BMJ Open

Protocol for an observational study investigating hormones triggering the onset of sustained lactation: the INSIGHT study

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ABSTRACT

Introduction Lactation is a hormonally controlled process that promotes infant growth and neurodevelopment and reduces the long-term maternal risk of diabetes, cardiovascular disease and breast cancer. Hormones, such as prolactin and progesterone, mediate mammary development during pregnancy and are critical for initiating copious milk secretion within 24–72 hours post partum. However, the hormone concentrations mediating lactation onset are ill defined.

Methods and analysis The primary objective of the investigating hormones triggering the onset of sustained lactation study is to establish reference intervals for the circulating hormone concentrations initiating postpartum milk secretion. The study will also assess how maternal factors such as parity, pregnancy comorbidities and complications during labour and delivery, which are known to delay lactation, may affect hormone concentrations. This single-centre observational study will recruit up to 1068 pregnant women over a 3-year period. A baseline blood sample will be obtained at 36 weeks' gestation. Participants will be monitored during postpartum days 1–4. Lactation onset will be reported using a validated breast fullness scale. Blood samples will be collected before and after a breastfeed on up to two occasions per day during postpartum days 1–4. Colostrum, milk and spot urine samples will be obtained on a single occasion. Serum hormone reference intervals will be calculated as mean±1.96 SD, with 90% CIs determined for the upper and lower reference limits. Differences in hormone values between healthy breastfeeding women and those at risk of delayed onset of lactation will be assessed by repeated measures two-way analysis of variance or a mixed linear model. Correlations between serum hormone concentrations and milk composition and volume will provide insights into the endocrine regulation of milk synthesis.

Ethics and dissemination Approval for this study had been granted by the East of England—Cambridgeshire and Hertfordshire Research Ethics Committee (REC No. 20/EE/0172), by the Health Research Authority (HRA), and by the Oxford University Hospitals National Health Service Foundation Trust. The findings will be published in high-ranking journals and presented at national and international conferences.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The investigating hormones triggering the onset of sustained lactation study comprises biological and comprehensive phenotype data.
⇒ Metabolites associated with the onset of lactation will be evaluated in serum, urine and milk samples.
⇒ Participants will benefit from breastfeeding support provided by the study investigators.
⇒ Risk of limited participant recruitment and retention during late pregnancy and the early postpartum period.

Trial registration number ISRCTN12667795.

INTRODUCTION

Lactation is tightly regulated by metabolic and reproductive hormones.[1] It plays a key role in infant health and neurodevelopment, and decreases infant mortality and morbidity from infections during the first year of life.[2 3] Longer-term benefits of breast feeding for the offspring include a reduced risk of obesity and type 2 diabetes, and higher performance in intelligence tests.[3–5] Lactation also confers benefits on breastfeeding women, and is associated with decreases in the risk of maternal diseases such as type 2 diabetes, cardiovascular diseases, and breast and ovarian cancer.[6–9]

Hormones influence the mammary gland from the onset of puberty until the end of pregnancy to induce formation of a branched epithelial milk-secreting organ. From puberty-onwards, oestrogen and the growth hormone/insulin-like growth factor (IGF-1) axis promote elongation and branching of the mammary ductal structure.[10 11] During pregnancy, prolactin, progesterone, oestradiol, insulin, growth hormone/IGF-1 and glucocorticoid hormones such as cortisol

Table 1 Major reproductive and metabolic hormones influencing mammary gland function in pregnancy and at the onset of lactation

Hormone	Role in pregnancy	Role at the onset of lactation	References
Reproductive			
Prolactin	Lobuloalveolar formation Milk synthesis	Tight junction formation Milk synthesis	16 19 20 26 27
Progesterone	Mammary ductal branching Lobuloalveolar formation	Withdrawal of progesterone initiates tight junction formation and increases milk synthesis	16 28 29
Oestradiol	Mammary ductal branching Lobuloalveolar formation	Not known	13
Metabolic			
Cortisol	Lobuloalveolar formation Milk synthesis	Tight junction formation	17 26
Insulin	Lobuloalveolar formation Milk synthesis	Milk synthesis	15 21 30
Growth hormone/IGF-1	Lobuloalveolar formation	Not known	12 14

