Study Protocol:

Fenumum

 A Nutritional Supplement for Better Breastfeeding?

Dr Niamh Keating

Academic Clinical Fellow

UCD Perinatal Research Centre

School of Medicine

University College Dublin

National Maternity Hospital, Dublin, Ireland

**Study Protocol**

**Title:**

Fenumum. A Nutritional Supplement for Better Breastfeeding?

**Principle Investigator:**

Dr Sharleen O’Reilly

**Other Investigators:**

Dr Niamh Keating, Dr Aifric O’Sullivan, Associate Professor Mary Higgins, Professor Fionnuala McAuliffe

**Authors and emails:**

Niamh Keating (niamh.keating@ucd.ie)

Mary Higgins (mary.higgins@ucd.ie)

Fionnuala McAuliffe (fionnuala.mcauliffe@ucd.ie)

Sharleen O’Reilly (sharleen.oreilly@ucd.ie)

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**Affiliation**

1. School of Medicine, University College Dublin, Ireland
2. National Maternity Hospital, Dublin, Ireland

**Language**

This study is to be made available in English

**Amendments**

This is currently version 2.0.

The date of each amendment, changes made and the rationale will be recorded here.

Changes made

14/9/20- Elimination of test weigh due to logistic issues. Primary endpoint will now be breastmilk composition at end of study duration

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**Introduction**

**Project Summary**

*Fenugreek is a herbal supplement, traditionally used to promote lactation in breastfeeding mothers. It is also used in Asia as a complementary supplement for diabetes mellitus due to its insulin sensitising properties. Women with insulin resistance as evident by gestational diabetes are more likely to suffer from low milk supply.*

Abstract

**Breastfeeding rates in Ireland are amongst the lowest in the world(Begley et al., 2008, Gallagher et al., 2016)**, despite the health benefits for Irish women (lower rates of breast cancer, diabetes, obesity) and children (lower mortality, SIDS, infections; higher IQ)(Victora et al., 2016, O'Reilly et al., 2011, Boney et al., 2005, Martens et al., 2016).

* UCD Perinatal Research Centre has shown that breastfeeding is associated with less childhood adiposity(Horan et al., 2016). Failure to breastfeed has real economic impact with losses running at 0.53% gross national income, in Ireland’s case €800 million (Victora et al., 2016). **Given these adverse impacts, strategies to improve breastfeeding rates are urgently needed.**
* There is evidence that breastfeeding improves insulin resistance in women with gestational diabetes (GDM). One in every seven Irish pregnancies are affected by GDM, which is caused by insulin resistance developing during pregnancy. **Women with previous GDM are at high risk of breastfeeding cessation due to low breastmilk production**.
* Fenugreek is a seed from the legume family thought to have insulin sensitising effects and is used as a traditional medicine for improving diabetes management in Asia. **Fenugreek is the most commonly recommended breastfeeding supplement for improving breastmilk production** but the mechanism is unclear. The efficacy of fenugreek is questioned due to previous conflicting low quality randomised controlled trials (RCTs).

**We hypothesise that a fenugreek supplement will improve breastmilk production in women with previous GDM through decreased insulin resistance**

**STUDY DESIGN**

**Pilot feasibility study:** The design of this study is to test the feasibility of a proposed RCT methodology and perform initial exploration of the transcriptome and metabolome markers.

**STUDY AIMS**

•To examine the **impact of fenugreek supplementation on breastmilk output** in postpartum women with previous GDM who are at risk of low breastmilk supply

•To **identify the potential mechanism through which fenugreek supplementation influences breastmilk output** (transcriptome and metabolomics analysis)

OUTCOMES

**Primary outcome**: Compliance with fenugreek supplementation, self-reported breastfeeding efficacy using Breastfeeding Self efficacy Scale- Short Form (BSES- SF), blood metabolomics and transcriptome changes

**Secondary outcomes:** Infant length, weight, head circumference; maternal BMI

**Our anticipated research outputs aim to demonstrate fenugreek supplement as a feasible treatment option to improve breastfeeding rates in Ireland and internationally**

**Hypothesis**

Fenugreek is a safe and acceptable galactagogue for women with a history of gestational diabetes who wish to breastfeed. This study aims to further analyse the effects of fenugreek on breastmilk composition and gene expression to test the hypothesis that fenugreek exerts its effect by improving the sensitivity of glandular breast tissue to insulin thereby increasing milk supply.

**Purpose of study protocol**

The purpose of this study protocol is to describe how this intervention study will be conducted and to ensure the safety of the participants and integrity of the data collected.

**Background**

Gestational diabetes is associated with an increased risk of Type 2 Diabetes Mellitus (T2DM) in later life. Around 10% of women with gestational diabetes will be diagnosed with T2DM within six months of delivery with a further 10% will be diagnosed within two years (Kim et al., 2002). Breastfeeding has been shown to reduce this risk. However despite the proven benefits breastfeeding rates of women with gestational diabetes are low. The reasons for this are multifactorial but low milk supply, either measured or perceived, is the most common reason why women stop breast feeding. It has been hypothesised that the state of insulin resistance in gestation diabetes may be the cause of low milk supply in this cohort. Insulin is required for mobilisation of glucose for breastmilk production.

Fenugreek (scientific name *Trigonella*) is a member of the legume family. It has been used for its galactogenic properties in traditional medicine for centuries. Despite this extensive popular use, fenugreek has not been well researched and it is not clear how it exerts its effect. One theory is that fenugreek increases sweat gland production and acts in a similar way on the mammary glands which are modified sweat glands (Betzold, 2004). It has also been demonstrated to have estrogenic properties. Despite its popularity, there remains limited scientific evidence of its benefit. Much of the reports of its benefit are anecdotal or from low quality studies. Information on doses is not consistently reported making it difficult to compare studies (Zapantis et al 2012).

