**Investigation of the Outcome of Pregnancies Complicated by Increased Fetal Movements and Their Relationship to Underlying Causes – A Prospective Cohort Study**

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**Conflicts of Interest statement**

The authors have no conflicts of interest to declare.

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**Abstract**

**Introduction:** Retrospective studies have reported an association between a single episode of significantly increased fetal movements (IFMs) and stillbirth after 28 weeks’ gestation. This prospective study aimed to report the outcome of pregnancies associated with maternal perception of IFMs and determine whether this symptom is associated with adverse pregnancy outcome, a pathological intrauterine environment or placental dysfunction.

**Methods:** Women reporting IFMs after 28 weeks’ gestation were recruited from St Mary’s Hospital, Manchester and Liverpool Women’s Hospital, UK between 01.11.2017-01.05.2019. Demographic and clinical information were obtained and an ultrasound scan was performed to assess fetal biometry, liquor volume and umbilical artery Doppler. Maternal serum samples were collected for analysis of placentally-derived biomarkers using ELISA. After delivery, maternal and fetal outcome data were collected and placentas and umbilical cord blood were obtained for analysis using immunohistochemistry and ELISA respectively. Placental and serum samples were matched by gestation and maternal characteristics to participants with normal fetal activity.

**Results:** Seventy-seven women presented with IFM representing 0.45% of the maternity population; 64 women consented to participate in the study of which 7 (10.9%) experienced adverse pregnancy outcome (birthweight <3rd centile, 2 (3.1%), pH≤7.10, 1 (1.6%), NICU admission, 4 (6.3%)). Women had IFM for varying lengths of time before presenting, 17.2% had IFM for less than 1 hour and 29.7% reporting IFM for greater than 24 hours. Four women (6.3%) had abnormalities of the fetal heart rate trace on assessment. Women with IFM had similar mode of birth to women giving birth in participating maternity units. There was no evidence of macroscopic placental or umbilical cord abnormalities, alterations in microscopic placental structure, placental endocrine dysfunction, or intrauterine hypoxia or infection in women with IFM compared to controls.

**Discussion:** This prospective study did not find evidence of an association between IFMs and adverse pregnancy outcome. It also did not find evidence of underlying placental dysfunction, cord anomalies, intrauterine hypoxia or infection in pregnancies with IFM. Further work is required to determine the strength of association between IFM and adverse pregnancy outcome and its origins. Presently, IFM cannot be used to identify fetuses at increased risk of adverse outcome.

**Keywords**

Exaggerated fetal movements; Excessive Fetal Movements; Placenta; Umbilical cord; Stillbirth; Adverse pregnancy outcome.

**Abbreviations**

BMI Body Mass Index

IFM Increased Fetal Movement

NNU Neonatal Unit

PBS Phosphate Buffered Saline

PI Pulsatility Index

RI Resistance Index

SNA Syncytial Nuclear Aggregates

**Key Message**

Maternal perception of increased fetal movements is an uncommon reason for consulting maternity services. In this prospective cohort study, this symptom was not associated with adverse pregnancy outcomes, abnormalities of the placenta and umbilical cord, inflammation or placental dysfunction.

**Introduction**

In high-resource settings, the majority of stillbirths occur before labour in normally-formed infants [1]. The disparity between stillbirth rates in different populations suggests that more can be done to reduce stillbirths [2]. One approach to stillbirth prevention is to identify pregnancies at increased risk in order that strategies can be developed to mitigate that risk. The UK Stillbirth Priority Setting Partnership identified the need to understand modifiable risk factors for stillbirth [3]; alterations in normal fetal activity patterns could be considered modifiable because maternal behaviour in response to altered fetal activity can be altered.

In recent years, evidence has emerged to suggest that maternal perception of a single episode of abnormally increased fetal movements (IFM) may be associated with stillbirth [4-8], with two-fold [4], four-fold [5] and seven-fold [6] risk of stillbirth respectively. Importantly, only a single episode of IFMs is associated with an increased risk of stillbirth, with multiple episodes of IFM being protective [4-6]. This has led to the suggestion that a single episode of IFMs could represent an acute in utero event [9].