IGF-1, insulin-like growth factor.

stimulate the formation of milk-producing lobuloalveolar structures at the end of mammary ducts (table 1).[12–17]

Prolactin is the principal hormone promoting milk synthesis, and circulating concentrations progressively increase throughout pregnancy and are correlated with the rate of lactogenesis, as assessed by urinary lactose excretion.[18] Prolactin signals via the STAT5 transcription factor to increase expression of mammary-specific genes mediating the production of lactose, milk proteins and lipids.[19 20] These lactogenic effects of prolactin are augmented by thyroid hormones, insulin and glucocorticoids, which enhance STAT5 activation and/or the transcriptional activation of milk synthesis genes in mammary cells.[21–23]

Hormones also trigger the postpartum onset of copious milk secretion, known as secretory activation, which typically occurs between 24 and 72 hours following childbirth (table 1).[1] Secretory activation is associated with an increase in milk volume, which leads to the maternal perception of milk 'coming in', characterised by breast fullness and swelling.[24 25] Secretory activation is mediated by a marked decrease in the circulating concentration of progesterone following delivery of the placenta, and requires a high circulating prolactin concentration in the presence of insulin and cortisol.[1] At the onset of lactation these hormonal changes increase milk synthesis and induce the closure of tight junction complexes between mammary cells, which establishes an osmotic gradient that stimulates water movement into the alveolar lumen (table 1).[26–30]

Secretory activation is critical for the establishment of lactation, and an absence or delay in onset of lactation is a major cause of inadequate and/or early cessation of breastfeeding.[31 32] Although the key hormones involved in secretory activation have been described, their circulating concentrations required for initiating lactation in the early postpartum period are not well defined.[33]

Furthermore, alterations in circulating metabolites associated with secretory activation remain to be fully characterised.

METHODS AND ANALYSIS
Objectives
Investigating hormones triggering the onset of sustained lactation is a single-centre observational study aiming to: (1) characterise the circulating hormone concentrations required for initiating postpartum milk secretion and (2) assess how maternal factors such as parity, pregnancy comorbidities, and complications during labour and delivery, which are known to delay lactation (table 2), may affect hormone concentrations.

Primary objective
Establishment of reference intervals (RIs) for hormones initiating postpartum onset of lactation.

Secondary objectives
► Measure longitudinal changes in circulating lactation hormones and metabolites during the first 4 days postpartum.
► Evaluate effects of maternal comorbidities, medications, mode of delivery and pregnancy complications on lactation hormone concentrations.
► Assess whether lactation hormone concentrations correlate with the timing of the onset of milk secretion.
► Evaluate the relationship between lactation hormones and breast milk volume.
► Characterise breast milk hormone composition.

Outcomes
Primary outcome
RIs will be established for lactation hormones during the early postpartum period before and after a breastfeed

Table 2   Examples of causes of delayed onset of lactation and association with endocrine disturbances

Cause	Effect on lactation	Associated endocrine abnormalities	References
Maternal factors/comorbidities			
Pituitary insufficiency, for example, Sheehan's syndrome	Absent or delayed onset	↓ multiple pituitary hormones	45
Polycystic ovary syndrome	Delayed onset and/or lactation insufficiency due to lack of prenatal breast enlargement	↑androgens, obesity-related endocrine abnormalities (see-below)	46 47
Obesity	Delayed onset	↓ mammary responsiveness to prolactin	50 51
Primiparity	Delayed onset	Possible ↓ mammary responsiveness to pregnancy-related hormones	48 49
Labour and delivery factors			
Medications, for example, epidural analgesia, i.v. oxytocin	Delayed onset	↓oxytocin	52 53
Caesarean section	Delayed onset	↓oxytocin, ↓prolactin	32 54

during the morning and afternoon, which will account for the effect of diurnal variation and infant suckling on hormone concentrations.

