. Specifically, the declining rates of mouth movement are evident for both the non-exposed and e-cigarette-exposed foetuses.

The aim of the research was to extend with a larger sample and differentiated exposure groups, the pilot study by Reissland et al.[5] In contrast to Reissland et al.[5] where non-exposed foetuses displayed a lower rate of mouth movement in comparison to smoke-exposed foetuses, in this study we found that once accounting for time of day the scan took place, the overall effect at 32 weeks of gestational age was borderline with a medium effect size. In contrast to prior research,[14] in the present study there is a negative correlation between frequency of foetal mouth movements and time of day the scan took place at 32 weeks of gestational age. At present, it is unknown how foetal mouth movements map onto general movements perceived by the mother and therefore impossible to compare our results directly to these studies.

It is important to note the large differences in the standard deviations between the e-cigarette-exposed group with greater variation in comparison to the heavy cigarette-exposed group with the smallest variation. One reason for the variability in the standard deviation for the e-cigarette exposure group most likely relates to the amount of nicotine consumed by the e-cigarette user, which is not controlled and cannot be classified by the number of times it is used a day as it is for the number of cigarettes smoked per day. Milligrams of nicotine in the e-cigarettes was self-reported in this study, and it is difficult for the mother to quantify her typical daily use. Future research should aim to obtain a biological and objective measure of nicotine.

Furthermore, the current results suggest that coding only mouth movements using the FOMS might not be sensitive enough for assessing subtle differences in foetal facial movement profiles of CO and nicotine-exposed foetuses. Hence, we conclude that coding foetal mouth movements using the FOMS alone cannot differentiate between exposure groups. Other facial and body movements may also need to be coded which were not accounted for in either the present study or the pilot study and a combination of assessment measures may be required,[1] such as the Fetal Neurobehavioral Assessment System (FENS)[18] or Kurjak's Antenatal Neurodevelopmental Scoring Test (KANET).[19]

The results support the hypothesis that overall, the rate of mouth movement per minute does significantly differ between 32- and 36 weeks of gestational age. This is in line with Reissland et al.,[5] whereby movement decreases as a function of gestational age.[20] Other research has also found a decline in foetal movements from 26 to 36 weeks of gestational age. It is thought that this is an indication of the developing neural systems and maturation process with movements becoming more precise and coordinated, possibly reflecting the function and development of the CNS.[21] In the current study, we only observed a significant decline in mouth movement frequency for non-exposed and e-cigarette-exposed foetuses. This might be an indication that exposure of nicotine and CO via cigarette smoking delays the normal decrease of mouth movement frequency, thus impacting CNS development.[5]

A range of studies has indicated that maternal mental health has an impact on foetal behaviour.[5,10] We found significant correlations at 36 weeks between frequency of mouth movement and depression, with heavy smokers scoring the highest. It could be the case that higher levels of depression offset the effects of CO, therefore leading to this group no longer having a lower level of frequency of mouth movement. Furthermore, the effects of stress may explain the higher levels of mouth movements for smoke-exposed foetuses in the pilot study by Reissland et al.[5]

Although the current study involved an adequate sample size overall, foetuses were unevenly distributed in the three exposed and non-exposed group which may be a contributing factor to the results and a limitation; thus, results need to be viewed with caution. There are a number of unmeasured sources of potential variance, for example caffeine intake and maternal fasting,[22,23] which should be assessed.

In conclusion because of the variability in foetal mouth movements observed in the present study, we argue that examining the frequency of mouth movements alone may not be the most appropriate method for assessing group differences. Rather we suggest that a combination of foetal behavioural assessments is needed to demonstrate how smoking status impacts foetal neurobehavioural development. The finding that mouth movements per minute decline as a function of gestation is in line with other research.

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CONFLICT OF INTEREST

None.

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