Potential reasons for the association between IFMs and stillbirth include fetal seizures, umbilical cord obstruction, intrauterine infection or maternal anxiety; evidence for these underlying causes is sparse [9]. Fetal seizures could reflect cerebral hypoxia, as hypoxic-ischaemic injury is the most common cause of seizures in term neonates [10]. This is supported by a case reports that describe the existence of in utero seizures observed on ultrasound; all of these infants had poor outcomes [11]. Similarly, cases have been described where a single period of painful, rhythmic fetal movement was associated with pathological fetal heart rate patterns in the presence of a tight nuchal cord [12].

All studies reporting an increased stillbirth risk associated with an episode of IFM are retrospective cohort or case-control studies, and are subject to a risk of recall and negativity bias [4-6], and in some instances selection bias [7, 8]. Thus, to further evaluate the association between IFMs and adverse pregnancy outcomes, and to characterise any underlying potential pathologies, prospective studies are needed. The only prospective study to date included 219 women who had IFM in the third trimester of pregnancy and found an association with large for gestational age infants, but no other adverse outcomes [13].

We aimed to investigate the pregnancy characteristics and outcomes of women reporting IFMs compared to the total obstetric population in the respective settings, and to determine whether there is any evidence of underlying pathology of the placenta or umbilical cord, or evidence of intrauterine hypoxia, intrauterine infection or inflammation, which could explain the previously observed association with adverse outcomes.

**Materials and methods**

A prospective cohort study was conducted at two large tertiary maternity units in the North-West of England from 01.11.2017 to 01.05.2019 - Investigation of the Outcome of Pregnancies Complicated by Increased Fetal Movements (INVEST Study). Women who reported a subjective significant increase in fetal activity patterns to their care provider after 28 weeks’ gestation were asked to participate in the study provided they also fulfilled the following criteria: maternal age ≥ 18 and ≤45 years with a singleton pregnancy with no known fetal anomaly. Women were excluded from the study if they were currently partaking in, or had previously been involved in, intervention studies to improve pregnancy outcome.

For comparison, aggregate anonymised data regarding all births occurring ≥28 weeks’ gestation at St Mary’s and Liverpool Women’s Hospitals during the recruitment period were obtained. Control samples were collected from women with no abnormalities of fetal movements recruited to prior studies or the Maternal and Fetal Health Research Centre tissue biobank. Placental samples were matched to the gestational age of samples with IFMs +/- 5 days, and maternal bloods were matched by gestational age +/- 7 days. Umbilical cord bloods were matched by gestational age +/- 5 days and mode of delivery to the IFM cord blood samples. Demographic characteristics of control and IFM samples used for analysis of placental structure and serum are shown in Table S1.

Participants had their demographic and clinical data collected at the time of presentation by trained midwives and clinicians. Information was also collected about the nature of IFM. Maternal blood pressure and urine dipstick testing were recorded, as were the results of ultrasound scans of fetal biometry and liquor volume, measured using the standard method used locally (amniotic fluid index or maximum pool depth). Doppler ultrasound was used to assess: umbilical artery pulsatility index (PI) and resistance index (RI); right and left uterine artery PI and RI; and middle cerebral artery PI and RI. Values were assessed in relation to published reference ranges [14-16] and <5th or >95th centile were classified as abnormal.

After delivery, outcome data were collected, including maternal presentation with episodes of reduced fetal movements (RFMs), onset of labour, birth outcome, birthweight, infant sex, gestation at delivery, mode of delivery, 5 and 7 minute Apgar score, umbilical artery pH, admission to neonatal unit (NNU) and whether the infant died in the neonatal period. Composite adverse pregnancy outcome (APO) was defined as one or more of any of the following: stillbirth, neonatal death < 28 days of age, Apgar score < 7 at five minutes of age, umbilical artery pH < 7.10, birthweight < 3rd centile or admission to NNU.

Placentas and umbilical cords were collected close as possible to the time of delivery. Photographs were taken of the umbilical cord and fetal and maternal surfaces of the placenta; the number of cord vessels and the trimmed placental weight was recorded. A full thickness sample (approx. 1cm3) of the umbilical cord at its insertion into the placenta was obtained and washed in sterile phosphate buffered saline (PBS). A minimum of three full thickness samples of placenta (~1cm3) were systematically sampled from the centre, middle and edge of the placental disc. The decidua and chorionic plate were removed and the remaining tissue washed in PBS. Tissue samples were fixed in 4% neutral buffered formalin for 24 hours at 4°C. Fixed tissue was then wax-embedded prior to histochemical and immunohistochemical analysis.