Secondary outcomes
Serial blood sampling during the early postpartum period will allow assessment of longitudinal changes in the concentrations of hormones during secretory activation. Correlation of hormone concentrations with maternal comorbidities, and complications during pregnancy and delivery, will help elucidate how these factors cause delayed onset of lactation. Furthermore, correlation of hormone concentrations with milk composition and volume will provide insights into the endocrine regulation of milk synthesis.

Study design
Pilot study phase
To assess whether sample collection will be feasible during the early postpartum period, an initial pilot study involving up to 100 women will be performed. Postpartum samples will be collected during a single study visit on postpartum day 4, either at the participant's home or in hospital. These samples will be used for an initial analysis of lactation hormone concentrations, and the data included in the final analysis.

Main study phase
To assess changes in lactation hormones during the early postpartum period, blood sampling will be performed at around 36 weeks' gestation, as a baseline measure and also before and after a breastfeed during the first 4 days after birth. Participants can provide blood samples on a single postpartum day, or opt for serial sample collection during postpartum days 1–4. Participants will record their breast fullness to determine the timing of lactation onset, as previously described.[32] Demographic and clinical characteristics such as participant age, comorbidities,

medications and complications occurring during pregnancy and delivery, will also be recorded. Infant birth weight will be recorded, and the infant weighed before and after a breastfeed to estimate milk volume. Up to 10 mL of milk will be obtained for biochemical analysis and a spot urine sample will be collected to assess lactation hormone metabolites. The study protocol is shown in figure 1.

Participants
Over a 3-year period, the study will recruit up to 1000 pregnant women attending maternity units within Oxfordshire, UK, who either have no risk factors for delayed onset of lactation (n~600), or one or more risk factors such as primiparity or obesity (n~400) (table 2).

Inclusion criteria
► Pregnant women, aged ≥18 years.
► Intention to fully or partially breastfeed.
► Willing and able to give informed consent for participation in the study.

Exclusion criteria
Participants may not enter or continue in the study if ANY of the following apply:
► Preterm birth (<37 weeks' gestation).
► Severe maternal illness, including postpartum depression or psychosis.
► Severe neonatal illness including major congenital abnormalities and infants who are only expected to live for a short period of time.
► Prolonged separation of infant from mother, for example, due to admission to the neonatal unit.
► Maternal or neonatal COVID-19 test positivity.
► Mother or infant infected with blood borne viruses such as HIV.
► Resides outside of Oxfordshire.
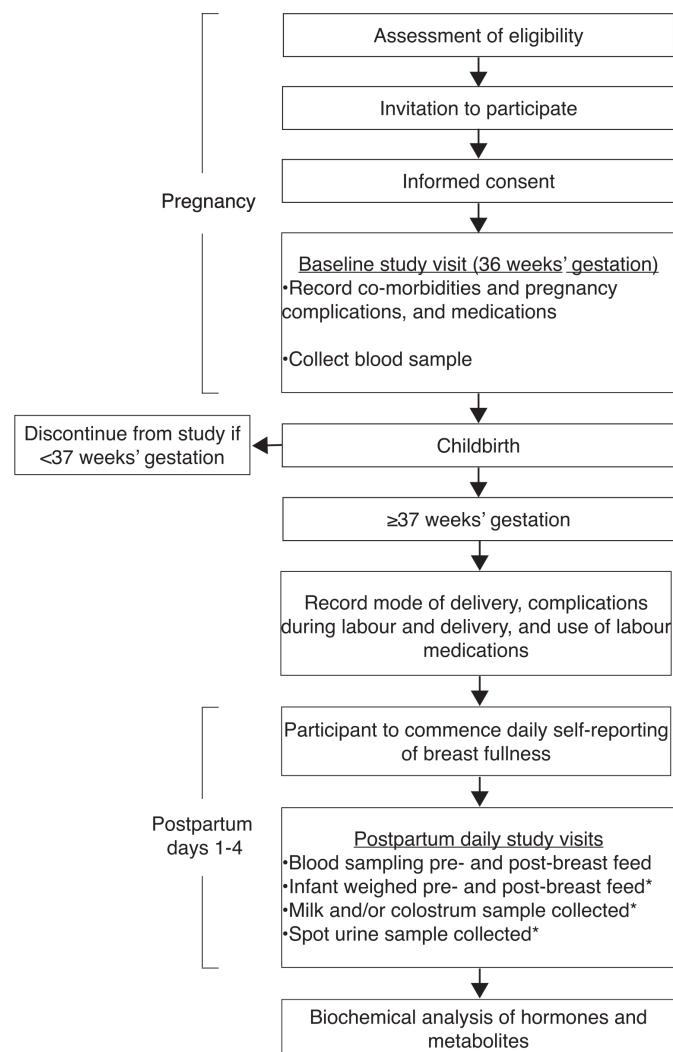
Figure 1 Flow chart of INSIGHT study procedures.
*Procedures undertaken only on a single occasion. INSIGHT, investigating hormones triggering the onset of sustained lactation.

