Early Provision of Maternal Colostrum by the Oropharyngeal route to Preterm Infants

By

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Dedication

This thesis is dedicated to the memory of my wonderful parents

Abstract

Preterm infants are those born before 37 completed weeks of gestation. Worldwide, about one million children die each year due to complications of prematurity and survivors may face lifelong disabilities. Approximately 50% of neonatal deaths and 17% of deaths among children under five are affected by prematurity. Colostrum is the first milk produced by the mother within the early few days after birth. Colostrum is very rich in immunological and growth factors that indicates its primary functions are protective and trophic. Oropharyngeal administration of colostrum (OPC) is a novel route that involves coating the infant's oropharynx with a small amount of colostrum (0.1 to 0.5 ml) during the early neonatal period. Immune and growth factors in colostrum might interact with the oropharyngeal mucosal-associated lymphoid tissues to modulate the infant's immune system and promote intestinal growth, potentially reducing infection and necrotising enterocolitis (NEC), improving survival and health outcomes. Ultimately OPC could provide a potential target to prevent mortality and morbidities of preterm and sick infants. This thesis aimed to investigate whether OPC administration during the early neonatal period prevents deaths, improves health outcomes and promotes the growth of preterm infants.

To achieve the aim of this thesis; initially, an online survey targeted neonatal professionals was performed to evaluate the current practice and perception of OPC administration in the UK neonatal units. Oropharyngeal colostrum has been introduced into UK neonatal practice despite a lack of high-quality evidence regarding its efficacy and safety. OPC practice was variable, frequently without written guidelines.

A Cochrane systematic review was conducted to synthesis and appraise the currently available randomised controlled trials (RCTs), which evaluated if early OPC given within the first 48 hours has a positive impact in preterm infants (< 37 weeks gestation) compared with control. Six RCTs were eligible for inclusion in this systematic review. Meta-analysis showed that early OPC could shorten the time to reach full enteral feeds

but did not reduce the incidence of late-onset infection (LOI), NEC and death nor the length of hospital stay. Available evidence is insufficient due to lack of participants and very low quality to demonstrate the benefits effects of OPC for preterm infants.

The third study, a matched case-control study evaluated the effects of OPC administration on the short-term health outcomes in preterm (≤ 32 weeks) infants. Eligible infants who were admitted to the Nottingham neonatal units after the implementation of OPC in the care of preterm infants, and received OPC, compared with those who were admitted before the use of OPC in the units. Preterm infants who received OPC within the first 96 hours of life achieved full enteral feeding (150 ml/Kg/day for consecutive 72 hours) earlier than those infants who did not receive OPC. A higher rate of receiving breast milk at discharge to home was also observed. However, the two groups had a similar length of hospital stay, weight Z-score at hospital discharge, and incidences of NEC, LOI and deaths.

Finally, a non-randomised observational study evaluated the response of gut hormones to OPC administration in preterm (< 37 weeks of gestation) and ill infants requiring neonatal intensive care (NIC). Preliminary results demonstrated a rising trend in plasma gut hormone concentrations in response to OPC administration in the participant preterm and full-term infants. This study is ongoing, and more infants are required before final conclusions can be elicited.

In conclusion, OPC administration is a potentially feasible intervention that shortens time to attain full enteral feeds in preterm infants. Given the high risk for preterm infants and the benefits of maternal colostrum, OPC may have preventive implications for improving the health outcomes of this vulnerable population. This work expands the current knowledge about the use of OPC in the care of preterm and sick newborn infants and could benefit efforts to improve preterm birth outcomes by informing guidelines, clinical decision and future research. Larger, well-designed, high-quality research with sufficient power are needed to assess the efficacy and safety of this intervention.

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Declaration

The work in this thesis was completed within the Academic Division of Child Health, Obstetrics and Gynaecology at the University of Nottingham (Queen Medical Centre campus), between October 2014 and March 2019.

Unless where specified, this report demonstrates my own work that achieved under the supervision of Dr Jon Dorling and Professor Helen Budge.

To the best of my knowledge, this thesis is an accurate illustration of the work performed, and no other study is reproducing this work, has been carried out within the University of Nottingham.

Amna Widad Ahmed Nasuf

March 2019

Presentations & Publications

Oral presentations

Nasuf A, Budge H, Ojha S, Dorling J. Oropharyngeal administration of mother's own colostrum to preterm infants: a survey of neonatal professionals. Trent Paediatric Society meeting on the 13th November 2015.

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List of abbreviations

Abbreviation	Definition
AGA	Appropriate for gestational age
BAPM	British Association of Perinatal Medicine
BER	Blinded Endpoint Reviews
CDSR	Cochrane Database of Systematic Reviews
CH	City Hospital
CI	Confidence interval
CLD	Chronic lung disease
CNG	Cochrane Neonatal Group
COG	Child Health, Obstetrics & Gynaecology
CPAP	Continuous positive airway pressure
CSR	Cochrane systematic reviews
CV	Coefficients of variation
DHM	Donor human milk
DHR	Digital Health record
DHR	Digital Health Records
DPP-IV	Dipeptidyl peptidase IV
EBM	Expressed breast milk
E-B-M	Evidence-based Medicine
EDTA	Ethylene diamine tetra-acetate
EGF	Epidermal growth factor
EHRs	Electronic Health Records
ELBW	Extremely low birth weight
ELISA	Enzyme-linked immunosorbent assays
EXP	extremely preterm
GA	Gestational age
GCP	Good Clinical Practice

GDPR	General Data Protection Regulation
GIP	Gastric inhibitory polypeptides
GLP	Glucagon-like peptide
GRADE	Grading of Recommendations Assessment, Development and Evaluation
GutH	Gut hormone
HR	Hazard ratio
HRA	Human Research Authority
HTA	Human Tissue Authority
IGF-1	Insulin-like growth factor-1
IL	Interleukin
IQR	Interquartile range
ISF	Investigator Site Folder
LBW	Low birth weight
Lf	Lactoferrin
LNU	Local neonatal unit
LOI	Late-onset infection
LOS	Late-onset sepsis
MBs	Magnetic beads
MECIR	Methodological Expectations of Cochrane Intervention Review
MLP	Moderate-late preterm
NEC	Necrotising enterocolitis
NHS	National Health Services
NIC	Neonatal Intensive Care
NICU	Neonatal Intensive Care Unit
NNAP	National Neonatal Audit Programme
NNRD	National Neonatal Research Database
NUH	Nottingham University Hospitals
OPC	Oropharyngeal colostrum

PIS	Parent Information Sheets
PN	Parenteral nutrition
PRESMA	Preferred Reporting Items for Systematic reviews and Meta-analysis
PSTIs	Pancreatic secretory trypsin inhibitors
PYY	Peptide tyrosine tyrosine
QMC	Queen's Medical Centre
RCPCH	Royal College of Paediatrics and Child Health
RCT	Randomised controlled trial
RM-ANOVA	Repeated Measure One-Way Analysis of Variance
ROB	Risk of bias
ROP	Retinopathy of prematurity
RR	Risk ratios
RTC	Research Ethics Committee
SCU	Special Care Unit
SD	Standard deviation
SEM	Standard error of the mean
SGA	Small for gestational age
sIgA	secretory immunoglobulin A
SoF	Summary of finding
TGF	Transforming growth factor
TLRs	Toll-like receptors
TNF	Tumour-necrosis factor
UNDGs	United Nations Millennium Development Goals
VLBW	Very low birth wei
VP	Very preterm
WAZ Score	Weight-for-age Z Score
WHO	World Health Organization

Chapter 1. Introduction

A newborn baby is a challenge but can be more challenging to families, health care system and societies if the baby born prematurely. Whilst being a newborn is not an illness or a disease, the neonatal period (first 28 days of life) is a critical stage of life. In 2016, the World Health Organization (WHO) reported that approximately 2.6 million babies died during the first month of life (1), which account for about 46% of deaths in children under five years of age (2).