In additional to its use as a galactagogue, Fenugreek also has insulin sensitising effects and it often used as a traditional medicine in Asian as a treatment for T2DM.

**Literature review**

Fenugreek (scientific name *Trigonella*) is a member of the legume family. It has been used for its galactogenic properties in traditional medicine for centuries. Despite this extensive popular use, fenugreek has not been well researched and it is not clear how it exerts its effect. One theory is that fenugreek increases sweat gland production and acts in a similar way on the mammary glands which are modified sweat glands (Betzold et al, 2004). It has also been demonstrated to have estrogenic properties. Despite its popularity, there remains limited scientific evidence of its benefit. Much of the reports of its benefit are anecdotal or from low quality studies. Information on doses is not consistently reported making it difficult to compare studies(Zapantis et al., 2012).

While fenugreek has been used for centuries in traditional practices as a galactagogue, the amount of evidence to support this is limited. Although most of the evidence is anecdotal Fenugreek is commonly recommended by lactation consultants (Sim et al., 2014). There can be a perception that as a herbal treatment it is safer than pharmacological galactagogues. Herbal remedies are often excluded from clinical trials. Breastfeeding studies are often difficult to run and use small sample sizes. Lactating women and women of childbearing age have traditionally been excluded from drug trials due to concerns about the effect of drugs on the fetus or newborn. As a result of this many licenced drugs may have not been dosed appropriately for women. Many studies have used compounds of herbal galactagogues so it is impossible to detect which component had the effect. Some of the studies that used controls are unblinded and vulnerable to bias.

A literature review was performed using Pubmed, Embase, CINAHL, Cochrane, TRIP, Web of Science, Lenus, ClinicalTrials.gov, ClinicalTrialsRegister.eu. Terms searched included Fenugreek, Trigonella, Galactagogue, breast feeding, lactation, lactation disorder, hypogalactia. Papers that looked at the non-galactagogue effects of fenugreek were excluded. 32 papers were identified. Of these, there were nine trials looking at the effects of fenugreek on breastmilk production.

Torbangun leaves (Coleus Amboinicus; CA) are used in traditional practice as a herbal galactagogue among the Bataknese people in North Sumatra in Indonesia (Damanik et al., 2006). In this study, participants were healthy women aged 20-40 who had delivered a healthy term infant with a birth weight more than 2.5kg and who intended to breastfeed for four months. This was a parallel randomisation intervention study with three arms; CA, fenugreek seed and a combination of Moloco + Vit B12 (human placental extract; used in traditional practice particularly in Japan but illegal in the EU).Human placental extract is a hydrolysate of human placenta, used in folk medicine in Asia. They recruited 75 women, with 25 randomised to each arm. Each participant took one month of the supplement, starting on day two postnatal. CA was provided as 150g per day in a soup which was prepared and delivered to the women daily. The dose of Fenugreek in this study was 1 capsule three times daily with each capsule containing 600mg of powdered fenugreek seed extract. Each participant carried out an observed 24 hour test weigh every two weeks. In this study they adjusted for density of breast milk by multiplying the result by 0.988ml. A sample of breast milk was also obtained for macro and micronutrient analysis on Days 8, 33 and 60. The researchers calculated the percentage increase in milk across the three groups. On Day 14 there was no statistically significant difference between milk production in the three groups. Of the three groups Fenugreek had the lowest milk production in 24 hours at day 56 (358ml vs 385ml with Moloco+ vit B12 vs 478ml with CA). This was an unblinded study using a group of people who may have had a bias in favour of CA as it is commonly used in traditional practice. It is not clear how compliance was measured in the fenugreek or Moloco reference groups. There was no difference in the nutritional composition of the breastmilk across the three groups.

Another study (Turkyilmaz et al., 2011) recruited 66 mother infant pairs into 3 even groups; fenugreek, placebo and control. Fenugreek was given in the form of a tea. Participants were given three 200ml cups a day although the amount of fenugreek in each cup was not reported. The placebo was an apple tea. The control group were given routine advice. Outcomes were measure by asking all women to use a standardised breast pump and pump both breasts for 15 mins on day three. Infants were weighed daily until they returned to birth weight. Infants in the fenugreek group had a lower weight loss in the first week of life and a shorter return to birth weight. The control and placebo groups had similar results. As with many breastfeeding studies the number of women recruited was small although a statistically significant difference was noted. The fenugreek use in this study was not pure and contained fennel and raspberry leaves, both of which may have mild galactagogue effects also.

A study looking at the effects of fenugreek on pre term infants (Abdou and Fathey, 2018) recruited women who had given birth at a gestation of less than 32 weeks. This was a case control study. Women in the treatment arm were given 200ml of fenugreek tea (containing 50g fenugreek seed extract) three times a day. They were asked to use a breast pump eight times a day. The age matched controls pumped eight times a day without fenugreek consumption. At baseline a medical history, clinical examination and blood sugar assessment were performed and a diet questionnaire was completed. There were three evaluation time points for breast milk evaluation, day 3, 8 and 15. Prolactin levels were checked on day 3 and 15. A difference in breastmilk production and prolactin levels was observed on day three in favour of fenugreek which did reach statistical significance. At 15 days there was no difference between the treatment and control groups. The authors concluded that fenugreek has a significant effect on early lactogenesis only. This is similar to the findings of the study by Turkylimaz et al. They also concluded that fenugreek may have a mild oestrogenic effect which may account for its effect.

