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Ultrasound observations of subtle movements: a pilot study comparing fetuses of smoking and non-smoking mothers

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Short title : Fetal subtle movements in smoking versus non-smoking mothers

ABSTRACT

Aim: One way to assess fetal health of smokers is to ask mothers to count perceived movements, an unreliable method hiding differences in prenatal development. The aim of this pilot study was to assess subtle fetal movements in ultrasound-scans and establish whether they differ in fetuses of mothers who smoked and non-smoking mothers.

Methods: This longitudinal pilot-study recruited twenty mothers (16 non-smoking; 4 smoking) scanned four times from 24-36 weeks gestation (80 ultrasound scans). Two types of fine grained movements were coded offline and analysed using a Poisson log-linear mixed model.

Results: Fetuses of smoking mothers showed a significantly higher rate of mouth-movements compared to fetuses of non-smoking mothers (p=0.02), after controlling for maternal stress and depression. As pregnancy progressed, these differences between the smoking and non-smoking groups widened. Differences between the two groups in the rate of fetal facial self-touch, remained constant as pregnancy progressed and was borderline significant (p=0.07).

Conclusion: Rates of fetal mouth-movement and facial self-touch differ significantly between smokers and non-smokers. A larger study is needed to confirm these results and to investigate specific effects, including the interaction of maternal stress and smoking. Additionally, the feasibility of this technique for clinical practice should be assessed.

Key words: fetal 4D ultrasound, fine grained fetal movements, smoking, maternal stress

Key notes

- This longitudinal pilot study investigated fetal subtle movements using 4D ultrasound scans of smoking and non-smoking mothers

- Results showed that fetal facial movements and self-touch appear to differentiate between fetuses of smoking and non-smoking mothers, with more movement in the fetuses of smoking mothers.

- The results of the proof-of-concept study warrant replication on a larger sample.

Introduction:

Cigarette smoking has proven harmful effects on fetal development (1). Despite anti-smoking advice to pregnant women having intensified over the past two decades, rates of maternal smoking have remained relatively constant, as shown in a Cochrane systematic review carried out by Lumley, Oliver,

Chamberlain & Oakley (2). A more recent review (3) cites differences in smoking prevalence between affluent classes and socially disadvantaged women. Although smoking rates have significantly declined for women of higher social classes, pregnant women from lower social classes still continued smoking with the same rate. The most recent statistics indicates that there is variability of smoking at delivery in the UK as a whole (12%) with the much higher percentage of 20.9% observed in Durham, Darlington and Tees Health authorities in the UK (4).

Decreased oxygenation of fetal blood because of fetal exposure to cigarette smoke affects the neurological development of the fetus in general (5) with atypical autonomous regulation at the cellular and organ levels (6). More specific effects of smoking during pregnancy include differential heart rate variability (7) and breathing movements (1). Hofhuis et al (1) reported that exposure to tobacco smoke reduced the frequency of fetal breathing movements and resulted in delayed maturation of the lungs, evident following birth.

Exposure to smoking is associated with anatomical differences. Lampl et al (8), comparing fetuses of 10 smokers and 24 non-smokers at 23, 27, and 32 weeks, found exposure was significantly associated with early growth acceleration in head and abdominal diameters, altered head shape, longer arms and shorter legs as well as a reduced tibia/femur ratio. These fetal body growth patterns were attributed to the fetus of smokers being in a state of chronic hypoxia (8). Habek (9) found, when comparing brisk to sluggish fetal movements, the fetuses of non-smoking mothers showing significantly more brisk movements. Fetuses of heavy smokers showed significantly fewer isolated head and arm movements. In contrast fetuses of relatively light smokers (around 10 cigarettes or fewer per day) and non-smokers did not differ significantly in their rate of spontaneous isolated head and arm movements. However, these results might have to do with the fact that the movements coded were relatively gross and an analysis of finer movements might reveal more specific data. The more fine grained analysis is especially important in the light of findings indicating that even relatively small quantities of nicotine affect brain development. Specifically, Garvey and Longo (10) found that even low level exposure to carbon monoxide affects the growth and development of the fetus. Hence the importance of comparing the

development of fine grained fetal movements which might not be perceived by the mother of fetuses exposed to cigarettes and those not exposed.