1.1 Definitions of Preterm Infants

Preterm birth is delivery of an infant before completed 37 weeks (259 days) of pregnancy, and according to the WHO, preterm infants are defined as infants born < 37 weeks of gestation (3). Based on the degree of immaturity preterm infants are classified into (3, 4) :

- Extremely preterm infant (EXP), born before 28^{+0} weeks of gestation.
- Very preterm infant (VP), born between 28^{+0} to < 32 weeks of gestation.
- Moderate-late preterm infant (MLP), born between 32^{+0} to <37 weeks of gestation.

As the growth and development of many of the body organs occur during the last trimester of pregnancy (5), preterm infants usually have low birth weight (LBW, birth weight <2500g) and immature functions of the major organs and systems. While prematurity is the most common cause for a baby being born with low birth weight, very low birth weight (VLBW, birth weight <1500g) or extremely low birth weight (ELBW, birth weight <1000g), preterm and LBW terms are not interchangeable (6). Some full-term babies have LBW or VLBW which are referred to as small-for-gestational-age (SGA) infants. SGA is defined as a baby born with a birth weight less than the 10th centile of his gestational age (GA) (7).

1.2 Preterm infants: a global challenge

Preterm birth is one of the most significant issues of perinatal and neonatal medicine creating a substantial global burden on diseases due to high mortality and morbidities in preterm population (8, 9). In 2015, the WHO reported that one in 10 babies are born preterm every year, and there were approximately 15 million preterm births across the world. Over one million children die each year due to complications of prematurity, and those who survive may face lifelong disabilities (8). The incidence of preterm birth varies between countries ranges from 5% to 18%. It was approximately, 7% in the UK, (10), 10% in the USA (11) and 5-9% in other developed countries (12) and it reached 18% n some African countries, that could be attributed to higher infection rates, maternal malnutrition, and inadequate antenatal care in these countries (13, 14). Approximately 60% of preterm births occur in developing countries, in Southern Asia and Sub-Saharan Africa (12) where the highest neonatal mortality also occurs representing 38 % and 39 % of neonatal deaths respectively (15).

With advances in reproductive technology and obstetric care, the rate of preterm birth has risen over the last 20 years in many countries (16, 17). Worldwide the WHO estimated a rise in preterm birth from 9.6% of live births in 2005 (18) to 11.1% in 2015 (8). The National Center for Health Statistics in the USA also reported a 4% increase in preterm births between 2014 and 2017 (19). This continuous rise in preterm births is an increasing burden on diseases.

Preterm infants have a high mortality rate; in the UK, in 2012, the preterm infants mortality rate was 23.6 deaths per 1,000 live births compared to 1.4 per 1,000 live births in full-term infants (20). Neonatal deaths contributed to approximately 50% of under-five deaths (2). Prematurity is the primary cause of neonatal death and the second leading cause of death in children under five years of age (8); accounts for approximately 35% and 16% respectively, Figure 1.1 (21).

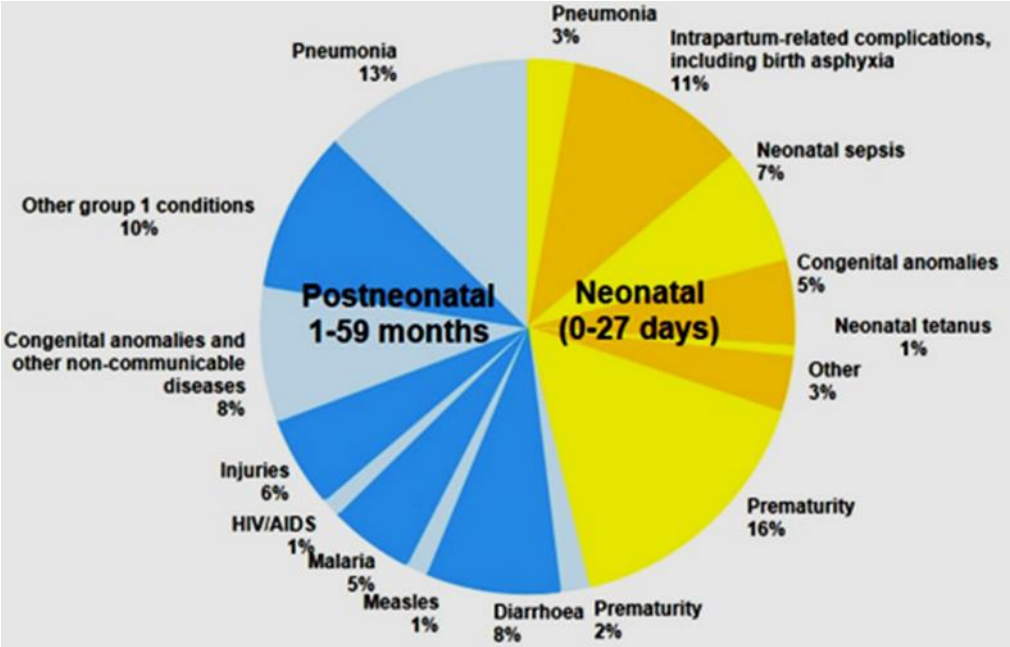


Figure 1.1 Leading causes of deaths among children under five years of age, 2016

Causes of death in children under five years of age. Each part is proportional to the percentage of total deaths in under five years.
Source: World Health Organisation, Global Health Observatory Data, Child Mortality and causes of death 2016 (21).

Advanced technology and collaborative work in perinatal and neonatal medicine, have led to an improvement in the survival rates of preterm infants, especially in developed countries. This is evident in the UK where the preterm infant mortality rate declined by approximately 15% from 2008 to 2012 (20). Improvement of survival rates was also reported in the USA where about 50% of infants born between 22 to 24 weeks survive and 80% to 100% in those born after 28 weeks gestation (22). Whilst, in developing countries, preterm infants have less chance to survive; and more than half of babies born between 28 and 32 weeks die. In 2014, in Nigeria, Iyoke et al. found a 16.9% prevalence rate and a preterm mortality rate of 46.1% for a population of babies with a mean GA of 32.6 ± 3.2 weeks (23).

Although early survival has improved, life-long morbidities showed a slight change that increases the burden on the health services, parents, societies and national economy. In the Global Burden of Disease Study 2010 (24), neonatal diseases contribute to 8.1% of

the Disability Adjusted Life Years (DALYs), and prematurity-related complications account for 3.1% of the DALYs (25). DALYs are measured by the sum of numbers of years lost due to early deaths and the years lived with impairments and disabilities (25). Therefore, prematurity was considered a significant cause of DALYs and continued a growing public health concern worldwide.

1.3 Preterm infants: a personal burden

The continuous surge in preterm births mean many people may experience the tragedy of being a parent of a critically ill baby or as family members or friends, thus may create substantial burdens on the life of the infants and their families (26-28).

1.3.1 Burdens on the infants

Preterm infants particularly those born before 32 weeks of gestation or LBW are at a higher risk for complications during the neonatal period and may require a prolonged hospital stay (29). Furthermore, the survivors may have long-term poor neurodevelopment and other chronic conditions (30, 31) that might be associated with functional deficiencies. It was reported that children who born preterm have a lower health-related quality of life which, is sufficient to influence their daily life compared to those born at term (32). For example, chronic lung diseases may increase the susceptibility to respiratory infections and reduce the functional capacity of the lung leading to exercise intolerance (33). Although the major neurodevelopmental disabilities such as cerebral palsy are diagnosed early, sometimes it is difficult to diagnose mild neurological impairments such as learning difficulty, behavioural and emotional problems, particularly in those infants born >32 to 36 weeks of gestation who are also facing an increased risk of long-term sequels such as unfavourable growth, neurological, behavioural and educational outcomes (34, 35). Additionally, children who are born premature may need frequent rehospitalisation due to their ongoing chronic conditions (36, 37).