► Safeguarding issues that may impede the safety of research staff carrying out home visits.
► Current participation in another research study involving investigational medicinal products.

Participant enrolment
Screening and recruitment
Potential participants will be screened by assessing their eligibility and intention to breastfeed. Introductory study information will be provided during antenatal clinic or ultrasound scan visits. Women can also identify themselves as eligible from advertising materials such as posters, social media advertising and the advertisement on the Larsson-Rosenquist Foundation Oxford Centre for the Endocrinology of Human Lactation (https://www.ocehl.ox.ac.uk/insight) website.

Informed consent
A trained member of the research team will obtain written informed consent. Written and verbal information will be provided to the participant detailing the exact nature of the study and any risks involved in taking part. Potential participants will be given the opportunity to discuss the study with the investigator, their general practitioner (GP) or other independent parties to decide whether to participate. It will be clearly stated that the participant is free to withdraw from the study at any time and for any reason without prejudice to her or her infant's future care, without affecting her rights, and with no obligation to give the reason for withdrawal.

Informed consent will be obtained electronically (e-consent) on a tablet device. If this is unavailable, then written informed consent will be obtained. The participant will be provided with a copy of the completed consent form and participant information sheet. The e-consent form will be stored on the trial specific database held on the University of Oxford High Compliance Server, and a copy kept in the medical records. The participant must complete the e-consent form or hard copy version of the informed consent form before any study procedures are performed.

Study schedule
Baseline assessment
The participant's comorbid conditions, medications and pregnancy complications will be recorded, and a blood sample collected.

Subsequent visits
Admission in labour or for caesarean section
The research team will check that participants are ≥37 weeks' gestation, as defined by the first trimester dating ultrasound scan.

Day 0–1 post partum
Continued consent will be confirmed with the participant. Delivery or caesarean section complications, and use of medications (eg, syntocinon, analgesia), will be recorded. Blood sampling will commence within 24 hours of birth and samples obtained pre-breastfeed and post-breastfeed. Self-reporting of breast fullness will also commence.

Day 1–4 post partum
The research midwife or research assistant will visit the participant in hospital, or at home. Blood samples will be taken pre-breastfeed and post-breastfeed. Breastfeeding support will be provided, if requested by the participant. A colostrum sample of ~1 mL and urine sample of ~20 mL will be obtained on a single occasion.

Day 4 post partum
A milk sample (~10 mL) will be obtained. Milk volume will be recorded by weighing the infant pre-feed and post-feed. Daily recordings of breast fullness will be collected on the final study visit.

Study procedures
Sample collection and handling
Around 10 mL of blood will be collected per sampling time point, and a maximum of four blood samples (two from a morning and two from an afternoon breastfeeding

session) will be obtained per participant over a 24-hour period. Thus, a maximum of 16 blood samples will be obtained from each participant over a 4-day period. Blood sampling will be undertaken at similar times during successive study visits to minimise the effect of circadian rhythm on hormone concentrations.

Colostrum and milk will be collected using an electric or manual breast pump, or by hand expression into a sterile container. On the day of collection, blood samples will be centrifuged and aliquoted centrally. Milk samples will be divided into skimmed milk (centrifuged) and whole milk aliquots (uncentrifuged). Samples will either be analysed freshly or frozen at −80°C for later analysis.