A study looking at the effects of mixed herbal supplementation on breast milk production used a combination of fenugreek, ginger and turmeric (Bumrungpert et al., 2018). This study was carried out in Thailand, where just 12% of women are breastfeeding at six months. This was a randomised double blind placebo controlled trial. 50 subjects were recruited. Inclusion criteria included women who were 1 month post-partum with exclusive breastfeeding. Participants were randomised to either treatment or placebo. The treatment arm of the study received a capsule containing fenugreek, turmeric and ginger three times a day for four weeks. Each capsule contained 200mg fenugreek, 120mg ginger and 100mg tumeric. The placebo was a capsule of corn starch.

Participants were taught to collect breast milk using a manual pump. They were asked to pump for two days at baseline and for two days before assessment at 2 weeks and 4 weeks.

It could be argued that by recruiting women at one month post partum this was likely to be a motivated cohort and less likely to have issues with breast milk production at this stage. A limitation of this study was its small sample size.

This study also looked at breast milk composition and found a higher concentration of vitamin A in the breastmilk of the treatment group. There was no significant difference in adverse effects between the two groups. Daily volume of breast milk produced was equal between the two groups at baseline. This study saw a statistically significant 49% increase in breastmilk production at 2 weeks and a 104% increase at 4 weeks in the treatment group compared with placebo. Both ginger and turmeric are also used as herbal galactagogues so from this study it is impossible to extrapolate that fenugreek had the galactagogue effect.

One study(Sakka AE, 2014) looked at the effect of Fenugreek herbal tea and palm dates. The outcomes measured were breastmilk production and infant birth weight recovery. The study comprised three groups- fenugreek, palm dates and a control. The dose of fenugreek was 1 cup of fenugreek tea three times daily. Each cup of fenugreek tea contained two tablespoons, approximately two grams. The palm dates group consumed 100 grams of palm dates three times daily. The control group had no galactagogue. The infants were weighed on days 3, 7 and 14. The women were asked to pump both breasts before the first feed in the morning of the third day. The control group saw the biggest drop in weight on day 3. The palm dates group had an increase in weight on day 7. There was no difference between group on day 14. Breast milk volume was greater in the fenugreek and palm groups on day 3 than in the control group.

(Ghasemi et al., 2015) looked at the effect of herbal tea containing fenugreek seed on signs of breast milk sufficiency in infants. This was a double blind randomised clinical trial with control groups. The infant parameters measured were wet diapers, frequency of defaecation, number of infant breastfeeding times. This study only included term female infants. The reason for this is unclear. Women with any contraindication to breast feeding, for example maternal HIV infection, were excluded. Fenugreek 7.5g plus 3g of black tea three times daily was provided. Women in the control arm had 3g black tea only, three times daily. The infants in the fenugreek saw a statistically significant increase in the pre-defined signs of breastmilk sufficiency- wet diapers, frequency of defaecation and frequency of infant feeding.

Ravi et al (Ravi and Joseph, 2020)looked at breastmilk production and weight gain in the first week of life. The objectives were to measure breastmilk production based on frequency in urination, the effect on weight gain. 60 women who delivered full term were recruited. 30 were assigned to the treatment arm and 30 to the control group. The inclusion criteria were healthy term infants, one week or less postnatal, poor milk supply reported by healthcare professional and willingness of the mothers to participate. The exclusion criteria were documented mastitis, breast engorgement, inverted nipples, currently consuming other herbals, clinically ill or previous breast surgery. Women in the treatment group were given 7.5g of fenugreek once daily in the morning. The control group received routine care with no intervention. Infant weights were taken on the 1st, 3rd, 5th and 7th days as well as the number of wet nappies. An increase in milk production from day 1 was observed. No side effects were reported. Fenugreek was noted to facilitate a faster return to birth weight. The infants had an increase in frequency of urination and weight gain in infants in the first week of life. The authors concluded that fenugreek was associated with enhanced breast milk production in the early post partum period.

Reeder et al 2013(Reeder et al., 2013) looked at the effect on fenugreek on milk production and prolactin levels in women who delivered pre term infants. A convenience sample of 26 mothers was recruited. Inclusion criteria included infants born at less than 31 weeks gestation. Women were randomised by a pharmacist to either fenugreek or a placebo. Prolactin was measured at baseline, day 5 postnatal. Three capsules of 575mg fenugreek was prescribed to be taken three times daily for 21 days. Women were asked to use an electronic breast pump eight to ten times per day for 21 days. Prolactin was measured every five to seven days. In a logbook the women were asked to record the minutes spent pumping, the breast milk volume, time spent in skin to skin and any side effects. Of note only 44% of participants completed this study.

One study looked at the safety and side effects of herbal galactagogues(Wagner et al., 2019) recruiting healthy women with no milk insufficiency. They used a herbal tea names Mother’s Milk which has been commercially available in the United States since 1978. The tea contains bitter fennel, anise, coriander, fenugreek seed and other herbs. This study used lemon verdana leaf as a placebo. The women who participated in the study were asked to keep diaries and self report any maternal or infant adverse effects. They also recorded the amount of tea consumed and perceived infant satisfaction. No adverse affects were reported up to 12 months of follow up.