Although women are generally advised to stop smoking during pregnancy (11), one study found that as a result of stress a sizable number of pregnant women smoke with increased frequency (12). Accordingly there may be an interaction between smoking, stress and fetal movements. Stress has been found to influence neurobehavioral development (13). Indeed one study of maternal stress and fetal movements (smooth and jerky upper limb movements) found that elevated stress in fetuses resulted in jerkier arm movements (14). High levels of prenatal maternal stress may be a risk factor for developmental disorders postnatally, since stress alters the biochemical equilibrium in the uterus (15). To summarise, maternal stress during pregnancy results in increased levels of maternal cortisol, and is a potential mechanism for perturbing fetal brain development which might lead to altered cortical laterality in the offspring (15). Similarly to stress, maternal depression has been implicated as a factor in fetal development and movement, with fetuses of depressed mothers being significantly more active in the fifth, sixth and seventh gestational months of gestation (16).

The proposed new method of coding fetal movements might highlight subtle differences which are missed with conventional methods investigating the effects of smoking. Although maternal *perception* of fetal movements has been used to establish whether fetuses are affected by maternal behaviours including smoking (17), these perceptions are not specific enough to compare movements of fetuses exposed to cigarette smoke (18). Indeed, a review of fetal movement counting, which asked mothers to quantify fetal movements they perceived during pregnancy, was inconclusive (17). This was because maternal counting of movements was not related in any studies to health outcome at birth. Furthermore, even when comparing Doppler measures of fetal movements with maternal perception of fetal movements, mothers were only able to report 16% of the movements which had been detected with Doppler measurements (18). In the present pilot study we suggest that the examination of fine-grained

movement using 4-D scans will provide a more objective measure of movement when specific areas (such as the face) of the fetus are examined. Frequency counts of specific types of facial movement can be determined and coded.

Findings regarding fetal movements indicate that the development of neurological functions can be inferred from the development of normal fetal movement, which include general movement, as well as more specific facial touch and fetal facial movement (19). The current longitudinal preliminary cohort study examines the effects of smoking upon two types of *fine grained* fetal movement, namely fetal mouth movements and fetal facial self-touch, during the second and third trimesters of pregnancy. Since stress and depression are identified in the literature as additional factors these were recorded at each scan and are adjusted for in the analysis.

METHODS

Participants

Mothers who had completed normal 20-week anomaly scans were invited to participate in this study. Twenty mothers were recruited, including four mothers smoking during pregnancy (2 boys and 2 girls) and sixteen non-smoking mothers (8 girls and 8 boys). All mothers who agreed to participate completed the study and attended all four scans. Participants were recruited through the midwives of the antenatal unit of the James Cook University Hospital, Middleborough, UK, following approved ethical procedures. During consent and before each procedure, mothers were made aware that additional scans were for research purposes and were not routine medical scans. No information was collected on those who did not respond to the invitation to participate.

Procedure

All participating mothers received four additional scans at 24, 28, 32 and 36 weeks gestational age, with fetuses being scanned in the morning for approximately 15-20 minutes. The scans took place in the radiography department, where mothers had previously undergone their routine 12 and 20 week medical

scans, with the mothers lying in a darkened room on their back or on their side, depending on the position of the fetus and how comfortable mothers were. The fetal face and upper torso were visualized resulting in full frontal or facial profile of the fetus by means of 4-D color ultrasound recordings. Additionally the sequences of traditional monochrome images were recorded in 2D. 4-D scans provide a detailed frame by frame view of a precise area of a fetus, specifically the face. Both were recorded for off line analysis with a GE Voluson E8 Expert Ultrasound System using a GE RAB4–8L Macro 4D Convex Array Transducer, with a frame rate of 2Hz, field of view of 70° and transducer frequency of 2-8 MHz. Frames are delivered every 0.5 of a second. Figure 1 provides selected images of a smoking and non-smoking fetus over a ten second period which illustrate the quality of the images and their ability to identify mouth movements and facial touch behaviour. Fetuses did not change uterine position during the length of the scan, but some fetuses changed uterine position from one gestational age to the next. Mothers were provided with a DVD copy of their scans.