Biochemical measurements

Lactation hormones, such as prolactin, progesterone, oestradiol, cortisol, insulin, growth hormone and IGF-1 (table 1), will be measured with validated immunoassay methods. Milk components and metabolites with potential as biomarkers for secretory activation will be assayed in blood, milk and urine samples using techniques including mass spectrometry. These molecules include: carbohydrates such as lactose, glucose, sialic acid and oligosaccharides; amino acids such as alanine, aspartate and glutamate; lipids such as free fatty acids and triacylglycerols; and intermediary metabolites involved in lipid synthesis such as citrate and oxaloacetate.[34] The biochemical and cellular composition of colostrum and breast milk will be assessed by flow cytometry, immunoassay and mass spectrometry.

Any residual sample will be stored long term for use in future ethically approved studies in accordance with University of Oxford guidelines and the Human Tissue Authority's Code of Practice. At the end of the study, with consent, samples may also be transferred to an ethically approved Research Tissue Bank for long-term storage. However, if consent has not been received then the samples will be destroyed according to local protocol.

Recording breast fullness and factors influencing the onset of lactation

Participants will record their perception of lactation onset on a daily basis from birth until postpartum day 4 using a validated breast fullness numerical scale of 1–5, anchored with 1=no change, 3=noticeably fuller and 5=uncomfortably fuller, as described.[32] The onset of lactation will be indicated by the time when breast fullness first reached scale point 3.[32] The following factors will also be recorded at each study visit: frequency and duration of mother–infant skin-to-skin contact; frequency and duration of breastfeeds, and use of formula milk or other feeds.

Infant weighing

The volume of the participant's breast milk expressed during a feed will be estimated by weighing her infant immediately before and after feeding using a calibrated portable electronic scale, as described.[35]

Withdrawal or discontinuation of participants

A participant may choose to withdraw from the study at any time, or choose to stop blood sampling at any time and remain on study follow-up. Participants who withdraw from the study can permit data and samples obtained up until the point of withdrawal to be retained for analysis. In addition, the chief investigator may discontinue a participant from the study at any time. Reasons include ineligibility (either during the study or retrospectively if overlooked during screening and the study), significant protocol deviation, significant non-compliance with study requirements, or as a clinical decision.

If the participant withdraws from the study before donating any samples then no data will be used. Withdrawn participants will be replaced. If the participant is withdrawn due to an adverse event, the chief investigator will arrange for referral to her GP or an appropriate healthcare professional involved in her care. In the event of a serious adverse event, the participant will be withdrawn from the study. The reason for withdrawal will be recorded in the case report form (CRF).

Statistics and analysis
Primary outcome analysis

The RI, which represents the middle 95% of measurements for a lactation hormone in a population of breastfeeding women with no risk factors for delayed onset of lactation (table 2), will be derived according to the Clinical and Laboratory Standards and International Federation of Clinical Chemistry recommendations by two methods.[36 37] Data will be log-transformed to achieve a parametric distribution. The mean and SD will be derived, and the RI calculated as mean±1.96 SD, with 90% CIs determined for the upper and lower reference limits. If hormone values cannot be normalised by logarithmic transformation, then a non-parametric percentile method will be used. This involves ordering measurements from smallest to largest, with the RI determined as being between the 2.5th and 97.5th centile of the ordered observations. For a sample size of 120, the 2.5th and 97.5th centiles represent the 3rd and 118th observations, respectively.

As samples will be taken pre-feed and post-feed, and at different times during the day, these RIs will consider the effect of diurnal variation and infant suckling on hormone concentrations.

Secondary outcome analysis

Differences in hormone values between participants with no risk factors for delayed onset of lactation and those with ≥1 risk factor will be assessed by repeated measures two-way analysis of variance or a mixed linear model. The log-transformed hormone value will be used as the outcome variable, and the two nominal variables will be (1) absence or presence of risk factors for delayed lactation and (2) the timing of the sample (pre breast feeding or post breast feeding).

Hormone values from a subset of participants willing to provide serial blood samples over a 4-day period will be used to assess longitudinal hormone changes. A mixed linear model, which incorporates factors such as breastfeeding status and sampling pre-feeds and post-feeds, will be used to analyse the temporal change in lactation hormone values over the study period while accounting for any missing data.