**Rationale for this study**

The benefits of breast feeding are well described. It is known that infants who are breast fed have improved immunity, reduced atopy, fewer respiratory tract infections and diarrhoeal illness, lower rates of obesity in later life. Benefits for the mother include a contraceptive benefit in the first few months, reduced risk in breast cancer, faster return to pre pregnancy weight, and improvement in glycaemic control and glucose tolerance. Human breast milk is the ideal food for the developing neonate. The WHO recommend that babies be exclusively breast fed for the first six months of life. Despite of the wealth of evidence of the benefits, breast feeding rates in the developed world remain low, particularly in Ireland. Of the Organisation for Economic Co-operation and Developmen (OECD) countries for rates of breast feeding, Ireland consistently ranks the lowest. The reasons for this are multifactorial and societal acceptance and encouragement is crucial. On an individual level, the most common reason for a woman who is motivated and willing to breast feed to stop is due to either a perceived or measured reduction in milk supply. In an Australian national survey carried out, “not enough breastmilk for my child” was self-reported as the reason for discontinuing breast feeding in 41%. Other reported reasons were a “child not attaching properly” in 14%, “having to return to work” in 12% and “too painful” in 11%(Welfare, 2011).

Factors which may negative impact on breastfeeding include flat or inverted nipples, mastitis, previous breast surgery including augmentation or reduction, infant neurological disorders, anatomical issues impacting on the ability to suck such as cleft lip or prematurity(NEIFERT, 2004).

Risk factors for readmission due to inadequate breastmilk intake in infants are infants born to mothers with no previous breastfeeding experience, lower level of education or did not attend breastfeeding classes(Edmonson et al., 1997).

Obesity is a major risk factor for insulin resistance and gestational diabetes. Obesity and diabetes mellitus both represent challenges to successful breastfeeding. Women with pre-gestational and gestational diabetes may experience impaired breast milk production. Factors that can interfere with breast milk production include maternal obesity, primiparity and pre term delivery. Obese women have consistently low breast-feeding rates independent of ethnicity and socioeconomic group(Nommsen-Rivers et al., 2010). There is significant overlap in the reasons for low breastfeeding rates in women with diabetes. Obesity can also contribute to difficulty positioning the infant at the breast. Obese women are also more likely to have a Caesarean Section or adverse perinatal event which may contribute to difficulty with breastfeeding initiation. Obese women are more likely to experience adverse perinatal outcomes which may interfere with breastfeeding initiation.(Nommsen-Rivers, 2016) .

There is growing interest in the role insulin plays in galactogenesis and the contribution that insulin resistance makes to low breast milk supply. This is of importance in improving breastfeeding rates as obesity and pre diabetes is a growing health concern. It is estimated that 23% of women of childbearing age in the United States are pre diabetic (Menke et al 2015). In one study looking at the effects of diabetes on milk supply, Odds of low milk supply increased by 2.6 fold after adjusting for Caesarean section, pre term delivery, PCOS, hypothyroidism and subfertility(Riddle, 2016)

Infants of women with gestational or pre pregnancy diabetes are at risk of neonatal hypoglycaemia. It is recommended that the infant be fed in the first hour of life in order to prevent this. Supplemental feeds which are sometimes used in the management of neonatal hypoglycaemia may affect milk production, as milk production improves with increased demand.

In countries such as Norway which have high breastfeeding initiation rates, obese women have lower rates of sustained feeding with 97% of women in the obese group (BMI >35) initiating breastfeeding but only 37% were still breast feeding at 4 months compared with 99% of the normal weight women (BMI <25) and 62% at 4 months (Winkvist et al., 2015).

Although women with gestational diabetes have challenges to breast feeding, they also stand to get the most benefit from it. Several studies have shown both long and short term benefits of breastfeeding on glycaemic control. In a study from the Atlantic Diabetes and Pregnancy study women who were lactating at 6 weeks post-partum were 60% less likely to have impaired glucose tolerance(O'Reilly et al., 2011).

The SWIFT study included 1035 women with a history of gestational diabetes who underwent a glucose tolerance test 6-9 weeks postpartum and annually for two years after delivery. 12% of women had developed T2DM at 2 years(Gunderson et al., 2012).

Women who exclusively breast fed had half the risk of developing type 2 diabetes. The lasting effects of lactation on glucose metabolism are unclear however and there is limited evidence on long term risk of T2DM. Confounders in this study were unmeasured determinants of lactation behavior. Reverse causation may explain the effect as women with an improved metabolic state may have increased lactation rates

While these studies have shown a protective effect of lactation on progression to diabetes they looked at short term follow up or relied on self-reporting of diabetes or retrospective reporting of breastfeeding duration. The 30 year Cardia study(Gunderson et al., 2018) was a prospective study looking at the relationship between lactation duration and progression to diabetes in a cohort of women followed up from 1986 to 2006. Retention at 30 years was 71%. There was a graded inverse association with duration of lactation and risk of diabetes in women of childbearing age. The risk reduction of breastfeeding was 25% for duration less than 6 months and 47% for more than 6 months. The protective effect of lactation was slightly stronger in the GDM group compared with the non GDM group. This protective effect was independent of race which suggests a biochemical effect rather than cultural or social influence. Black women in the United States have lower breastfeeding initiation rates and higher rates of type 2 diabetes compared with white.

Lactation appears to have a sustained protective effect on maternal metabolic risk factors and changes in maternal metabolic risk factors with 50-89% reduction in metabolic syndrome in the 8 years after delivery(Gunderson et al., 2007).