1.3.2 Burdens on the parents

1.3.2.1 During hospital stay

Preterm birth and the hospitalisation of the infants are very stressful experiences to parents. Preterm birth is often unexpected, therefore could be considered as traumatic events, which affect the daily lives of the parents (38) and increase the risk of post-traumatic symptoms and preventing the development of normal parenthood (39). Parents of preterm infants are susceptible to emotional problems compared to parents of term infants (40). When the baby is born, parents are shocked by the event and the clinical condition of their baby that may prevent the parents from caring for their baby (41), this may make them feel powerless and helpless (42).

In the Neonatal Intensive Care Unit (NICU), the initial reaction of the parents to their preterm infant is often that of a guest or foreigner and a feeling of uncertainty about parenthood (43) especially the mothers who experience more anxiety and poor adaptation (44). Parental stress is due to physical and emotional separation from their infant, their infant's health, the uncertainty of survivals and the future of their infants. Some parents are also affected by the appearance of the infants especially EXP infants that may further worsen the first relationships between parents and their babies (45). The infant clinical status and environment of the NICU may also prevent skin-to-skin contacts that may adversely affect the mother's bonding to her baby (43), which might has a potential impact on the infant's long-term outcome (46). Since preterm infants might require a prolonged hospital stay (47), parents may have to suspend their normal life and spend extended time in the NICU; these may have great stressful effects on the families (48, 49).

1.3.2.2 Beyond the hospital

Parental stress may continue after hospital discharge and during the first few years, as they need to adapt to the independent role and safeguard high-risk fragile infant. Additionally, parents' uncertainties about the future growth and development of their infants may continue after discharge to home particularly with the mothers who are more

prone to depression and anxiety (50). Due to the ongoing problems of preterm infants especially EXP infants, parents have to manage, further medical and developmental necessities. The daily care of preterm infants needs more efforts from the parents and possibly more time consuming than the need for full-term infants. Such as, preterm infants are more likely to experience feeding difficulties (51); approximately 31 to 45% of preterm infants suffer from feeding complications during the first two years of life (52).

Moreover, long-term complications, particularly those associated with poor neurodevelopment (30), may lead to persistent increased stress and burden on the families (26, 53), through functional impairments and disabilities of the diseases such as cerebral palsy. Securing medical care and rehabilitation programmes for the child are also time-consuming as well as requiring extra expenses. Preterm infants are likely to be re-hospitalised and require frequent outpatient visits (37) that may overburden the parents (27, 54). Furthermore, financial concerns are other factors that might exaggerate parental stress. The unpaid leave from work, reducing hours of work and out-of-pocket budgets contribute to the financial burden on the parents and their quality of life (55). Given the negative impacts of a disabled child on the families , improving health outcomes of preterm infants might minimise the adverse effects of having preterm infants on the parents (27).

1.4 Prematurity is a research priority

Healthy births and reduction of LBW and VLBW are one of the National Leading Health Indicators (NLHIs) recommended by the American Institute of Medicine (56). Reducing child mortality is one of the United Nations Millennium Development Goals (MDGs) (57). Though there was a global reduction in child mortality, there was a slighter decline in neonatal mortality (8). Prematurity contributes to about 35% of neonatal deaths and represents a significant share of deaths in children under five years of age (2). The Office of National Statistics 2016 data for England and Wales also showed an increase in the

neonatal mortality rate between 2015- 2016 and complications of prematurity were the leading cause of neonatal and infant deaths, Figure 1.2 (58).

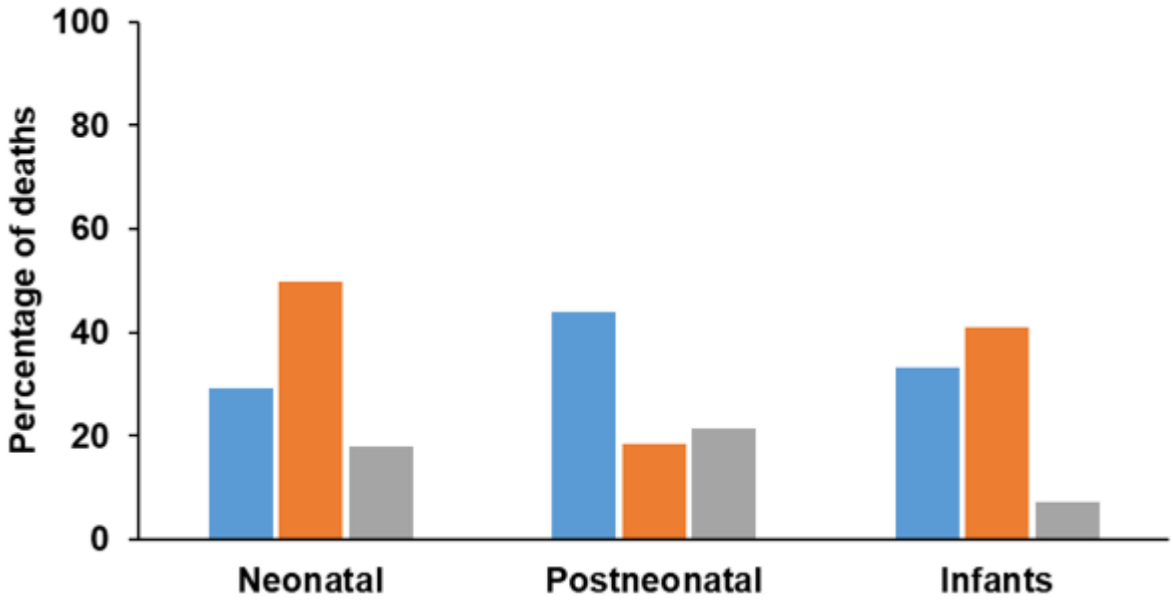


Figure 1.2 Causes of neonatal, post-neonatal and infant deaths in England and Wales, 2016

Columns represent percentages of the causes of infants' deaths. Neonatal: < 28 days of life; Postneonatal: 28 days-one year; Infants: under one year. Light blue column: Congenital anomalies; Orange column: prematurity complications; Grey column: Sudden infant deaths. Source: data downloaded from the website of the Office for National Statistics, UK (58).

The continuous increase in preterm births creates more burden on diseases (12). In the UK, the EPICure study, population-based studies of EXP infants (22 to 26^{+6} weeks) demonstrated that there was a rise in the rate of infants born at or less than 26 weeks by 44% between 1995 and 2006 and survivors are also increased, however, morbidities were unchanged among the survivors (59).

Although MLP infants have better outcomes compared to VP and EXP infants, however, compared to full-term infants, they may experience problems during the neonatal period, and long-term health, educational and behavioural issues (60, 61). Since, MLP infants compromise a majority of preterm infants, in the UK, they accounted for 85% of the 7% of preterm births (62) and in the USA, accounted for 7.17% of live births (19). Therefore,

even a slight increase in health problems may create a significant burden to health care services and societies (63).

Preterm infants require extended time in the hospital (47), which will be longer if the infant had complications such as infection or/and necrotising enterocolitis (NEC). Infants with NEC had been hospitalised 60 days longer than preterm infants without NEC (64). In the UK, the median length of hospital stay for infants born at 24 weeks and between 30 and 31 weeks, were 123 and 44 days respectively (65). In the USA, in 2000, the mean daily cost was $1535 for infants born ≤32 weeks compared to $700 for infants born between 33 and 36 weeks (66).

Moreover, the costs of preterm infants rise further in childhood and adulthood from survival with ongoing problems (67). The Chief Medical Officer 2012 data for the UK, reported that the annual cost of preterm infants from birth throughout childhood was £1.24 billion and the total cost for societies was £2.48 billion (68). This is likely to translate into economic costs for health services, families and communities. Hence, research emphasis on preterm infants could assist and guide public health policies and decide financial priorities.

To accelerate the progress towards reducing child mortality (MDG4), the United Nation (UN) (69) highlighted that focusing on prematurity is critical for improving child survival, and outcomes (70-72). Adapting and innovating innervations to improve preterm outcomes, were also addressed as an urgent priority research agenda by the WHO (3) and the Preterm Birth Research Priority Setting Group (73).