Placental measurements (area, diameter, radius, cord insertion) were obtained from placental photographs using Image Pro Plus v.7.0 software. The maternal surface of the placenta was examined for infarcted tissue [17]. The cord was examined for true and false knots, and the umbilical coiling index was calculated (no. coils/total length of cord cm). [18]. Microscopic placental structure was analysed by immunohistochemistry to identify structures of interest as previously described [17, 19] using the following primary antibodies: anti-CD31 (Dako 4µg/ml), anti-Ki67 (Dako, 0.2 µg/ml), anti-M30 (Roche, 13.2 µg/ml) or a matching concentration of non-immune mouse IgG (negative control) (Dako, Cambridge, UK). For vascularisation, the number of capillaries in each terminal villus were counted in ten fields of view and the mean value per tissue section was calculated. Avascularity was calculated as the number of terminal villi with no CD31 positive staining as a percentage of the total number of terminal villi per field of view. Ki67 (proliferation) and M30 (apoptosis) staining was analysed using QuPath software (v.0.2.0). Ki67 positive nuclei were expressed as a percentage of the total haematoxylin stained nuclei and M30 positive staining was expressed as the proportion of nuclei surrounded by cytoplasmic M30 positive staining to give an apoptotic index. The apoptotic and proliferative index was calculated in centre, middle and edge pieces of placental tissue then pooled to give an overall result for each placenta.

Maternal blood samples for serum and plasma were collected at the time of presentation. Umbilical venous blood samples for serum and plasma were collected by venepuncture of the umbilical vein as close as possible to the time of delivery, blood samples were centrifuged at 2400*g* for 10 minutes at 4°C, the supernatant removed, and stored at -80°C. hPL, hCG, PAPP-A and PlGF were measured in maternal venous serum and EPO was measured in umbilical venous serum using enzyme-linked immunosorbent assay (ELISA) according to the manufacturers’ protocol. Standard curves were generated and linear regression was used to interpolate unknown values. C reactive protein was measured by automated chemiluminescence on maternal and umbilical venous samples.

Statistical Analyses

Power calculations based on previous data regarding placental apoptosis in women with RFMs [17] estimated that for a 90% power and alpha value of 0.05, 12 placental samples would be required to show a significant difference in apoptosis between control and IFM pregnancies ending in the composite adverse outcome. It was anticipated that 10-20% of IFM pregnancies would end in adverse outcome [20]. Consequently, between 60 and 120 women with IFMs would be required for an adequately powered study.

Participants demographic characteristics, details of presentation and pregnancy outcomes in women reporting IFMs were analysed using STATA (Version 14, Statacorp, TX, USA). All remaining data were analysed using GraphPad Prism (Version 7.04, CA, USA). Data regarding pregnancy characteristics and outcomes in the background obstetric populations at St Mary’s and Liverpool Women’s Hospitals were not compared to the INVEST cohort due to large differences in group sizes. Data were tested for normality using the D’Agostino-Pearson test, normally distributed variables were analysed using an unpaired t-test and non-normally distributed data were analysed using the Mann Whitney-U test. Categorical data were analysed using Fisher’s exact test. Data were considered statistically significant at p<0.05.

Ethical Approval

A favourable ethical opinion was obtained from Greater Manchester or North West Research Ethics Committees (Refs 15/NW/0829, 13/WM/0288, 17/NW/0229, 08/H1011/83, 08/H1010/55+5) and the Health Research Authority (IRAS 220808).

**Results**

During the study period 77 women presented with IFM, 7 of whom presented out of hours, 3 potential participants could not be contacted by the research team and 3 declined to participate. Therefore, 64 women agreed to participate in the study (Figure 1). 50 participants gave maternal serum and 36 placentas and 13 umbilical cord bloods were collected after birth. This gives a frequency of IFM of 0.45% of births (95% Confidence Interval (CI) 0.35%-0.55%). Participants appeared to have similar characteristics to the total population of women giving birth at the recruiting maternity units during the same timeframe, although fewer participants smoked cigarettes (Table 1). Women with IFM had symptoms for varying lengths of time before presentation, with 17.2% having IFM for less than 1 hour and 29.7% reporting IFM for greater than 24 hours (Table 2). Participants reported IFM over a wide gestational age range from 27 through to 41 weeks. IFM was not associated with maternal hypertension and was rarely associated with abnormalities of the cardiotocograph (CTG), with 4 traces (6.3%) classified as abnormal. IFM was infrequently associated with abnormal findings on ultrasound scan, with 2 cases of oligohydramnios and 3 cases with an estimated fetal weight <10th centile; no fetuses had an umbilical artery pulsatility index ≥90th centile (Table 2). 23 (35.9%) women with IFM subsequently presented with RFM before the end of pregnancy (Table 3).