In the Nurses Health study(Ley et al., 2020), a longer duration of lactation was associated with a reduced risk of T2DM, estimated at a decrease of 15% per year of lactation for women who have given birth in the previous 15 years. Confounders in this study may include other health behaviours as women with longer duration of breastfeeding were more likely to be of healthy BMI, have a lower risk dietary score and higher multivitamin use less likely to have ever smoked. Women with a longer lifetime duration of lactation also had a more favourable glucose biomarker profile.

In a systematic review of the relationship between duration of lactation and risk of type 2 diabetes, a longer duration of breastfeeding was associated with a 32% reduction in the relative risk of type 2 diabetes (Aune et al., 2014) when compared with no breastfeeding. This was independent of other risk factors for type 2 diabetes including BMI, physical activity, education, income, parity and positive family history for type 2 diabetes. There was a 9% reduction in relative risk for each 12 month increase in duration of breastfeeding across a woman’s lifetime.

Biochemical factors that might explain the benefits of breastfeeding on glycaemic control include the higher glucose utilization by the mammary gland, increased lipolysis to accommodation the metabolic demands of lactation, increased metabolic rate and mobilisation of fat stores. Breastfeeding is associated with a reduced risk of the metabolic syndrome which is strongly associated with type 2 diabetes (Gunderson et al., 2010). Other metabolic effects of breastfeeding include increased maternal adipokines, including ghrelin which is involved in satiety(Stuebe et al., 2011). The effect of breastfeeding on weight loss has been the subject of a systematic review and found to have a poor association(Neville et al., 2014), however the location of the fat loss may contribute to improved glucose tolerance with women who breastfeed demonstrating less visceral fat that women who never breastfed(McClure et al., 2011). Lactating women demonstrate relative insulin sensitivity with lower glucose and insulin levels(Tigas et al., 2002).

The average daily volume of breast milk produced is 800ml. This provides the infant with approximately 560kcal per day, 40% of which is from lactose(Neville et al., 1988). The primary source of milk lactose in plasma glucose and lactating women provide approximately 60g of glucose to provide this. This demand is met by increasing carbohydrate intake, decreasing endogenous glucose production or reducing glucose storage.

Despite a wealth of anecdotal evidence on the efficacy of fenugreek as a galactagogue, previous studies have shown conflicting results. Any positive benefit is small. Many studies recruited women without evidence of or risk factors for breast milk insufficiency, such as gestational diabetes. Often a compound of traditional herbal galactagogues is used. Studies are often unblinded with small numbers recruited. Often traditional medicines are studied in populations where the reliance on such medicines is already strong, increasing the potential for bias in these studies. While some studies have looked at the effects on prolactin no studies have identified a clear mechanism of action for the herbal galactagogues. There is need for further well-designed studies to evaluate the effectiveness and acceptability of fenugreek as a herbal galactagogue in women at risk of low breastmilk supply.

**Study Objectives**

**Primary aims**

Compliance and tolerability of Fenugreek as a herbal galactagogue. Breastmilk composition at the end of treatment duration.

**Secondary aims**

Secondary outcomes are infant head circumference, length, weight and maternal BMI.

**Rationale for outcomes measured**

The design of this study is to test the feasibility of a randomised control trial (RCT) methodology. Outcomes measured include breast milk production. Low milk supply has been identified as the biggest cause of breastfeeding cessation and our study population is at particular risk of this. Compliance is an important measure of the acceptability of the fenugreek supplement which is an important consideration when recommending it to lactating women. The side effect profile will advise the tolerability of this treatment. Fasting glucose at six weeks will be used as a surrogate marker of glucose homeostasis. Breast milk composition and transcriptome analysis will provide initial exploration as to how fenugreek exerts its effect.

**Study Design**

1. **Overview**

This is an open label study of fenugreek in women in the immediate postnatal period who have had a pregnancy with gestational diabetes. This feasibility study will test methodology that will be used to run a future randomised controlled trial (RCT). Figure 1 outlines the trial design and data collection time points. Recruitment will occur in two stages. Women with gestational diabetes will initially be invited to participate through their antenatal clinic and then followed up on the postnatal ward. Eligibility criteria are women with gestational diabetes in their current pregnancy of age greater than 18 who intend to breastfeed for the study duration of four weeks. Exclusion criteria are pre-existing diabetes mellitus, allergy to peanut or fenugreek, an infant with congenital abnormality, low birth weight, prolonged admission to the neonatal intensive care, maternal infection that precludes breastfeeding such as HIV.



1. **Subject Selection**
2. **Inclusion criteria**
	1. Gestational diabetes mellitus
	2. Immediate postpartum period
	3. Intending to breastfeed for the study duration of four weeks at a minimum
	4. Age >18 years
3. **Exclusion criteria**
	1. Known pre-existing diabetes mellitus- with the provision that a proportion of women may have pre-existing diabetes but have never previously been tested outside of pregnancy
	2. Allergies to fenugreek, peanuts or other legumes
	3. Infant factors that may interfere with breastfeeding eg a congenital anomaly such as cleft palate, aneuploidy which may contribute to poor feeding due to hypotonia, low birth weight, prolonged admission to neonatal intensive care
	4. Maternal factors in which breastfeeding would be contraindicated for example maternal human immunodeficiency virus (HIV) infection, use of a prescribed medicine or drug in which breastfeeding is not recommended.
4. **Ethical considerations**

Under the hospital research ethics committee guidelines, patients are classed as a vulnerable group. The women included in the study will have a diagnosis of gestational diabetes, while this is medically treated complication of pregnancy, the women will generally be healthy volunteers. Their only vulnerability will relate to their pregnancy and subsequent breastfeeding. Participation is completely voluntary and if they choose not to participate or to withdraw at any stage it will have no impact on their care.