1.5 Prematurity-related complications

During foetal life, through the placenta, the mother provides essential nutrients to the foetus and excretes metabolic products. The placenta also produces hormones for foetal growth and protects the foetus against infection by its mucosal macrophages and transfer of maternal IgG (74). Maturation of the foetus occurs throughout the pregnancy to

accomplish development and growth of organs and systems to adapt for extra-uterine life. Preterm birth disrupts these physiological processes. Therefore, the foetus is born with underdeveloped organs and systems that impair their functions, which manifested in prematurity-related complications. Those immature fragile organs are susceptible to injuries during the perinatal and neonatal periods; they are also influenced by the aetiology of the preterm birth and maternal risk factors such as maternal infection (75).

Moreover, some of the treatment modalities, medications and procedures, which are required for life support, are additional factors that might influence the responses of the immature systems. For example, mechanical ventilation and high oxygen therapy contribute to the pathogenesis of chronic lung disease (CLD) (76). The risk for complications increases with decreasing gestational age, reflecting the degree of immaturity; an infant born between 22 and 25 weeks have the highest risk of mortality and complications (28, 77, 78). The response of the infant's organs to the extra-uterine environment (including the NICU) and the treatment strategies have a significant impact on the short and long-term health outcomes of the infant.

1.5.1 Short-term complications

Short-term complications manifested in the neonatal period and during first hospital admissions. These complications might prolong hospital stay, increase the risk of infections and adverse long-term outcomes. Table 1.1 summarises the foremost prematurity-related complications.

Table 1.1 Prematurity-related complications

System	Complications	Mechanisms
Respiratory	RDS (79) Perinatal asphyxia Pneumonia CLD (80)	Surfactant deficiency Poor postnatal adaptation Ventilator associated pneumonia Oxygen toxicity Barotrauma/Volutruama
Cardiovascular	Hypotension/bradycardia (16) Patent ductus arteriosus (81)	Hypovolemia/sepsis/cardiac Hypoxaemia
Gastrointestinal	Feeding intolerance (82) NEC SIP (83) Gastroesophageal reflux (84)	Immature suckling/swallowing Immature GIT/ enteral feeding/sepsis
Nutritional	Parenteral nutrition and its sequelae (85, 86) Growth impairment	Poor store and intake/ rapid growth
Neurological	Perinatal asphyxia IVH (87) Apnoea of prematurity (88)	Fluctuating cerebral blood flow/mechanical ventilation Immature respiratory centre
Haematological	Hyperbilirubinemia Anaemia of prematurity (89) RBCs transfusion (90)	Immature live enzymes/sepsis Blood sampling/erythropoietin deficiency
Metabolic	Hypoglycaemia (91) Hyperglycaemia Hypocalcaemia	Poor glycogen store/decreased production Stress/immature glycogenolysis Poor response to PTH
Renal	Poor handling of water, salts and acids	Low glomerular filtration rate
Temperature	Hypothermia (92) hyperthermia	Large surface area/ low brown fat/ thin skin Central/sepsis/dehydration
Immunological	High risk of infections	Deficient cellular and humoral immunities
Eye	ROP (93) Refraction errors (16)	Oxygen toxicity ROP

RDS: respiratory distress syndrome; CLD: chronic lung disease; NEC: necrotising enterocolitis; GIT: gastrointestinal tract; IVH: Intraventricular haemorrhage; PTH: parathyroid hormone; ROP: retinopathy of prematurity. RBCs; red blood cells

1.5.2 Long-term complications

Preterm survivors are at increased risk of long-term consequences on health and development such as:

- Neurodevelopmental disabilities and global developmental delay (30, 31, 94, 95).

- Chronic lung diseases (96, 97) persistent vulnerability to respiratory infection and a higher risk of asthma during childhood (98, 99).
- Retinopathy of prematurity (100), higher risk of refraction errors and late retinal detachment especially in ELBW infants (101).
- Growth failure (102, 103).
- Hearing impairment and the need for hearing aids (95, 104).
- Rehospitalisation due to a substantial risk of illness during childhood (37, 105).
- Short bowel syndrome (95, 106).
- Higher risk for developing non-communicable diseases like diabetes and hypertension (107, 108).
- Emotional and psychological stress on the family (55).

1.6 Preterm infant's immune system

The immune system is composed of innate and adaptive responses. The innate immunity is the first host, non-specific defence mechanism that provides immediate protection against pathogens. This immunity does not have persistent immune responses (109) but it presents antigens to the adaptive immune system (110). It comprises physical barriers like the skin, mucous membranes of respiratory and gastrointestinal tract (GIT), and a cellular component including neutrophils, mast cells, macrophages, monocytes and natural killer cells; in addition to humoral factors such as complements and cytokines (111, 112).

The adaptive immune system includes lymphocytes (T and B), antibodies and cytokines; it develops during the first few years of life through environmental interactions at the skin and mucosal surfaces (109). The adaptive immunity is the second line of defence, based on antigen receptors represented on T and B lymphocytes (113) and usually triggered if the innate responses failed to fight pathogens, thus, requires more time to being activated (112). It is more potent and efficient in targeting pathogens and yield a long-lasting immunity.

After birth, the newborn infants face complex immune needs to fight infection, prevent harmful inflammatory processes and balance the transition from sterile intrauterine life to an extrauterine environment that rich in pathogens and antigens (110).

1.6.1 Neonatal innate immunity

Newborns are mainly dependent on innate immunity while the adaptive immune system is underdeveloped and its linking with the innate immunity is also impaired (114), these physiological processes are more compromised in preterm infants depending on the degree of maturity (113, 115).

Preterm infants have fragile, gelatinous skin that is very vulnerable to injury, especially in EXP infants (116). Similarly, their protective mucosal and epithelial barriers of the respiratory tract and GIT are underdeveloped; these immature natural mechanical barriers increase the susceptibility to invasion by pathogens (117). The cellular component (neutrophils, monocytes, macrophages, natural killer cells) is also deficient (118). Preterm infants have minimal storage pools of neutrophils, which often results in a rapid depletion of the circulating neutrophils (neutropenia) particularly during infection (119). Additionally, their neutrophils have limited migratory capacity, impaired phagocytosis and degradation of ingested pathogens (120); these factors render preterm infants susceptible to infections and increase the risk of septicaemia. Moreover, the cytotoxicity of the neonatal natural killer cells is weak that could increase the risk for viral infections (121).

The complement system is one of the principal, potent immune mechanisms that regulates inflammatory damage. It composed of a variety of proteins that promote a non-antibody dependent opsonisation and facilitate phagocytosis (114) of microorganisms such as group B streptococci and Gram-negative bacteria, which are common pathogens colonise infants admitted to neonatal units (122). In neonates, the levels complement proteins were about half of older children, and further reduced in preterm infants, reaching the normal levels by 6 to 12 months of age (123, 124). Although preterm infants can

activate the complement cascade, the complement proteins are rapidly depleted during infection (124).

Fibronectin is another essential glycoprotein in the immune system; it is an opsonic factor for bacteria, such as Staphylococcus aureus and B streptococci. It enhances chemotaxis of neutrophils, stimulates the production of inflammatory cytokines by macrophages and plays a role in T-cell activation and endothelial function (125). Newborn infants also have low fibronectin levels compared to children and adults and are more reduced in preterm infants (126).

Moreover, Preterm infants have low pattern-recognition receptors such as Toll-like receptors (TLRs) e.g.TLR4 which has a fundamental role in the inflammatory process and could be activated by Gram-negative bacteria (127). Preterm infants expresse high TLR4 in the intestinal mucosa and lack the capability for suppressing TLR4 signalling that may explain their increased risk of NEC (128).