Outcomes for women with IFM were similar to the overall population, although there was a higher rate of induction of labour (50% vs. 40.4%, Table 3). Six infants (9.4%) had a birthweight <10th centile and the same proportion were >90th centile; 4 infants were admitted to the neonatal unit and one had an umbilical artery pH <7.05 (Table 3). Overall, 7 infants (10.9%) had the composite adverse neonatal outcome.

When compared to placentas from women with normal fetal movements, women reporting IFM did not have any differences in macroscopic placental size or structure (Table 4); all umbilical cords examined had three vessels and none had true knots or strictures. The umbilical cord coiling index was consistent with previous reports and not different in IFM. There was no difference in proliferation, apoptosis or the number of blood vessels in the villi or the proportion of avascular villi (Figure 2). There was no difference in placentally-derived proteins hCG, hPL, PAPP-A and PlGF in maternal serum (Figure S1) or in CRP which was measured as a marker of infection. Analysis of umbilical cord blood from infants with IFM found no difference in CRP, which were all beneath the limit of detection of the assay, or erythropoietin compared to women with normal fetal movements.

**Discussion**

This study identified, recruited and followed women presenting with IFM at two large UK tertiary maternity units. In this cohort, IFM was an uncommon reason to present to maternity services and was not associated with increased rates of adverse perinatal outcome. We were unable to demonstrate evidence for underlying abnormalities of the placenta or cord that have been hypothesised to lead to IFM [9].

The strength of this study is its prospective design and collection of a wide range of clinical data together with blood and placental samples. Selection or reporting bias was minimised because the majority of eligible women approached agreed to participate and there was no loss to follow-up. The participating maternity units serve an ethnically and socially diverse population suggesting these findings may be generalizable. However, the study recruited fewer women than anticipated, with an incidence of 0.45% of women being much lower than 9.6% reported in a prospective study from China [13] and 6.9% of controls in MiNESS [4]. As women perceiving IFM are not routinely asked to contact maternity services in the UK, women perceiving this symptom may not have been recruited. Furthermore, this study did not include a qualitative description of IFM which is potentially important in determining their significance with regards to stillbirth [7]; Sadovsky described “a history of sudden, strong, vigorous fetal movements with an increased rate followed by cessation, as almost invariably a sign of acute fetal distress and fetal death” [21] and Linde et al. reported that 10% of mothers who experienced a late stillbirth described IFM as a “death-jerk” and “intense” [8]. However, we observed no relationship between the duration of IFM and the frequency of IFM, with no adverse outcomes in women reporting <1 hour of IFM.

There are few other prospective studies of increased or excessive movements for comparison of outcomes. In their larger contemporaneous cohort study, Huang et al. also observed no increase in the frequency of small for gestational age infants, neonatal unit admission or evidence of neonatal compromise [13]. They reported increased frequency of infants with birthweight >90th centile which was not evident in our cohort. Whitehead et al. propose that umbilical cord pathology may underpin some presentations with IFM [12], but there were no cases of umbilical cord knots or tight nuchal cord or raised umbilical artery PI in our cohort, which given its size is not unexpected. Our findings with IFM contrast with prospective studies of women with reduced fetal movements which demonstrate increased rates of adverse outcome (including perinatal death and birthweight <10th centile) [20, 22] and underlying abnormalities of placental structure and function [17, 23]. Similarly, we did not see abnormalities of fetal heart rate trace or evidence of abnormal fetal growth on ultrasound scan in women with IFM, whereas these have been seen in women with reduced fetal movements [20, 22, 24]. Even though some women had high numbers of perceived movements (with 10% women perceiving >115 movements in 30 minutes) we did not see abnormalities in fetal heart rate traces as described in infants with seizure activity [25, 26]. Therefore, the reasons for the observed association between a single episode of increased or excessive fetal movement with late stillbirth in retrospective studies remain unclear. Thus, additional prospective studies are needed which also incorporate women’s description of fetal activity to differentiate between women’s experiences of IFM [8, 27]. These studies should also consider alternative reasons for the observed association including increased maternal anxiety and concern for fetal wellbeing or reduced knowledge of normal fetal activity [28].