Measures

Scan recordings were used to code mouth movements and facial touch behaviors using an adaptation of the Facial Action Coding System (20) found to be reliable in previous research (19, 21). Following established procedures 11 types of mouth movements were identified: upper lip raiser, lip pull, lip corner depressor , lower lip depressor, lip pucker, tongue show, lip stretch, lip pressor, lips parting, mouth stretch and lip suck (21). Because of variations in these movements, with some occurring rarely in the analysis, we did not distinguish between mouth movements but analysed them as generic mouth movements. The number of face touches by either hand of the fetus was also recorded.

Mothers completed the Perceived Stress Scale (PSS) questionnaire, assessing stress levels at each scan (22). The PSS is a widely used valid and reliable 10 item five-point Likert-based scale (ranging from 0='no stress' experienced during the last month to 4='very often' stressed, measuring the degree to which mothers perceive their life as stressful. The PSS score ranges from 0 (minimum) to 40

(maximum). Additionally, mothers completed the Hospital Anxiety and Depression Scale (HADS) (23). The scale has seven items and ranges from 0 (minimum) to 21 (maximum). Following established methods, smoking status at each scan was assessed by self-reported maternal report and verified in the hospital notes.

Ethics

Ethical permission for the study was obtained from the County Durham and Tees Valley 2 Research Ethics Committee (REC Ref: 08/H0908/31 and REC Ref: 11/NE/03/61) and the research and development department of James Cook University Hospital, as well as the Durham University (Department of Psychology ethics committee). All mothers gave informed written consent.

Reliability

Reliability of scan coding was assessed using Cohen's Kappa (κ), with 44% of scans of nonsmoking mothers and 33% of scans of smoking mothers assessed independently by an independent trained coder. Reliability estimates for mouth movements were very high: for non-smokers $\kappa = 0.90$, (range 0.70 to 1) and for smokers $\kappa = 0.87$, (range 0.70 to 1). Similarly reliability estimates for facial touch behaviors were very high: for non- smokers $\kappa = 0.88$, (range 0.70 to 1) and for smokers $\kappa = 0.90$ (range 0.85 to 1).

Statistical analysis

Reflecting the longitudinal, repeated structure of the measurements, a Poisson log-linear mixed effects analysis (24) was used to assess differential changes in development, expressed as the rate of movement over gestational age between the smoking and non-smoking groups. The analysis models the number of *events* – of fetal mouth movement events or facial self-touch events - as a count variable adjusted by the length of scan as an exposure variable, with covariates of gestational age, sex, maternal age, stress and

depression, together with an interaction between gestational age and smoking status to allow for differential development, and a random individual fetus effect. The individual random fetus effect essentially allows for individual variability between fetuses in their propensity to the event and is assumed to be normally distributed.

Formally, we can write the model as

$$\log(\lambda_{it}) = \log(\text{scan length}_{it}) + \beta_0 + \beta_1 \text{ gestational age}_{it} + \beta_2 \text{ smoker}_{it} + \beta_3 \text{ stress}_{it}$$
$$+ \beta_4 \text{ depression}_{it} + \beta_5 \text{ sex}_i + \beta_6 \text{ maternal age}_i$$
$$+ \beta_7 (\text{gestational age * smoker})_{it} + u_i$$
$$C_{it} \sim \text{Poisson}(\lambda_{it})$$
$$u_i \sim \text{Normal}(0, \sigma_f^2),$$

where C_{it} are the event counts for fetus i at gestational age t, λ_{it} is the underlying Poisson rate, β_0 to β_7 are unknown regression coefficients and σ_f^2 is the individual fetus variance. The indices to the individual covariates show which of them vary over time in our model. Thus gestational age, stress and depression are measured at each scan, whereas the others are measured once.