1.6.2 Neonatal adaptive immunity

The adaptive immunity is also underdeveloped in preterm infants; it has inadequate lymphocyte activation, and cytokines and immunoglobulin productions (129). Therefore, preterm infants are deficient in secretory immunoglobulin A (SIgA), which is the main mucosal protective factor, due to poor functions of the B-cells and delay in plasma cell maturation. Neonatal T-lymphocytes also respond ineffectively to physiological stimuli with poor cytokine productions, antigen processing and degradation (130). Though, newborn infants have a passive immunity that obtained from their mothers by trans-placental transfer of immunoglobulin (IgG). This passive immunity is mostly transferred after 28 weeks of gestation; hence, preterm infants have a lower IgG level compared to full-term infants; 250mg/dl at 28 weeks versus 1500 mg/dl (131, 132).

1.7 Necrotising enterocolitis (NEC)

NEC is a well-known devastating gastrointestinal disease, one of the leading causes of deaths and morbidities among preterm infants (133). The incidence of NEC varies between counties, from 0.3 to 5.0 per 1000 live births (134), comprising 1-7.5% of NICU admissions (135). NEC is inversely related to the GA and birth weight; 90% of cases occur in preterm infants (136, 137). In the USA, NEC is high reaching 13% in infants <28 weeks (138, 139), in Canada, 5.1% among infants <32 weeks gestation (140) while was lowest in Japan 1.6% in VLBW infants (141). In the UK, a multicentre randomised controlled trial (RCT) found that NEC affected 10% of infants <31 weeks (142); recently, severe NEC (confirmed at laparotomy, post-mortem, or causing death), is estimated to affect 3.15% of infants <32 weeks (143). Improvement of preterm survival especially of EXP has led to an increase in the incidence of NEC from 3% to 11% between 1997 and 2000 and 5% to 15% between 2003 and 2007 in infants born between 22 to 28 weeks (136, 137). However, variation in NEC prevalence could be attributed to underdiagnosis of less severe cases and inconsistent case-definitions.

Although NEC was described a long time ago (the 1950s) and a considerable amount of literature has been published on its aetiology and pathophysiology, the definite cause remains to be defined. However, prematurity and low birth weight are the main consistent risk factors (95, 135, 144, 145). Enteral feeding including, the type of milk and the feeding regimens also influences the infant's susceptibility to NEC (146-149). Other factors, with conflicting evidence, could increase the risk of NEC such as, maternal prolonged rupture of membrane and chorioamnionitis (95, 150), hypoxic-ischemic insults (151), congenital heart diseases, patent ductus arteriosus (152), packed red cell transfusion (139, 153), use of H2 blocker (154) and vasoactive agent such as dopamine, indomethacin (155), polycythaemia, and umbilical vessels catheterisations (156).

NEC is a complicated multifactorial process that involves inflammation and bacterial invasion of the immature mucosa of the GIT with resultant mucosal necrosis (157, 158).

Immaturity of the GIT, weak immune responses and enteral feeding are the main contributing factors for the pathogenesis of NEC (159, 160). Hypo-perfusion and/or hypoxia of the bowel stimulate the release of mediators of inflammation from the ischemic gut like tumour necrosis factor, which triggers an inflammatory process leading to mucosal injury and damage to the intestinal barrier. The injured immature intestinal mucosa, use of antibiotics (161, 162) and enteral fasting (147, 148) are participating factors that act synergistically to promote intestinal atrophy and abnormal bacterial colonisation of the bowel (163); this could lead to overwhelming septicaemia, septic shock and death.

The onset of NEC is variable from non-specific clinical signs to sudden fulminant course and deaths within hours, depending on the GA of the infant (140, 164). Therefore, a high index of suspicion is a keystone for managing NEC. Diagnosis of NEC is mainly based on the clinical signs and some radiological findings such as pneumatosis intestinalis (gas within the bowel wall), portal vein and free peritoneal gases, which are more life-threatening signs (165). Based on the clinical presentation the severity of NEC is classified according to modified Bell's criteria, commonly used definition (166, 167), into three stages; namely I, II and III depending on the clinical, and radiological signs as demonstrated in Table 1.2. Based on the treatment approached, NEC is classified into medical and surgical NEC, which has a poorer outcome (168). However, NEC can only be confirmed by inspection of gangrenous necrosis, which mainly affects the terminal ileum or colon, at laparotomy or histopathological findings of the resected tissues.

Table 1.2 Modified NEC criteria (adapted from Kliegman 1987 (167)

Bell stage	Systemic signs	Gut signs	Radiographic findings
Stage I Suspected NEC	History of risk factors non-specific/Reluctant to feed lethargy/apnoea/bradycardia temperature instability	High gastric residual (pre-feed) vomiting ± bilious mild abdominal distension occult blood in the stool	Normal/mild intestinal distension
Stage II Definite NEC: IIA: mildly ill	Increased desaturations and/or bradycardia temperature instability lethargy	High gastric residual (pre-feed) definite abdominal distension abdominal tenderness possibly bloody stools absent bowel sounds.	Ileus, fixed dilated bowel loops, pneumatosis intestinalis
IIB: moderately ill	As IIA with thrombocytopenia and/or mild metabolic acidosis	Same as previous Abdominal distension with definite tenderness possible abdominal wall oedema and/or discolouration. mass at lower right abdomen	As IIA and portal vein gas, ascites (possible)
Stage III Advanced NEC: severely ill - IIIA: bowel intact	As IIB plus hypotension, bradycardia, apnoea, sever metabolic acidosis, respiratory acidosis disseminated intravascular coagulopathy, neutropenia	Same as previous, plus severe abdominal distension and tenderness with abdominal wall induration (sings of peritonitis)	As IIB with definite ascites
IIIB	As IIIA	As IIIA	Perforation, Pneumoperitoneum

NEC: necrotising enterocolitis

Despite aggressive and optimal medical and surgical management, NEC is still one of the leading causes of death in NICU. The mortality rate for NEC ranges from 10 to 30% and may reach 50% for surgically treated infants (169). It is inversely related to the weight and GA of the infants, reaching up to 100% in extremely preterm infants (136, 164). NEC survivors may face further complications during the recovery that may lengthen their hospital stays and negatively impact on outcomes and cost to health systems (134). In the USA, it was estimated that infants with NEC stay in the

hospital 60 days longer than their counterparts without NEC with additional costs of $216 666 per survivor and $6.5 million per year for treating infants with NEC (64).

Moreover, NEC associated with long-term consequences for the survivors especially those who have surgical interventions (157, 170); approximately 10 to 30% of survivors face long-term complications including intestinal strictures, short bowel syndrome, failure to thrive and neurodevelopmental abnormalities (168, 171). Despite the proposed measures to prevent NEC, such as modulating enteral feeding regimens, use of prebiotics and probiotics and antibiotics, NEC remains a common gastrointestinal emergency in NICU with high mortality and long-term sequels, though, mother's milk, due to its immune-protective and growth factors, has been linked with a reduction in risk of NEC (149, 172, 173).

1.8 Late-onset infection (LOI)

Preterm infants are vulnerable to infection due to the immaturity of their immune systems. This susceptibility to infections is influenced by the intensive care that needed for life support such as mechanical ventilation, parental nutrition, and intravascular catheterisations.

Infection is one of the leading causes of neonatal mortality especially in preterm infants (174). Late-onset infection (LOI) is defined as a blood culture proven microbial growth after 72 hours of life (174). The incidence of LOI is approximately 8-9/ 1000 live births in VLBW and 26 /1000 live births in ELBW preterm infants. Its rate varies between regions and countries from 0.6%-14.2% among neonatal admissions and is inversely related to the GA. It was estimated that 36.6% of preterm infants born < 28 weeks GA had at least one episode of LOI in comparison to 29.6% and 17.5%, of preterm infants born between 28-32 weeks GA and 33-36 weeks GA respectively (175). In contrast to respiratory distress syndrome, which has been reduced since the surfactant era and with advances in neonatal medicine, infections related mortality and morbidities have increased (133).