**Conclusion**

Presently there is insufficient evidence from prospective studies to suggest that women reporting increased or excessive fetal movements have increased risk of adverse perinatal outcomes; this may be due to challenges for mothers differentiating active healthy babies from those with abnormal excessive activity. Current methods of antenatal surveillance did not detect evidence of fetal compromise, thus are not indicated for this presentation other than to provide maternal reassurance of fetal viability.

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**Tweetable Abstract**

Increased Fetal Movements was reported in 0.45% of pregnancies after 28 weeks’ gestation. This prospective study found no association with adverse pregnancy outcomes, abnormalities of the placenta and umbilical cord, inflammation or placental dysfunction.

**References**

1. Draper, E.S., et al., *MBRRACE-UK Perinatal Mortality Surveillance Report, UK Perinatal Deaths for Births from January to December 2016* 2018, The Infant Mortality and Morbidity Studies, Department of Health Sciences, University of Leicester.: Leicester:.

2. Flenady, V., et al., *Stillbirths: recall to action in high-income countries.* Lancet, 2016. **387**(10019): p. 691-702.

3. Heazell, A.E., et al., *Research priorities for stillbirth: process overview and results from UK Stillbirth Priority Setting Partnership.* Ultrasound Obstet Gynecol, 2015. **46**(6): p. 641-7.

4. Heazell, A.E.P., et al., *Alterations in maternally perceived fetal movement and their association with late stillbirth: findings from the Midland and North of England stillbirth case-control study.* BMJ Open, 2018. **8**(7): p. e020031.

5. Heazell, A.E.P., et al., *Stillbirth is associated with perceived alterations in fetal activity - findings from an international case control study.* BMC Pregnancy Childbirth, 2017. **17**(1): p. 369.

6. Stacey, T., et al., *Maternal Perception of Fetal Activity and Late Stillbirth Risk: Findings from the Auckland Stillbirth Study.* Birth, 2011. **38**(4): p. 311-316.

7. Warland, J., et al., *An international internet survey of the experiences of 1,714 mothers with a late stillbirth: the STARS cohort study.* BMC Pregnancy Childbirth, 2015. **15**: p. 172.

8. Linde, A., K. Pettersson, and I. Radestad, *Women's Experiences of Fetal Movements before the Confirmation of Fetal Death--Contractions Misinterpreted as Fetal Movement.* Birth, 2015. **42**(2): p. 189-94.

9. Heazell, A.E.P., et al., *Excessive fetal movements are a sign of fetal compromise which merits further examination.* Med Hypotheses, 2018. **111**: p. 19-23.

10. Tekgul, H., et al., *The current etiologic profile and neurodevelopmental outcome of seizures in term newborn infants.* Pediatrics, 2006. **117**(4): p. 1270-80.

11. Usta, I.M., A.M. Adra, and A.H. Nassar, *Ultrasonographic diagnosis of fetal seizures: a case report and review of the literature.* BJOG, 2007. **114**(8): p. 1031-3.

12. Whitehead, C.L., et al., *Are increased fetal movements always reassuring?* J Matern Fetal Neonatal Med, 2019: p. 1-6.

13. Huang, C., W. Han, and Y. Fan, *Correlation study between increased fetal movement during the third trimester and neonatal outcome.* BMC Pregnancy Childbirth, 2019. **19**(1): p. 467.

14. Ciobanu, A., et al., *Fetal Medicine Foundation reference ranges for umbilical artery and middle cerebral artery pulsatility index and cerebroplacental ratio.* Ultrasound Obstet Gynecol, 2019. **53**(4): p. 465-472.

15. Gomez, O., et al., *Reference ranges for uterine artery mean pulsatility index at 11-41 weeks of gestation.* Ultrasound Obstet Gynecol, 2008. **32**(2): p. 128-32.

16. Kurmanavicius, J., et al., *Reference resistance indices of the umbilical, fetal middle cerebral and uterine arteries at 24-42 weeks of gestation.* Ultrasound Obstet Gynecol, 1997. **10**(2): p. 112-20.

17. Warrander, L.K., et al., *Maternal perception of reduced fetal movements is associated with altered placental structure and function.* PLoS One, 2012. **7**(4): p. e34851.

18. Pergialiotis, V., et al., *Umbilical cord coiling index for the prediction of adverse pregnancy outcomes: a meta-analysis and sequential analysis.* J Matern Fetal Neonatal Med, 2019: p. 1-8.