1. **Subject recruitment plans and consent process**

Recruitment will be a 2-step process:

Step 1 will involve approaching women attending diabetes in pregnancy clinics at NMH, confirming their diagnosis of gestational diabetes and intention to breastfeed. Participants will have their contact details taken at this stage with their consent

Step 2 will occur on the maternity wards after participants have delivered their baby. Researchers will approach the participant during their 2-3 day postpartum stay and confirm that breastfeeding initiation and the intention to continue breastfeeding for the duration of the trial. Signed consent will be obtained at this time and signal a woman’s formal recruitment into the trial.

1. **Risks and benefits**

The fenugreek tablets may cause the urine of the woman to smell sweeter than normal and alter the colour slightly. There is no physical harm being caused but women will be advised of this possible effect. It may cause mild gastrointestinal upset such as flatulence or diarrhoea. Patients who are allergic to peanut, soybean, chickpea may be allergic to fenugreek and it should be avoided in those people.

Individual benefit will potentially occur with increased breastmilk production leading to improved breastfeeding outcomes. The women will also have access to additional breastfeeding support services through the National Maternity Hospital (NMH) drop- in lactation consultant clinics.

1. **When and how to withdraw subjects**

Participants can withdraw at any stage in the study. Information on how to withdraw will be provided in the information leaflet and can be done by phone or email.

1. **Data collection and follow up for withdrawn subjects**

Data collected will be in the form of electronic recruitment log, hard copies of patient consent forms, breastfeeding questionnaire. Personal data collected with be pseudo-anonymised and stored on password protected computer in line with UCD and NMH data protection requirements. Hard copies of consent forms will be kept in a locked office in the UCD Perinatal Research Centre at NMH which requires authorised swipe card access. For participants who wish to withdraw from the study we will confirm whether data already collected can be used for the analysis. If this is not the case this data will be removed.

1. **Data Management**

Data collected will include an electronic recruitment log, hard copy of participant consent form. Information from breastfeeding questionnaire, biometric data on mother and infant including maternal weight, height, BMI, and infant head circumference and birth weight, serum fasting glucose result at baseline and six weeks will be stored electronically and be pseudo- anonymised. Data will be stored on secure, encrypted, password protected computers.

1. **Intervention**

This is an open label study of the food supplement Fenugreek to assess its tolerability and efficacy as a herbal galactagogue. Fenugreek is considered a herbal product from the plant family Fabacae. The botanical species is Trigonella foenum- graecum. The plant part used is the seed. Our fenugreek will be sourced from Vegavero, (https://shop.vegavero.com/uk/p/Fenugreek-Organic) a Germany based food supplement that partners with the AGROLAB group (https://www.agrolab.com/en). The seeds are harvested organically in France and processed in Germany. A capsule contains 625mg Fenugreek seed extract (FSE) powder provided as 8:1 concentrated FSE to capsule shell (hydroxypropyl methylcellulose) which equates to 5000mg fenugreek clover.

The extraction solvent is water and the extraction method is heat. The

Drug: extract ratio is 8:1. Capsules will be provided in convenient weekly tablet boxes. The return of used tablet boxes and counting missing capsules will monitor compliance. In addition regular weekly contact will be maintained by phone, text message or email with participants to encourage adherence. A symptom diary will be provided to all participants in addition to lactation support contact information.

1. **Outcome measured**

Outcomes measured will be breastfeeding self-efficacy as measured by the Breastfeeding Self-Efficacy Scale- Short form after a 28 day course of fenugreek supplement. Other outcomes will be tolerability and side effect profile, fasting glucose as a marker of maternal glucose homeostasis, breast milk composition and transcriptome analysis. With their consent the women’s experience of their participation will be recorded as part of a semi structured interview.

1. **Sample size**

This study aims to recruit a sample size of 40 women for the pilot. In addition, 10 women who meet the same inclusion criteria will be recruited to no intervention but will be asked to provide a breastmilk sample when their baby is four to six weeks old and complete a questionnaire at that time also.

**Study Procedures**

1. **Screening for eligibility**

Women attending the gestational diabetes clinic will be approached by a clinician who is not directly involved in their clinical care. The clinician will be a member of the research team and will identify themselves as such. The clinician will ask women if they have gestation diabetes and whether they would be interested in participating in the study. If the woman indicates interest the clinical will go through the consent process and provide a patient information leaflet. Once the women is consented we will verify her eligibility by checking her medical records.

1. **Schedule of intervention**

Once trial registration is complete, we will start the recruitment process. The anticipated study end date is February 2022.

1. **Safety and adverse events**

Fenugreek is a common food ingredient used in cooking in Asian countries and commonly consumed as a tea. Consultation with the Food Safety Authority of Ireland was undertaken to clarify fenugreek’s status as a food product rather than a medicine. Fenugreek meets their definition of a food supplement which is as follows:

*“‘food supplements’ means foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities".*

The Food and Drug Administration (FDA) of the United States classify fenugreek under Generally Regarded as Safe (GRAS), Title 21 Food and Drugs Subchapter B (Food for Human Consumption)

An initial consultation with the HPRA (ref: 44295) stated that fenugreek would not generally be considered a medicinal product

Formal classification was sought on fenugreek from the Health Products Regulatory Authority (HPRA) which does not fall into their definition of a medicinal product.

Patients will be provided with a contact number should they have any concerns or develop any side effects. Fenugreek is a food product and generally well tolerated. It should be avoided in those with peanut allergy due to the relationship between Trigonella species and peanuts and the potential for cross reactivity. Peanut allergy is one of the exclusion criteria. Mild side effects may occur including gastrointestinal upset and a sweet odour to sweat, urine and breastmilk, described as a maple syrup odour.