The immature immune system is the main risk factor. However, other factors could raise the risk of infection in preterm infants such as the need for invasive interventions, which may disrupt the fragile mucosal barrier, increase the risk of infection especially in extremely preterm infants (118). Additionally, delay to start enteral feeding and to achieve full enteral feeds that may lead to more extended stay in a microbial environment at NICU.

The clinical presentations of LOI are subtle and non-specific, delaying early diagnosis, which may have devastating consequences. Therefore, clinical care providers have a low threshold to treat and start empirical antibiotics. However, prophylactic antibiotics therapy may increase the emergence of drug resistance and influence the intestinal bacterial colonisation of the infant (176), providing opportunities for potentially pathogenic bacteria to colonise the gut, that may translocate to other organs and tissues, increasing the risk of invasive systemic infection (163).

With the increasing rate of preterm birth (177) and growing survival of VLBW and ELBW infants, the morbidity burden will increase. Thus the length of hospital stays and the risk of LOI will continue to be a challenge to the neonatal care (2, 178). Despite the use of infection control protocols, infections are still the most leading cause of neonatal death even in high-income counties (179). A large cohort study by Stoll et al. found that 21% of VLBW infants who survived more than three days had at least one episode of LOI. The authors also indicated that infections among ELBW infants were associated with poor neurodevelopmental and growth outcomes in early childhood (174).

Thus, new policies are needed to decrease the incidence of nosocomial infections. One intervention that might protect against infection is the mother’s colostrum (180, 181). It was reported that the incidence of infections in preterm infants is lower in breastfed than in formula-fed infants; therefore breast milk is recommended as the "golden measure to prevent infection" (182).

1.9 Mother's colostrum and milk

Breast milk is the natural fluid secreted by mammalian mammary glands to feed their offspring and to provide them with the essential nutrition and protection for good health. Human milk is produced by the mother during late pregnancy and continues after delivery; it offers the optimal early nutrition for growth and healthy development of the infants. The benefits and importance of breastfeeding were identified a long time ago and exclusive breastfeeding for at least six months is recommended by the WHO and the American Academy of Pediatrics (AAP) (181, 183). A unique feature of human milk in contrast to other species like cows and rodents is that its composition is less influenced by maternal nutrition (184).

Moreover, human milk has significant immune protective advantages due to various biologically active components that have the potential to modulate the immune system of the infants and enhance growth and development (185, 186). The antibacterial nature of breast milk was first reported in the literature in the late eighteenth century; when Paul Ehrlich, a German physician proved that maternal antibodies could be transmitted to offspring through breast milk. Such was the importance of this work that Ehrlich was awarded the Nobel Prize in Physiology or Medicine in 1908 for his impact on immunology (187). Furthermore, the immunological features of milk assisted in the advancement of the understanding of immunology (188, 189).

Human milk is secreted in three stages, colostrum, transitional milk and mature milk. Colostrum is the first milk produced during late pregnancy until 3-5 days after birth. It is followed by an increasing volume of transitional milk which is in turn replaced by mature milk by the end of the second week of life (190). Human milk has great nutritional, developmental and protective benefits for newborn infants. The constituents of human milk are dynamic, adapt over lactation, diurnally, within a feed and differ between lactating women and populations (191, 192). However, the nutritional properties of human milk are preserved despite these variabilities. The main nutritional constituents are lactose (6.7 to

7.8 g/dl), protein (0.9 to 1.2 g/dl), and fat (3.2 to 3.6 g/dl) with 65 to 70 kcal/dl in addition to many micronutrients such as vitamins, iodine (193).

For this thesis, I focused on the bioactive compositions of human milk and colostrum, as these bioactive factors can influence the biological functions of the body and consequently affect the health outcomes of the infants. The following section reviews some of the bioactive components of colostrum and milk.

1.9.1 Immunological constituents

The principal immune components of human milk and colostrum are as follows:

1.9.1.1 Secretory immunoglobulin A (SIg A)

SIg A is the primary immunoglobulin in human milk. It is the first line of defence against microbes, and is the predominant Ig in human milk, in contrast to other mammalian species (cattle and sheep) where IgG is the main (188). IgA levels change over different stages of lactation, the highest concentration presents in colostrum, which contains more than 1.5g/L (194, 195).

IgA is the most crucial defence factors for mucosal surfaces especially the intestinal mucosa. It possesses a secretory component, which affords IgA resistance against proteolytic enzymes in the gut and helps fixation to the mucosa (196). By a process called “immune exclusion”, it prevents bacterial attachment to the mucosal cells, which is a necessary process for invasive disease. The Ig A captures the bacteria then, embedded in mucus thus facilitating elimination of the bacteria by peristalsis (197). Recently it has been recognised that it can reduce the virulence of bacteria, influence the intestinal flora and decrease the inflammatory responses associated with pathogenic microorganism and potential allergens (198). In addition, colostrum contains IgG but in a lower concentration than mature milk. However, it was reported that there were no differences in the level of IgG in colostrum from women who delivered preterm or term infants (199). Conversely,

colostrum is deficient in IgM, and no significant difference has been reported with GA (200, 201).

1.9.1.2 Lactoferrin

Lactoferrin (Lf) is an iron-binding glycoprotein. It is present on mucosal surfaces and in biological fluids like tears, saliva, seminal fluid, and milk. Lf is the chief whey protein in human milk throughout lactation. Human milk has a higher level of Lf in comparison to other species; for example, bovine colostrum contains 5mg/ml while mature bovine milk has only 20-200µg/ml, in contrast, to mature human milk, which has a minimum of 1mg/ml of Lf (202).

Lf is a fundamental component of the innate immune system, it has a broad antimicrobial activity and is one of the primary mucosal defence factors (203). As iron is an essential substrate for bacterial growth, due to its high iron-binding capacity, Lf inhibits the growth of potentially pathogenic bacteria by decreasing iron availability. Lf also acts by disrupting microbial cell membrane, inhibiting adhesion to host cells and preventing biofilm formation (204, 205). Lf can resist intestinal enzymes, and this may facilitate its action in the gut lumen as a prebiotic that promotes the growth of beneficial bacteria, therefore, might inhibit colonisation of the gut by pathogenic microorganisms (206).

Moreover, Lf has anti-inflammatory and immunomodulatory properties in the gut by activating T-lymphocytes and cytokine expression (204). Recently, it was suggested that Lf has a possible antiviral and antibacterial action by direct interaction of its molecules with microorganisms (203, 207). Lf is being investigated as a potential immune therapy in the prevention of NEC and infection in preterm infants (142, 208).

1.9.1.3 Oligosaccharides

Oligosaccharides are biologically active carbohydrates, one of the most vital components of human colostrum and milk and present in high concentrations approximately ranging from 7 to 12 g/L (209, 210). Human milk oligosaccharides are complex molecules

classified into four groups, and more than 100 oligosaccharides have been identified (211) in human milk; this might explain their diverse functions. Oligosaccharides' structural complexity is a distinctive feature of human milk compared to bovine milk, which contains only a trace of oligosaccharides and less complex structure (212). Oligosaccharides have been considered to be the furthermost crucial human milk component that influences the intestinal flora in breastfed infants (213). Oligosaccharides are resistant to the digestive enzymes of the GIT and act as prebiotics to promote the growth of non-pathogenic bacteria like Bifidobacterium bifidum in the gut (214, 215). Oligosaccharides also function as analogues to inhibit the attachment of microorganisms to mucosal cells of the respiratory and gastrointestinal tracts, an essential stage for microbial invasion (216, 217). It was proposed by in vitro studies that oligosaccharides can activate T-lymphocytes and cytokines production (210). Recent evidence suggested that oligosaccharides in human milk protect against NEC due to their antimicrobial, anti-inflammatory actions and their potential role in the maintenance of intestinal integrity, healing and maturation of intestinal wall (210, 218, 219). Moreover, oligosaccharides have the potential as signalling molecules and nutrients for the growing brain, therefore, might contribute to postnatal neurological development (220). Wang et al. reported that there were higher levels of brain gangliosides and glycoprotein sialic acid, which are essential for brain development and cognition, in breastfed infants than formula-fed infants (221).