19. Ptacek, I., et al., *Quantitative assessment of placental morphology may identify specific causes of stillbirth.* BMC Clin Pathol, 2016. **16**: p. 1.

20. Higgins, L.E., et al., *Antenatal placental assessment in the prediction of adverse pregnancy outcome after reduced fetal movement.* PLoS One, 2018. **13**(11): p. e0206533.

21. Sadovsky, E. and W.Z. Polishuk, *Fetal movements in utero: nature, assessment, prognostic value, timing of delivery.* Obstet Gynecol, 1977. **50**(1): p. 49-55.

22. Dutton, P.J., et al., *Predictors of poor perinatal outcome following maternal perception of reduced fetal movements--a prospective cohort study.* PLoS One, 2012. **7**(7): p. e39784.

23. Winje, B.A., et al., *Placental pathology in pregnancies with maternally perceived decreased fetal movement--a population-based nested case-cohort study.* PLoS One, 2012. **7**(6): p. e39259.

24. Daly, N., et al., *Cardiotocography as a predictor of fetal outcome in women presenting with reduced fetal movement.* Eur J Obstet Gynecol Reprod Biol, 2011. **159**(1): p. 57-61.

25. Ingemarsson, I. and J.A. Spencer, *Fetal seizure activity associated with lethal cerebral damage at birth: two cases.* Acta Obstet Gynecol Scand, 1998. **77**(1): p. 127-9.

26. Landy, H.J., A.N. Khoury, and P.S. Heyl, *Antenatal ultrasonographic diagnosis of fetal seizure activity.* Am J Obstet Gynecol, 1989. **161**(2): p. 308.

27. Heazell, A.E., et al., *Stillbirth is Associated with Perceived Alterations in Fetal Activity – Findings from an International Case Control Study.* BMC Pregnancy and Childbirth, 2017. **17**(1): p. 369.

28. Warland, J., et al., *"They told me all mothers have worries", stillborn mother's experiences of having a 'gut instinct' that something is wrong in pregnancy: Findings from an international case-control study.* Midwifery, 2018. **62**: p. 171-176.

**Figure Legends**

**Figure 1.** Flowchart showing details of participant recruitment and sample collection.

**Figure 2**. Assessment of placental vascularity and turnover. (A) Proliferation measured as the number of Ki67 positive nuclei as a percentage of total nuclei. There were no significant differences between IFM (n=36) and NFM (n=28) groups (p=0.27, Mann-Whitney U test). (D) Apoptotic index, measured as the number of cells with cytoplasm stained positive for M30 as a percentage of total cells, showed no significant differences between IFM (n=36) and NFM (n=29) groups (p=0.66, Mann-Whitney U test). (G) Vascularity assessed by CD31 immunostaining in samples from IFM (n=36) and NFM (n=30) placentas demonstrated no difference in vascularity between the two groups (p=0.28, Mann-Whitney U test). The black line shows the median. Representative images of Ki67 (B-C), M30 (E-F) and CD31 (H-I) staining. The red arrows point to positive staining. Scale bars = 50μm. IFM, increased fetal movements; NFM, normal fetal movements.

**Tables**

|  |  |  |  |
| --- | --- | --- | --- |
| **Characteristic** | **INVEST Cohort****(n=64)**  | **Liverpool Women’s Hospital**  **(n=8131)**  | **St Mary’s Hospital, Manchester (n=8941)**  |
| Age (years)  | 30 (21-43) | 30 (15-54) | 31 (14-54) |
| BMI (kg/m2) | 27 (17-50) | - | 26 (14-58) |
| Gravidity  | 3 (1-13) | 2 (1-33) | 3 (1-18) |
| Parity  | 1 (0-11) | 1 (0-11) | 1 (0-14) |
| Previous pregnancies ending before 24 weeks’ gestation | 1 (0-9) | - | - |
| Ethnicity (n,(%))White British UnclassifiedPakistaniEastern European Chinese South East Asian Western European Southern European Middle Eastern East African Caribbean  | 44 (68.8)5 (7.8)5 (7.8)2 (3.1)2 (3.1)1 (1.6)1 (1.6)1 (1.6)1 (1.6)1 (1.6)1 (1.6)  | - |  |
| Cigarette Smoking (n,(%)) | 2 (3.1) | 1262 (15.5) | 782 (8.9) |
| Alcohol Use (n,(%)) | 3 (4.7) | - | - |
| Administration of IM Steroids (n,(%)) | 1 (1.6) | - | - |
| Past Medical and/or Surgical History (n,(%)) | 29 (45.3) | - | - |
| Prescribed Medications (n,(%))Ferrous Sulphate InhalersAspirinFolic Acid Other  | 24 (37.5)4 (6.3)4 (6.3)3 (4.7)2 (3.1)11 (17.2) | - | - |