To determine whether lactation hormone concentrations are associated with breast milk volume or the timing of onset of lactation, bivariate correlations will be assessed using the Pearson or Spearman rank correlation tests. Adjustment for potential confounding variables will be undertaken using a multivariate linear regression model.

Sample size determination for primary outcome

Around 120 participants with no risk factors for delayed onset of lactation are required to establish an RI with acceptable 90% CIs for the upper and lower reference limits.[37] This study involves establishing RIs at a single time point in the third trimester and for up to 16 different time-points over a 4-day period. Each participant is expected to provide blood samples for an average of four time-points, and therefore 480 participants will likely be required to provide samples for all 16 time-points. A total of N=1920 postpartum blood samples (120 blood samples at each of 16 time points) are required for the primary outcome. The pilot study is also expected to contribute around 100 samples from 50 participants (two samples per participant) to the primary outcome. Therefore, 455 participants providing an average of 4 samples each (total of 1820 postpartum samples) will be recruited to the main study. Thus, N=505 participants (50 from pilot study and 455 from main study) will be recruited in total for the primary outcome analysis.

Sample size determination for secondary outcomes

A major secondary outcome is alteration in lactation hormones for participants at risk of delayed onset of lactation compared with participants with no risk factors. Prolactin is critical for secretory activation, and sample size has been determined using published prolactin values for breastfeeding women.[38] The effect size (d) for a 10% change in serum prolactin is 0.57, given that a 10% change is 18.3 µg/L and pooled SD is 32 µg/L.[38] G*Power statistical software was used to calculate sample size with this effect size and the following parameters: a two-tailed t-test for difference between two independent means, alpha error probability of 0.05, power of 0.95 and a 1:1 allocation ratio. This calculation indicates that a sample size of 81 participants with ≥1 risk factor for delayed onset of lactation is required at each time point for secondary outcome analysis. Assuming each participant provides samples for an average of four time points, then 324 participants are required to provide samples for all 16 time points. A total of N=1296 postpartum blood samples (81 blood samples at each of 16 time points) are required for the secondary outcomes. The pilot study is also

expected to contribute ~100 samples from 50 participants (two samples per participant) to the secondary outcome. Therefore, 299 participants providing an average of 4 samples each (total of 1196 postpartum samples) will be recruited to the main study. Thus, N=349 participants (50 from pilot study and 299 from main study) will be recruited in total for the secondary outcome analysis.

Accounting for participant withdrawal

In total, 505 participants with no risk factors for delayed onset of lactation, and 349 participants with ≥1 risk factor are required for the primary and secondary outcome analyses. Assuming a 20% drop-out rate, then 632 participants with no risk factors and 436 participants with ≥1 risk factor for delayed onset of lactation (1068 participants in total) will need to be recruited.

Decision points and end of study

The protocol will be evaluated in a pilot study involving ~100 participants. Depending on the results, a decision will be made whether to limit recruitment to certain participant groups, such as multiparous women with no breastfeeding issues who are more willing to participate in the study and provide multiple blood samples. In addition, if sample collection from participants at home proves difficult, then the protocol will be amended to collect blood samples while participants are in hospital following child birth, and only collect limited samples during home study visits.

The end of the study is the point at which all data have been collected and analysed, and the database is locked. If it is not possible to collect sufficient blood samples to establish RIs at any of the time points, then the study will be stopped.

Procedure for accounting for missing, unused and spurious data

If there are insufficient (ie, N<120) samples to calculate the RIs at each time-point using log-normal distribution or the centile method, then a bootstrap calculation method will be used to derive RIs. Bootstrapping involves random re-sampling of the original data observations to generate replacement values and establishment of a 'pseudosample' from which RIs can be derived.[39] This method requires normally distributed data obtained from a minimum of 40 samples.[40] The derivation of hormone RIs can also be affected by values lying above or below the assay detection limit. For very high values, sample dilution will be undertaken prior to biochemical analysis.