To fit the model, we centre the gestational age variable at the midpoint of 30 weeks, so that the effect of smoking which is measured by the parameter β_2 is assessed at the midpoint of the study. The parameter β_7 measures the extra change in slope in gestational age of the participant being a smoker.

Mixed effects modelling was carried out using the *glmer* function (25) in the *lme4* package of the statistical package R. Testing between models was carried out using likelihood ratio tests, allowing differences in deviance to be compared to a chi-squared distribution on the appropriate number of degrees of freedom. Parameter estimates were interpreted by exponentiating them, with the resultant values interpreted as multiplicative effects on the event rates. Confidence intervals for σ_f^2 are typically non-symmetric and so a 95% confidence interval is reported for this parameter rather than a standard error.

RESULTS

Demographics

Non-smoking participants had a mean age of 29.4 years (range 19 - 40 years), and smoking participants had a mean age of 27.0 years (range 20-36 years), with no significant difference between the groups (t=0.67; df=18, p=0.51). Smoking status was measured at each scan but there was no change over time in this variable - all smokers remained smokers, and all non-smokers remained non-smokers. Smoking mothers smoked a mean of 14.25 cigarettes a day over the four scans; this is similar to the reported mean of 12 cigarettes a day for women in 2012 in Great Britain reported by *Action on Smoking and Health* (26). The first research scan of smoking mothers was performed at a mean age of 24.2 weeks (range 23.4 to 25.2 weeks); the second at 28.0 weeks (range 27.4 to 28.6 weeks); the third at 32.0 weeks (range 31.2 to 33.2); the fourth at 35.9 weeks (range 35.2 to 36.7 weeks). For non- smoking mothers research scans were performed at nearly identical times; the first at a mean age of 24.1 weeks (range 23.5 to 25.6 weeks); the second at 28.1 weeks (range 27.5 to 28.5 weeks); the third at 32.0 weeks (range 31.1 to 32.5 weeks); the fourth at 36.1 weeks (range 35.6 to 36.5 weeks). In total, 77 scans were analysed over 20 fetuses (the position of the fetus in three scans did not allow data to be extracted).

All fetuses were clinically assessed and declared to be healthy after birth. Fetuses of non-smokers were born at a mean of 39.6 weeks (range 37 to 42 weeks) gestational age, similar to non-smokers, with a mean of 39.9 (t=0.29 on 18 df, p=0.18). Apgar scores were measured at 1 and 5 minutes were 9 in both smokers and non-smokers. There was no significant difference in the mean birth weight of fetuses of non-smokers (3313 grams; SD 488 grams) and smokers (3255 grams; SD 512 grams) (t= 0.21 on 18 df, p=0.83). There was also no significant difference in the head-circumference (smokers: mean = 33.5 cm; non-smokers: mean = 34.9 cm; t=0.922 on 17 df; p=0.37).

Analysis of mouth movements

In total, 4,528 mouth events were recorded. Table 1 shows the estimates of the parameter estimates for the Poisson mixed effects analysis for the mouth movement events. Model 1 shows the estimates when all covariates are included in the model. All parameter estimates are significant except for the sex of the fetus and maternal age. Model 2 shows the adjusted model excluding sex and maternal age. A likelihood ratio test comparing Model 2 and Model 1 gave a change in -2 log likelihood of 2.54 on 2 df, which was not significant when compared to a chi-squared distribution (p=0.28).