**Table 1.** Demographics of women recruited to INVEST compared to the background obstetric populations.Continuous data are presented as the mean (range). Categorical data are given as the total number of participants (percentage). Past medical and/or surgical history is defined as the presence of one or more current or previous surgical or medical conditions. Cells with a dash represent demographics in the background obstetric population where information was not available. BMI, body mass index.

|  |  |  |
| --- | --- | --- |
| **Characteristics of Presentation** | **Number** | **Result** |
| Duration of IFMs  | < 1 hour ≥1 < 6 Hours≥6 < 12 Hours≥12 < 24 Hours ≥24 HoursUnknown | 64  | 11 (17.2) 12 (18.8) 8 (12.5) 9 (14.1) 19 (29.7) 5 (7.8)  |
| Time Passed Since Episode of IFMs  | Ongoing ≤ 12 Hours>12 ≤24 Hours> 24 hours Unknown  | 64  | 16 (25) 24 (37.5) 14 (21.9)8 (12.5)2 (3.1) |
| Gestation at Presentation (days) | 64 | 242 (194-287) |
| Blood Pressure on Admission with IFMs | Systolic (mmHg) Diastolic (mmHg) | 61 | 116 (84-137)68 (56-90) |
| Cardiotocography findings:  | Baseline (bpm)Variability (bpm)Accelerations present (n (%))Decelerations present (n (%)) | 60565654 | 139 (121-156)12 (4-20)56 (100)3 (5.6) |
| Number of Fetal Movements per 30 minutes | 50 | 48 (2-227) |
| Amniotic Fluid Index at Presentation: (n (%)) | Normal Oligohydramnios  | 49 | 47 (95.9) 2 (4.1) |
| Maximum Pool Depth at Presentation: (n (%)) | Normal Polyhydramnios Oligohydramnios | 53 | 50 (94.3)2 (3.8)1 (1.9) |
| Abnormal Placental Scan Appearance (n (%)) | 52 | 7 (13.5) |
| Estimated fetal weight centile | 54 | 58.5 (5.9-98.7) |
| Estimated fetal weight < 10th centile (n (%))  | 54 | 3 (5.6) |
| Doppler Ultrasound Investigations  | Umbilical artery PI Middle cerebral artery PIMean uterine artery PI | 544749 | 0.92 (0.51-2.71)1.84 (0.97-3.35)0.72 (0.38-1.26) |
| Uterine Artery Notching (n (%)) Present | 49 | 3 (6.1) |

**Table 2. Presenting characteristics of pregnancies with increased fetal movements.** Continuous data are presented as mean (range). Categorical data are given as number of participants (percentage), bpm, beats per minute; IFM - increased fetal movements; PI, pulsatility index; RI, resistance index.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Outcome** | **INVEST (n)** | **INVEST Cohort** | **Liverpool Women’s Hospital**  | **St Mary’s Hospital, Manchester**  |
| Presentation with RFMs before the end of pregnancy? (n (%)) | 64 | 23 (35.9) | - |  |
| Obstetric problems before the end of pregnancy? (n (%))Yes Fetal growth restriction Hypertension Gestational diabetes Threatened preterm labour Large for gestational age Oligohydramnios Antepartum haemorrhageOther  | 64 | 15 (23.4)3 (4.8)1 (1.6)1 (1.6)1 (1.6)1 (1.6)1 (1.6)1 (1.6)5 (8) | - | - |
| Fetal sex: (n (%))Male Female  | 64 | 26 (40.6)38 (59.4) | - | - |
| Gestation at delivery (days)  | 64 | 39+1 (35+4- 41+5) | 39+0 (28+1 – 42+2) | 39+2 (28+0 – 43+5) |
| Preterm births (<37 weeks) (n (%)) | 64 | 5 (7.8) | - | - |
| Induction of labour (n (%)) | 64 | 32 (50) | 3511 (43.2) | 3391 (37.9) |
| Mode of Delivery:  | Normal vaginal delivery Instrumental vaginal Caesarean section:  | 64 | 36 (56.3)11 (17.2)17 (26.5) | 4689 (57.7)1078 (13.3)2364 (29.1) | 4879 (54.6)1379 (15.4)2683 (30.0)  |
| Birthweight (grams) | 64 | 3410 (2492-3930) | - | 3270 (490 – 5158) |
| Birth weight centile  | 64 | 54.5 (1.9-99.7) | 45.1 (0-100) | 57.4 (0-100) |
| Birth weight centile thresholds (n (%)) | <3rd centile <10th centile >90th centile  | 64 | 2 (3.1)4 (6.3)6 (9.4) | - | - |
| Birth Outcomes  | Apgar 1 minute Apgar 5 minuteArterial pH Arterial BEVenous pH Venous BEAdmission to neonatal unit | 63633534333564 | 9 (6-10)10 (9-10)7.2 (7.02-7.34)-7.6 (-10 - 14.1)7.3 (7.16-7.44)-5.2 (-1.1 -11.7)4 (6.3) | 8 (0-10)10 (0-10)7.23 (6.78-7.81)-5.27 (-22 – 40.7)7.31 (6.8-7.97)-3.75 (-19 – 18.2)711 (8.7) | 9 (0-10)10 (0-10)7.20 (6.52-7.55)-5.92 (-25.3 – 13.4)7.30 (6.62-7.56) -4.77 (-23.4 – 15.5)1133 (12.6) |
| Total composite adverse outcomes  | 64 | 7 (10.9) | - | - |