1. **Study outcome measurements and ascertainment**

Outcomes measured will consist of

1. Fasting glucose at at six weeks post-partum
2. Information on breastfeeding history collected by questionnaire at time of recruitment
3. Anthropometric measures of
	1. Mother- weight, height, calculated BMI
	2. Infant- birth weight, length, head circumference plotted on WHO centiles
4. Maternal symptom diary
5. Returned pill boxes to measure compliance
6. 30ml expressed breastmilk sample for transcriptome and metabolomic analysis
7. Maternal experience interview
8. Breastfeeding Self-Efficacy Scale- Short Form (BSES-SF) at six weeks postpartum- in place of test-weigh

The gold standard for measuring breast milk volume uses stable isotope analysis methods (Butte 1988). The test weigh performed pre and post feed has been shown to yield comparative results. It is a safe, non-invasive method that the woman can perform in her own home, hence this was the method chosen for this study. Due to COVID-19 restrictions and logistic challenges around home visits this was eliminated from the pilot study.

Women were to be trained in the use of this scales, with this training supplemented by written material and online secure access videos. This process involves the mother weighing the infant before and after every breast feed for one day (24 hours). The difference in weights is equivalent to the amount of milk consumed by the infant in that feed and this recorded by the mother on a record sheet provided. The introduction of restrictions due to COVID-19 resulted in shorter postnatal stays which meant women may not have the opportunity to complete the test weigh in full. In addition, it was felt to be inappropriate to have researchers on the postnatal ward due to shortages of personal protective equipment and concerns about possible contamination. This was also an extremely stressful time for new mothers and it was felt that the test weigh would be an additional burden. The decision by the research team was to eliminate the test weigh from the baseline investigation. Breast feeding sufficiency would be self -reported at six weeks postnatal using the Breastfeeding Self Efficacy Scale- Short Form, a standardised questionnaire used to assess breastfeeding confidence(Dennis and Faux, 1999). Items are self-reported on a five-point Likert scale with higher ratings indicated higher levels of breastfeeding self-efficacy.

Provision of a “*top up*” formula feed or glucose gel was also recorded. Following this a four-week supply of fenugreek capsule was provided in pre prepared bill boxes. The women will be supplied with a symptom diary and weekly phone calls will take place to check if there are issues or questions the woman may have.

Following the treatment course, the 24-hour pre and post feed weigh was to be repeated. This was to be done using the same standard scale and performed in the woman’s own home. The symptom diaries and precision scales were then to be collected. Compliance was measured by collecting the pill boxes. A breast milk sample was to be obtained for transcriptome analysis. Again, due to restrictions around COVID-19, home visits were initially not permitted and the check-up at four weeks was eliminated. Instead the participant will be requested to freeze the milk sample at home (-20 degrees Celsius) . At six weeks postpartum the study participants will attend for an oral glucose tolerance test. The frozen milk sample will be brought to this visit where it will be thawed and re-frozen at -80 degrees Celsius. Breast feeding sufficiency will be self -reported at six weeks postnatal using the Breastfeeding Self Efficacy Scale- Short Form, a standardised questionnaire used to assess breastfeeding confidence(Dennis and Faux, 1999). Items are self-reported on a five-point Likert scale with higher ratings indicated higher levels of breastfeeding self-efficacy.

**Anthropometric measurements**

**Maternal**

Maternal weight and height will be recorded on the post-natal ward, after birth. Weight of the participants was recorded in light clothing by a member of the research team using a calibrated weighing scales. Height is recorded without shoes using a wall mounted stadiometer. BMI is calculated as weight in kg divided by height in metres squared.

**Neonatal**

At birth, infant weight is recorded using a calibrated weighing scales. Infant length and head circumference will be recorded on the postnatal ward recorded and plotted on WHO growth centiles. The Ponderal Index is a marker of the nutritional status of the neonate. It is low in malnourished infants and high in obese ones. This is calculated as the neonatal weight in grams x 100/ neonatal length in cm3.

**Assessment of maternal glucose homeostasis**

Gestational diabetes in NMH is screened for by a glucose challenge test (GCT) in at risk women between 24 and 28 weeks (Antenatal Screening for Gestational Diabetes, 2014). Universal screening is not performed. Risk factors for gestational diabetes are family history, previous macrosomic fetus >4.5kg, African or South East Asian ethnicity. Women with a diagnosis of gestational diabetes in a previous pregnancy are automatically referred to the gestational diabetes team for glucose monitoring. Women may be screened during their pregnancy if there is a suspicious of fetal macrosomia, unexplained polyhydramios, persistent glycosuria or symptoms of hyperglycaemia such as excessive thirst and polyuria. A positive GCT result is defined as a blood glucose greater than or equal to 7.8mmol/L. Women with a positive result are referred for a glucose tolerance test. This involves a fasting plasma glucose sample followed by a 100g carbohydrate load and hourly blood glucose for 3 hours. A diagnosis of gestational diabetes mellitus is based on two or more results outside of the normal range and women are referred to the Diabetes clinic. This is repeated at six weeks post partum for women with an antenatal diagnosis of gestational diabetes.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time | Fasting | 1 hour | 2 hours | 3 hours |
| Normal plasma glucose range in mmol/L | <5.3 | <10 | <8.6 | <7.8 |

An additional serum sample will be obtained at the 6 week OGTT. This will be retained for future analysis as an anonymised sample at UCD.