1.9.1.4 Cytokines

Cytokines are pluripotent peptides secreted by several various (immune and inflammatory) cells. They are present throughout the body as well as in amniotic fluid, colostrum and milk. Cytokines collectively act in a network like fashion by interacting with distinctive cellular receptors to enhance and regulate the immune system. Human milk contains many cytokines, which are secreted by the mammary glands and milk cells and have the potential to modify the immune system (222, 223).

Many varieties of cytokines have been recognised like interleukin (IL), interferon (IFN-γ), tumour-necrosis factor (TNF-α), transforming growth factor β (TGF-β), granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF), and granulocyte-macrophage-colony-stimulating factor (GM-CSF) (224, 225). Human milk cytokines can pass through the intestinal mucosal barrier to interact with the cellular receptors to modulate the immune responses. Cytokines modulate the immune system by variable actions; pro-inflammatory like IL6, TNF-α and INF-γ that stimulate the immune response and the differentiation of B-lymphocytes into IgA plasma secreting cells, therefore, might protect against infections. Other cytokines have anti-inflammatory properties such as IL10 and IL13 (225). Cytokines have a vital role in response to injuries and protection of mucosal surfaces, which are the primary access point for an invasion by microorganisms (226). TGF-βs are the principal cytokine in human milk that play an essential role in cellular proliferation, intestinal homeostasis and tolerance (227). TGF-β involves in T-cell activation and can, via its effect on B-cells, in conjunction with other cytokines, initiates production of IgA at the mucosal surfaces to potentiate mucosal immunity (228).

1.9.1.5 Pancreatic secretory trypsin inhibitors (PSTI)

PSTI is a peptide, which protects the pancreas from auto-digestion. PSTI has also been found in the gastric secretion and in intestinal mucosal cells where it protects excessive digestion of the mucosa (229). Recently PSTI was found in a significant concentration in human milk especially during the first few days after birth (230). During this early neonatal period, with the introduction of feeding, there is a rapid increase in the secretion of acid and digestive enzymes of the GIT (231). These changes may increase the risk of intestinal injury and could rationalise the higher levels of protective factors such as PSTI in colostrum compared to mature milk. Therefore, PSTI might have the potential to prevent mucosal damage, facilitate gastric mucosal repair, promote intestinal wall healing and create a mucosal defence (232, 233).

1.9.2 Growth factors in human milk

Mother's milk has various effects on the growth of some of the body organs such as the GIT, nervous and vascular systems. These effects are mediated through its numerous growth factors, such as epidermal growth factor (EGF), transforming growth factor (TGF) and insulin-like growth factor-1 (IGF-1) which have significant effects on cellular differentiation, proliferation and maturation and repair of the tissues (234, 235). It was reported that most of the growth factors are present in higher concentrations in colostrum compared to mature milk that may indicate their importance for maturation during early life (236, 237). Table 1.3 summarises the main growth factors present in human colostrum.

Table 1.3 Colostrum growth factors

Growth factors	Role
Epidermal growth factor (EGF)	EGF presents at a very high level in colostrum (237). EGF stimulates proliferation and maturation of the enterocytes (238). Main intestinal protective factor from hypoxic-ischemic injuries (239, 240).
Heparin-binding growth factor (HB-EGF)	Protects against hypoxic and ischemic injuries and has an important role in the repair of post-hypoxic-ischemic injuries, such as resuscitation and NEC injuries (240).
Neuronal growth factors (NGF)	Have a critical role in the development of the nervous system. Promote neuronal maturation of the gut and enhance GIT motility (241-243).
Insulin-like growth factor (IGF)	Higher level in colostrum. IGF has a role in tissue growth (244). Stimulate erythropoiesis
Vascular endothelial growth factor	Higher level in colostrum. Regulates vascular endothelial and may play a role in reducing the risk of ROP (245, 246).
Erythropoietin	Stimulate RBCs production and may prevent anaemia of prematurity. It also has a trophic effect on the intestinal mucosa. (247, 248).

NEC: necrotising enterocolitis; GIT: gastrointestinal tract; ROP: retinopathy of prematurity; RBC: red blood cell.

1.9.3 Cellular components

Human milk and colostrum also possess cellular components. They are rich in macrophages, lymphocytes, granulocytes, epithelial cells. The presence of maternal cells in an active motile form in human milk suggests that these cells could have continuous

functions in breast milk feed infants (249, 250). In colostrum, neutrophils and macrophages are the predominant cell type; they represent about 40% to 60% and 30% to 50% of the total leukocytes respectively. Lymphocytes account for 5%-10% and T-lymphocytes are the predominant type of breast milk lymphocytes (251, 252). These cells act by different mechanisms; neutrophil and macrophages act by direct killing of microorganisms, and lymphocytes (activated-T-lymphocytes) by producing cytokines (253). It was proposed that the infant's infectious status influence leukocyte and macrophage counts in breast milk (254) that may suggest additional potential support of breast milk in response to infection. Recently, undifferentiated stem cells have been identified in human milk (255).

1.9.4 Other protective factors

In addition, to the previously described elements, human milk contains many other factors such as nucleotides, lysozymes, free fatty acids, Mucins, soluble CD14, TLRs agonists and antagonists (256). Human milk has many enzymes such as lipase an enzyme that enhance fat absorption by the intestine (257). Nucleotides represent about 20% of non-protein nitrogen in human milk (258) and are suggested as central factors in GIT maturation and development of the immune function of the infant. Nucleotides also have a potentially favourable effect on the gut microflora (259). Long-chain polyunsaturated fatty acids (PUFAs) and gangliosides present in higher levels and distinctive form in breast milk compared to formula milk; long-chain PUFAs are essential for the cell membrane structure, especially in the neuronal and retinal cells (260). Human milk gangliosides have also been involved in neuronal development, cellular growth and prevention of infection (261, 262). These bioactive components of human colostrum and milk act synergistically to promote the growth and the immunity of newborn infants and provide effective development and protection against serious illness such as NEC and infections.

1.9.5 Colostrum versus mature milk: compositions and immune activity

During lactation, milk constitutes change between, colostrum, transitional and mature milk. While the three stages of human milk have enormous nutritional, developmental and protective benefits for the newborn infants, they vary in their physical characteristics and compositions.

Colostrum is the first stage of lactation; produced in low volume and it has unique characters in comparison to mature milk. Colostrum is rich in immunological constitutes, growth factors, minerals, fat-soluble vitamins and proteins but has a relatively low lactose level, and lipids (193, 263-265). This unique composition of colostrum indicates that its primary functions are protective and trophic. For example, SIgA is approximately 100 times higher in colostrum than mature milk (266). Lactoferrin is highly concentrated in colostrum reaching almost to 7g/ml in contrast to 1g/ml in mature milk; moreover, colostral lactoferrin has a higher affinity for iron chelation which explains its bacteriostatic function (264) as described in section 1.9.1.2. IgA and Lf constitute approximately 10% of the colostrum weight compared to only 1% in mature milk. However, the lower level in mature milk is compensated by increasing milk volume (267).

Similarly, the concentration of oligosaccharides is higher in colostrum reaching 20g/L that decline over the periods of lactation; dropping to 5g/L in mature milk (268). Higher levels of bioactive cytokines in colostrum are also reported (185). Growth factors like EGF, TGF-α and PSTI, which are peptides with a significant healing effect on the injured intestinal mucosa, were found in higher concentrations in colostrum (234, 238). Additionally, colostrum is more abundant in cells than mature breast milk particularly leucocytes (251). These compositions of colostrum seem to compensate for the deficiency of infant's immune responses especially mucosal immunity during the early life.