**Table 3. Pregnancy outcomes in women recruited to INVEST compared to the background obstetric population giving birth after 28 weeks’ gestation.** Continuous data are presented as mean (range). Categorical data are given as the total number of participants (percentage). Cells with a dash represent outcomes in the background obstetric population where information was not available. BE, base excess; RFMs, reduced fetal movements.

|  |  |  |  |
| --- | --- | --- | --- |
| **Macroscopic Characteristic** | **Control****Median (Range) Or****Mean (Standard Deviation)****Or Number (%)** | **IFM****Median (Range) Or****Mean (Standard Deviation)****Or Number (%)** | **P value** |
| Untrimmed placental weight (grams)\* IFM n=31 | 648.5 (454.4-952.7) | 606 (404.4-1003) | 0.50 |
| Trimmed placental weight (grams) ∆Control=25 | 541.4 (±116.9) | 494 (±95.87) | 0.10 |
| Mean diameter (cm) ∆ | 17.6 (±2.3) | 18.39 (±2.1) | 0.20 |
| Maximum radius (cm)\* | 9.9 (7.3-16.7) | 10.5 (8.4-14.2) | 0.30 |
| Minimum radius (cm) ∆  | 7.4 (±1.0) | 7.9 (±1.0) | 0.05 |
| Placental surface area ∆ | 251.5 (±69.5) | 271.8 (±67.2) | 0.26 |
| Placental roundness\*  | 1.5 (1.4-1.8) | 1.5 (1.4-1.9) | 0.71 |
| % total of abnormally white maternal placental surface\*  | 0 (0-38) | 0 (0-3.5) | 0.16 |
| Cord Insertion Distance from Nearest Placental Edge (cm) ∆IFM n=31 | 5.3 (±2.3) | 4.9 (±2.5) | 0.52 |
| Ratio of Cord Insertion Distance:Max Radius ∆IFM n=31 | 0.5 (±0.2) | 0.5 (±0.2) | 0.48 |
| Number of cords with false knots (%) ᶧ | 1 (3.8) | 3 (9.4) | 0.62 |
| Coiling Index\*  | 0.18 (0-0.5) | 0.16 (0-0.3) | 0.39 |
| Number of Cords with an Umbilical Coiling Index >0.3 (%) ᶧ  | 3 (11.5) | 5 (15.6) | 0.72 |

**Table 4. Macroscopic features of placentas and umbilical cords from pregnancies with increased fetal movements and control pregnancies with normal fetal movements**. Control n=26, IFM n=32 unless otherwise stated. ∆ = Unpaired t-test, data shown as mean (±SD); \* = Mann-Whitney U test, data shown as median (range); ᶧ = Fisher’s exact test, data shown as n (%). Circular objects will have a roundness = 1; other shapes will have a roundness > 1. Cord insertion distance:max radius values closer to 1 indicate greater centricity of umbilical cord insertion whereas values closer 0 indicate more marginal insertion. Coiling index calculated as total number of umbilical cord coils/total length of cord (cm).