The final model (Model 2) can be interpreted as follows. The estimate for gestational age is significant and negative, showing a declining rate of mouth movements for the non-smoking group (the reference group) as the fetus matures. The multiplicative effect for gestational age is 0.970, showing a 3.0% decline for each extra week of age. The estimate for the smoker variable is positive, and the multiplicative effect is 1.583, showing that at 30 weeks, fetuses of smoking mothers have 58% more mouth movements than non-smokers (p=0.021). The interaction between fetal age and smoker is also significant, and estimates the *change* in fetal age slope between smokers and non-smokers. Fetuses of smoking mothers decline more slowly in their rate of mouth movements than fetuses of non-smokers – the multiplicative effect for fetuses of smokers is estimated by (0.970*1.1016 = 0.985) or a 1.5% decline for each extra week of fetal age –about half the decline of fetuses of non-smokers. Finally the effect of stress is positive, suggesting increasing rate of mouth movements with increasing stress. The multiplicative effect is 1.009, suggesting around a 1% increase for a unit increase in stress score, or a 9% increase for a change of ten stress units.   In contrast, the depression estimate is negative, indicating that increasing depression is associated with a decreasing rate of mouth movement.

Reparametrisation of Model 2 using different centering values for fetal age can be used to assess the difference between fetuses of smoking and non-smoking mothers at different fetal ages. Centering at 24 weeks, the difference in slope is non-significant (p=0.072); at 28 weeks it becomes significant

(p=0.030) and at 36 weeks is highly significant (p=0.006).   The differences in mouth movement rate between fetuses of smokers and those of non-smokers, therefore, becomes wider as the fetus matures and becomes significant at around 28 weeks. Figure 2 shows the fitted developmental model for fixed values of stress (12.0) and depression (5.0) for smoking and non-smoking participants on the log scale. The increasing separation of the two trajectories is evident.

Analysis of facial self-touch movements

A similar analysis was carried out on the number of facial self-touch events. In total, 1,114 facial touch events were recorded.   Table 2 shows the estimates of the parameter estimates for the Poisson mixed effects analysis. Model 3 shows the estimates when all covariates are included in the model. In this analysis, the estimates for the smoking by age interaction and the main effects of depression, gender and maternal age are not significant. The effect of smoking is borderline significant. Model 4 shows the estimates once the above four non-significant terms have been excluded from the model, with smoking status retained in the model. The effect of excluding the three variables and the interaction was tested using a likelihood ratio test. The change in -2 log L (649.74-648.97) is 0.77 on 4 degrees of freedom which is not significant (p=0.94).

The facial self-touch statistical model is essentially similar to the model for the analysis of mouth movements. The estimate for age is significant and negative, showing (with the absence of an interaction effect) an *identical* declining rate of mouth movements for both the non-smoking and smoking groups as the fetus matures. The multiplicative effect for age is 0.959, showing around a 4% decline for each extra week of age. The estimate of maternal smoking, although just failing to reach formal statistical significance, is similar in effect to the previous analysis; the multiplicative effect is in fact slightly larger at 1.688, suggesting that the facial self-touch rate for fetuses of smoking mothers is raised by around 69% compared to non-smokers. Finally the effect of stress is again positive, suggesting increasing rate of touch movements with increasing stress. The multiplicative effect of 1.028

suggests a 2.8% increase in touch movement for a unit increase in stress score, or a 31% increase for a larger change of ten stress units. Figure 3 shows the estimated fitted developmental model (Model 4) on the log scale for a fixed value of stress of 12.0 for smoking and non-smoking participants, showing the common declining rate for both groups.

Discussion

This is the first study which compares longitudinally two types of movements, namely facial movements and touch behaviours of fetuses of mothers smoking during pregnancy and fetuses of mothers who did not smoke during pregnancy. Similar to other studies analysing general body movements (17), mothers who reported higher levels of perceived stress had fetuses who showed more of these subtle movements.

Maternal smoking is a crucial risk factor for poor psychological, behavioral and physical outcomes in infant development (1), with increasing evidence of the harmful effect of smoking on fetal development. This study found that the rate of fetal facial movements was associated with maternal smoking: within a general pattern of declining movement with gestational age, fetuses of smoking mothers showed more movements at 30 weeks compared with non-smoking mothers, after controlling for maternal stress and depression. Additionally, the rate of decline in movements was significantly slower for fetuses of smoking mothers. An analysis of facial self-touch showed similar effects. The difference between the rate of movement of fetuses of smoking and non-smoking mothers was larger in magnitude, and which was marginally significant (p=0.07). There was a common decline in the rate of movement by gestational age in both the smoking and non-smoking groups.