Data management

Data will be recorded using an electronic CRF (eCRF), and then transferred to the study specific database. This holds the unique participant study numbers, sample numbers, screening log, e-consent forms, as well as clinical and laboratory data. Any hard copies of the CRFs and consent forms will be kept in a locked filing cabinet in the key-coded locked office of the Lead Research Midwife. The consent form will be retained to meet the UK Human

Tissue Authority traceability requirements, and also as the basis for retention of details and approach for any future ethically approved studies.

Quality assurance procedures

The study may be monitored or audited in accordance with the current approved protocol, Good Clinical Practice, relevant regulations and standard operating procedures.

Patient and public involvement

Healthcare professionals such as community midwifes reviewed the study protocol and participant-facing information. In addition, breastfeeding women on the hospital postnatal ward, and in the community, reviewed the study advertising and participant-facing information, and provided feedback on the appeal and comprehensibility of this information.

ETHICS AND DISSEMINATION
Study conduct

The study will be conducted in accordance with the principles of the Declaration of Helsinki and with Good Clinical Practice. The chief investigator will submit an annual progress report to the Research Ethics Committee, Health Research Authority (HRA) host organisation, sponsor and funder. An end-of-study notification and final report will be submitted to the same parties. The findings will be published in high-ranking journals in the field and presented at national and international conferences.

Participant confidentiality

The study will comply with the General Data Protection Regulation and Data Protection Act 2018, which require data to be deidentified as soon as it is practical to do so. Participants will be identified only by a participant study number on any electronic database.

Other ethical considerations

Incidental findings are not expected from the sample analysis. Hormone values are likely to vary substantially within a healthy mother during the early postpartum period, and such alterations will not require referral for clinical investigations or management.

DISCUSSION

This protocol has been established to investigate hormone concentrations triggering secretory activation, and ascertain whether alterations in lactation hormones are associated with a delay in the onset of copious milk secretion. Delayed onset of lactation, defined as occurring >72 hours postpartum, affects between 17% and 40% of breastfeeding women, and is reported to cause excessive neonatal weight loss during the first postpartum week, and lead to increased use of formula supplementation and decreased duration of breast feeding.[31 32 41–44] A range of maternal factors, which potentially contribute to endocrine dysregulation, may lead to delayed onset of lactation (table 2). For example, endocrine disorders due to pituitary or ovarian abnormalities have been reported to delay lactation onset or cause lactation failure (table 2).[45–47] Furthermore, parity influences the timing of secretory activation, with primiparous mothers experiencing a significantly increased duration of time from parturition until onset of lactation compared with multiparous mothers.[48] Such effects may have an endocrine basis, as rodent studies have shown an effect of parity on mammary hormone responsiveness. Thus, mammary glands from parous mice exhibit a more rapid expansion of ductal structures and earlier onset of milk protein synthesis when exposed to pregnancy-associated hormones, compared with the mammary glands of hormone-treated nulliparous mice.[49] In addition, maternal obesity, occurring either at the pre-pregnant stage or at the time of delivery, is reported to delay onset of lactation,[50] and a mouse study has revealed that obesity-induced lactation insufficiency is associated with mammary gland resistance to prolactin.[51] Medications administered during labour may also influence lactation hormones and cause delayed onset of lactation.[52 53] The administration of infused oxytocin with epidural analgesia to women in spontaneous labour is reported to decrease postpartum plasma concentrations of oxytocin, which is required for stimulating milk let-down during a breastfeed.[53] Moreover, caesarean section is an independent risk factor for delayed onset of lactation. Delivery by emergency Caesarean section decreases the pulsatility of oxytocin secretion and impairs prolactin secretion during a breastfeed in the early postpartum period.[32 54]

The characterisation of hormone and metabolite concentrations at the physiological onset of lactation, as well as in women at risk of delayed onset of lactation, will improve understanding of the factors involved in the initiation of successful lactation. The study also has the potential to facilitate development of hormonal biomarkers that can predict those likely to suffer from low-volume or delayed breast milk production, and help identify individuals who may benefit from galactagogue therapy to stimulate lactation. In addition, the study will provide biological insights into how lactation hormones influence the volume and composition of breast milk.

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