**Pre and post feed weigh**

Mothers test weighed their infants before and after every feed for a 24-hour period at baseline and again after the four-week treatment course. This was done using a Soenhle precision digital scales. The scales has a setting that calculates the difference between the pre and post feed weigh. This eliminates human calculation error. The amount calculated in grams equates the amount of milk in millilitres that the baby consumed in that feed. Milk is slightly denser than water so counters the insensible losses during feeding.

**Maternal experience interview**

Semi-structured interview will be digitally recorded and interviewer notes taken while the woman is waiting to complete the second step of her oral glucose tolerance test (two hour waiting period). The recordings will directly coded using NVivo and thematically analysed to better understand the women’s breastfeeding experience and the impact supplementation had on that experience.

**Breastmilk sample**

 Participants will be provided with a manual breast pump (Haakaa Generation 3 Silicone Breast Pump) in order to obtain a 30ml sample for analysis. This can be taken from either breast and collected in the tube provided. The collected sample is stored in the fridge until collection. The remaining milk collected can be given to the infant. The sample once collected the sample will be retained for the duration of the data retention. The samples will be retained at UCD in -80c frozen state.

Breastmilk metabolome analysis: Comprehensive metabolomic analysis will be performed on participant expressed breastmilk samples. Metabolomic profiles will be acquired using a 600 MHz NMR spectrometer. NMR allows detection and quantification of the most abundant metabolites present in biological samples. MS coupled with liquid or gas chromatography techniques will be used to target specific groups of metabolites including but not limited to fatty acids and amino acids. The transcriptome analysis and the NMR metabolomics will inform the targeted approach for MS metabolomics. The combined use of NMR and MS techniques allows more comprehensive coverage of the breastmilk composition.

Blood biomarkers analysis: Glucose and blood lipids will be measured using the Randox Daytona autoanalyzer. Insulin and adiponectin will be measured using ELISA techniques.

1. **Statistical plan**

**Sample size determination and power**

We propose to recruit 50 women into this pilot. We know from previous research(da Costa et al., 2010) examining breastmilk volumes produced over time across 12 countries using standardised stable isotope analysis methods (gold standard measurement) that 100ml is a clinically meaningful difference and that 600ml output in 24hr is standard for the first month. The test weighing technique yields similar results to the stable isotope method (20) but has the advantage of being non-invasive. Twenty women will give a reasonable standard deviation for breastmilk production and other variables; it is a commonly reported recruitment figure for breastmilk supply studies. We will aim for 10 women in the control group.

**Analysis Plan and Statistical Methods**

Statistical analysis will be performed using a combination of statistical programs including the most recent version of SPSS, SIMCA and R statistical suites. All are available through UCD. Differences in categorical data will be examined using chi- squared tests. Continuous data will be explored using a combination of t-tests and other multivariate approaches such as regression, principal component analysis and partial least squares discriminant analysis. All of the research team members are experienced in performing such analysis. Qualitative data will be analysed using thematic analysis methods. Interviews will be carried out until such a time as thematic saturation has occurred. Member checking will be used to assess the validity of this analysis.

1. **Data handling and record keeping**

All data will be initially pseudo anonymised and coded and each participant will be assigned a unique code. Any identifying information will be removed from data sheets before any analysis. Data will be stored on secure, password protected, encrypted computers. This will enable analysis to take place to achieve the objectives of the research. The data collected will not be processed in such a way that damage or distress in caused to the participants. There will be no disclosure of personal data unless that disclosure is required by law or the data subject has given his or her explicit consent to the disclosure. Once the analysis has been completed for this research project the pseudo anonymisation will be changed to full anonymisation through the destruction of the file with the identifiable information linking the coded data. The principle of generalisation will also be applied to the coded data.

Patient data will be coded and pseudo anonymised on collection. The database will be password protected and the computer will be encrypted. All paper information will be kept in a locked office. Once data entry is verified into the electronic database we will securely shred the paper. All electronic data will be kept on a password protected encrypted computer until anonymised

1. **Training**

Research Integrity Training

Good Clinical Practice (GCP) Certificate

1. **Record retention**

Access to data collected will be strictly limited to the data controller and data processors. Only the authorised researchers within the team will have access to data which will be kept securely in locked offices and filing cabinets, on password protected and encrypted computers and encrypted portable devices. Files containing patient data will be stored at The National Maternity Hospital on a secure password-protected computer. All data will be pseudonymised and coded, and each participant will be assigned a unique code, therefore individuals involved in the study will not be identified. Once the analysis has been completed for this research project the pseudo anonymisation will be changed to full anonymisation through the destruction of the file with the identifiable information linking the coded data. The principle of generalisation will also be applied to the coded data. Permission will be sought within the consent process to anonymise the pseudo anonymised data upon completion of this research project. Participants will be made aware that following anonymisation their data will not be possible to withdraw their personal data and sensitive health data

1. **Study administration**

**Organising and Participating Centres**

The National Maternity Hospital, Dublin, Ireland

University College Dublin, Ireland

**Funding and Conflicts of Interest**

Not applicable

There are no conflicts of interest

**Subject Stipends or Payments**

Subjects will not be paid or reimbursed for their participation in this study.

1. **Publication and Dissemination**

This study is part of an MD for Dr Niamh Keating. It will be prepared for publication and assessment. We plan to use data from this study to write a manuscript for publication in a peer reviewed journal. Depending on study findings this will be in the field of Obstetrics, Diabetes Mellitus or Clinical Nutrition. Results will be presented at a relevant conference

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