Although anti-smoking advice to pregnant women has intensified over the past two decades (11), quit rates have remained relatively low. Ebert and Fahi (11) found that smokers perceive health risks associated with smoking as abstract constructs too far removed from their understanding, when set against the immediate gratification from smoking. Hence, more concrete measures of observable movements need to be introduced. Our pattern of fetal fine grained movements in relation to smoking and gestational age indicate some differences to other studies analysing more gross fetal body movements (9, 27). The first of these studies (27) found that fetuses (less than 37 weeks gestation) of mothers who smoked did not differ in their general movements when compared with similarly aged fetuses of non-smoking mothers. A second study (9) reported that fetuses of smoking mothers showed less frequent gross movements. These results relate to general fetal movement and derive from cross sectional rather than longitudinal data. In contrast, the present study examined the frequency of two types of specific subtle fetal movements observable in ultrasound scans, namely mouth movements and facial self-touch behaviours. We argue that these fine grained movement observations provide a more sensitive measure for studies of fetal movements. In addition, the type of movement needs to be considered. Self-touch has been found to be used by young infants to soothe themselves during stressful situations (28); thus stressed fetuses may use self-touch more frequently compared with non-stressed fetuses. Our finding of maternal stress and frequency of fetal facial touch supports this hypothesis.

Previous research has identified a smoking dose-dependent difference in fetal general movements. Habek (9) found a significant difference between non-smokers and mothers smoking over 20 cigarettes per day but not 10 cigarettes per day. In the present study pregnant women smoked about 14 cigarettes per day, and an increase of subtle fetal movements was recorded. Similarly to our findings, Dieter et al (16) found increased movements of fetuses exposed to smoking before 8 months gestational age compared to fetuses of non-smokers. It needs to be noted that stress also had a positive impact on frequency of movements supporting findings of other research cited above. The effect of a ten unit increase in stress for mouth and touch movements of 9% and 31% increases respectively, was however *less* than the changes for being a smoker (58% and 69% increases respectively). Smoking appears to be more important than stress in our pilot analysis.

Thus, over and above the effect of stress, smoking status had a significant effect on frequency of fetal mouth movements and a marginally significant effect on facial self-touch. We can suggest one possible mechanism. The difference in fine grained fetal movement analysis might reflect the fact that the fetal central nervous system maturation is affected by maternal smoking (29) and hence fetuses of smokers declined more slowly in their rate of mouth movements than fetuses of non-smokers. Specifically fetuses of smokers showed a significant delay compared with the normal declining rate of movements. Such delay has been reported for example in relation to speech processing abilities in infants exposed to smoking during pregnancy (30). Key et al (30) examined event related potentials (ERPs) 34 hours after birth in response to six consonant-vowel syllables in healthy newborn infants of smokers and non-smokers and found that not only did infants of smokers discriminate between fewer syllables but also that they were slower in their reaction times. Huizink (29) discusses a number of mechanisms which bind nicotine to nicotinic acetylcholine receptors in the brain leading to premature cell differentiation and ultimately to premature brain cell death. Such mechanisms might play a role in the delay of declining movements in the current sample which indicates that fetuses of smoking mothers show less mature behavioral patterns compared with fetuses of nonsmoking mothers (29).

In sum, this exploratory pilot study indicates that fetal facial movement patterns differ significantly between fetuses of smoking compared with non-smoking mothers with fetuses of smoking mothers showing a raised rate of movement compared to non-smoking mothers, with a slower rate of decline. There is also some evidence that similar effects exist for facial self-touch although failing to reach significance. Our findings concur with others that stress and depression have a significant impact on fetal movements (12, 27) and need to be controlled for, but additionally these results point to the fact that nicotine exposure *per se* has an effect on fetal development over and above the effects of stress and depression.

Finally, we conclude with some caveats to our results. Firstly, this is a pilot study, and larger studies are required to confirm reported associations and further understand the relationship between maternal smoking, stress, depression and fetal development. The lack of significance in the fetal facial self-touch results may be caused by low power. Secondly, given the computational limitations of the Voluson E8 which delivers a new image every ½ second, we might miss some very fast movements. However, given that one of the most common movements observed (mouth stretch) lasts on average 3.1 seconds (range 1.6-4.1) the frame rate would not significantly affect our ability to observe fine grained mouth movements. Thirdly, we have not attempted to control for social class in our analysis, and it is possible that differences in social class between the smoking and non-smoking mothers may account for some of the differences in fetal movement between the two groups. Similarly, we did not include information on paternal effects, including paternal smoking behaviour. In the statistical analysis, we were unable to consider additional interaction effects beyond the interaction of smoking with gestational age due to small numbers of cases. Future studies should consider both the inclusion of these additional controlling factors and the inclusion of further interactions in the statistical model to fully understand the relationship between maternal smoking and fetal fine-grained movement.

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Table 1 Analysis of mouth movement rate showing developmental age effects of smoking, stress and depression.

Variable	Model 1 -2 log L = 1177.63			Model 2 (excluding sex and maternal age) -2 log L = 1180.17			
	Estimate	s.e.	p-value	Estimate	s.e.	p-value	Exp (estimate)
Intercept (β_0)	-1.8878	0.3886		-2.3425	0.1002		
Fetal age	-.0306	0.0043	<0.001	-0.0305	0.0043	<0.001	0.970
Smoker(yes)	0.4292	0.2033	0.035	0.4596	0.1984	0.021	1.583
Stress	0.0089	0.0033	0.008	0.0087	0.0033	0.009	1.009
Depression	-0.0465	0.0104	<0.001	-0.0458	0.0103	<0.001	0.955
Sex (male)	-0.0128	0.1715	0.456				
Maternal age	-0.0133	0.0137	0.331				
Fetal age by smoker interaction	0.0160	0.0081	0.048	0.0160	0.0081	0.048	1.016
σ_f^2 and 95% confidence interval	0.1164 (0.0578, 0.2815)			0.1030 (0.0602, 0.2367)			

Table 2 Analysis of facial self touch rate showing developmental effects of smoking and stress.

Variable	Model 3 -2 log L = 648.97			Model 4 (excluding sex, depression, maternal age, age by smoker interaction) -2 log L = 649.74			
	Estimate	std. err.	p-value	Estimate	std. err.	p-value	exp (estimate)
Intercept (β_0)	-4.3937	0.5978		-4.3407	0.1546		
Fetal age	-0.0407	0.0085	<0.001	-0.0421	0.0071	<0.001	0.959
Smoker(yes)	0.5942	0.3063	0.052	0.5238	0.2899	0.070	1.688
Stress	0.0281	0.0065	<0.001	0.0273	0.0064	<0.001	1.028
Depression	-0.0126	0.0193	0.516				
Sex(male)	-0.1503	0.2556	0.556				
Maternal age	0.0057	0.0210	0.787				
Fetal age by smoker interaction	-0.0003	0.0159	0.985				
σ_f^2 and 95% confidence interval	0.2434 (0.1290, 0.5111)			0.2485 (0.1317, 0.5211)			

Acknowledgement

Figure 1. Illustrative 4D scan frames of a 32 week old fetus of a smoking mother (top line) and a 32 week old fetus of a non-smoking mother (bottom line) over a ten second period of observation.

Figure 2. Final fitted model for rate of fetal facial mouth movements by maternal smoking status and gestational age.

Figure 3. Final fitted model for rate of fetal facial self touches by maternal smoking status and gestational age.

Author Contributions

Conceived and designed the experiments: NR. Wrote protocol and was responsible for ethics: NR, KK. Performed the experiments: NR Designed the statistical analysis and analyzed the data: BF. Wrote the paper: NR BF JM KK.

estimated number of facial movements in 600 seconds

gestational age of fetus
non-smoking = grey solid, smoking = black dashed

estimated number of self touches in 600 seconds

gestational age of fetus
non-smoking = grey solid, smoking = black dashed