1.10 Preterm infants and mother's milk

Mother's milk has been recommended as the primary food for preterm infants. However, from the nutritional aspect mother's milk is not the optimal feeding alone for preterm infants, especially those who are EXP and LBW infants. Preterm infants have a high risk of nutritional deficiencies and growth impairment, and they need higher protein, caloric, minerals and vitamin intakes to support their growth rates; mother's milk may not meet some requirements such as calcium, phosphorous and iron. (269, 270). Therefore additional nutrients may be needed to fortify the mother's milk (271). However, mother's milk has a unique nutritional advantages over formula milk for preterm infants, due to its higher content of cysteine and taurine, long-chain polyunsaturated fatty acids, nucleotides, and gangliosides compared to formula milk (217). Mother's milk also has a high level of lipase; an enzyme which, improves fat absorption (193).

Moreover, the mother's milk is more tolerable by preterm infants than formula milk and promotes gastric emptying. While from the nutritional aspect for the long run it is not the optimal feeding alone for preterm infants, mother's milk has substantial protective benefits. The AAP recommended the early introduction of mother's colostrum and milk for feeding preterm infants, and donor human milk (DHM) is the preferred alternative, to formula, when mother's milk is unavailable or inadequate (272). From a nutritional point of view, preterm milk has higher levels of protein, amino acids, fat and sodium compared to full term milk, while it contains similar concentrations of minerals as term milk except for the calcium which, is lower in preterm milk (193, 273).

In comparison to mothers who have term infants, colostrum secreted by mothers who have preterm infants has higher concentrations of biologically active immune factors, such as IgA, lactoferrin and some interleukins, and growth factors (192, 195, 201, 274). The levels of these biofactors are inversely related to the duration of pregnancy. Growth factors like EGF, TGF-α and PSTI were also found in higher concentrations in colostrum of mothers of EXP infants compared to MLP infants (236). These-gestational age

associated concentrations in the bioactive factors suggest that preterm colostrum has been distinctively expressed to support extra-uterine adaptation and protect the preterm infants against infection, during the early postnatal weeks.

The high concentration of bioactive substances in colostrum produced by mothers of preterm infants is linked to the open tight junctions of the mammary epithelium, which allow para-cellular transport of immune factors from maternal circulation to the milk (184, 274). These tight junctions fuse steadily over the first few days following birth and close entirely with the production of mature milk. Additionally, some studies have postulated that colostrum of mothers who have preterm infants may continue longer than mothers with full-term infants that may extend for almost seven days (274, 275).

Nevertheless, during foetal life, the growth of the foetus occurs throughout the third trimester of pregnancy, and approximately 15% depends on the nutrients and growth factors from the swallowed amniotic fluid (276). The amniotic fluid has an essential role in the development and growth of the GIT, which occurs mainly during late pregnancy (158). Similarity between the compositions of colostrum and the amniotic fluid has been established (277), therefore, providing colostrum to preterm infants during the early neonatal period may substitute the infant with the trophic effects of the amniotic fluid on intestinal growth and development (278) and may support these vulnerable infants against the higher risk of NEC and infection.

In summary, preterm infants had a deficient immune response and delayed production of immune and growth factors, the higher levels of bioactive components in mother's colostrum and prolonged colostrum phase of the lactation period could compensate this deficiency. Mother's milk has been linked with lower risks of some of the prematurity-related morbidities such as sepsis (279, 280), NEC (173, 281) retinopathy of prematurity (282, 283) and neurodevelopmental outcomes (284, 285). Therefore, it is evident that colostrum could protect the preterm infants especially extremely preterm infants during the critical early neonatal period.

1.11 Enteral feeding challenges for preterm infants

Enteral feeding is the provision of food into the GIT, whatever the route used. Feeding preterm infants safely and adequately is one of the major challenges in the care of these infants. During the early postnatal period, preterm infants especially those EXP and ELBW are critically ill, clinically unstable and cannot tolerate enteral feeding. Thus, commencement of enteral feeding will be delayed, leading to prolongation of parenteral nutrition (PN), which requires placement of indwelling intravenous catheters leading to increased risk of invasive sepsis (286). Moreover, prematurity associated conditions that affect intestinal perfusion commonly prevent enteral feeding. Despite the evolution of perinatal and neonatal medicine that improved preterm survival, the introduction of enteral feeding and the provision of adequate enteral feeds remain a continuing challenge for neonatal professionals, patients and parents (287).

During the neonatal period, the objective regarding nutrition of preterm infants is to achieve a postnatal growth by a rate similar to the intrauterine growth of a foetus with the same GA (288) with adequate functional development. Preterm infants are a heterogeneous group with a variable degree of immaturity that is influenced by many factors such as the cause of preterm birth, maternal illnesses, intrauterine and postnatal environments (16). Therefore, introducing enteral feeds requires a balanced clinical decision between these factors and the extent of the immaturity of the organs and the infant's clinical status. The concern of serious diseases such as NEC further complicates the provision of enteral feeds to these vulnerable infants (289).

1.11.1 Strategies for feeding preterm infants

Enteral feeding is the best and safest way for providing nutritional requirements and is preferable to PN, which has been linked to severe complications such as sepsis and liver disease (85, 86). However, PN is an essential adjunctive or exclusive therapy to optimise the nutritional needs in critical cases especially for EXP and ELBW infants (290, 291). Enteral feeding is also considered as one of the modifiable risk factors for NEC in preterm

infants (292). Yet, there is still variability in the practice of enteral feeding for preterm infants internationally and between neonatal units within a country (293, 294).

To achieve full breastfeeding or/and bottle feeding, preterm infants usually pass through different stages before they start to swallow, coordinate and then gather appropriate attachment and sucking. Different practices have been used to provide enteral feeding to preterm infants during the neonatal and transitional periods. However, there is still no consensus about an approach for feeding preterm infants, particularly during the neonatal period, due to the lack of sufficient evidence for the optimal time to start, type of milk to use, the safest volume to begin with and the speed of advancement (147, 291, 293, 295).

In the subsequent four sections, I review some of the challenges that may face the feeding of preterm infants

1.11.1.1 Type of milk

Human milk is a central constituent of any strategy for enteral nutrition of all infants including preterm infants. There is robust and consistent evidence that mother's own milk is associated with a reduction in prematurity-related complications (296-298). Therefore, feeding mother's own milk was highly recommended by the WHO and the AAP and the European Society of Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) as the primary option for feeding preterm infants (181, 183, 270). Early provision of human milk has been linked with decreased mortality, morbidities, especially protection against NEC (172, 173, 281), and better neurodevelopmental outcomes (284). However, human milk needs to be fortified to meet the necessary nutritional requirements for optimal growth and development of preterm infants (295). Human milk fortifiers are usually indicated in infants born before 32 weeks of gestation and VLBW infants. Improvement in health outcomes that associated with the use of mother's milk is related to the volume of mother's milk received by the infants especially during the first two weeks of life (173, 281). Pasteurised DHM is an alternative if mother's milk is unavailable or insufficient (299). However, DHM has lower energy, nutritional constituents such as fat, protein and

less protective factors such as IgA and lactoferrin (300) that might be related to the effect of pasteurisation; additionally, DHM usually collected after months of birth (301, 302). Formula milk is another option for feeding preterm infants if the mother's milk is unavailable or insufficient and DHM is also unavailable, or the infant is unable to feed DHM (303). Although formula milk feeding resulted in better short-term postnatal growth, it is associated with a higher risk of NEC compared to human milk (304). However, the benefits of DHM over formula milk still uncertain (143, 295).

1.11.1.2 Trophic feeding

Feeding preterm infants is commonly initiated as minimal enteral feeds or trophic feeding. Trophic feeding is defined as providing a small volume of milk (10-20ml/kg/day) without increasing the rate for 5 to 7 days (305). Trophic feeding was initiated in the late 1980s; it is hypocaloric non-nutritional feeds that have been recommended to stimulate the development of the immature gut of preterm infants without worsening the severity of diseases (306). Other terms have been used; gut priming, minimal enteral nutrition and hypocaloric feeding. Trophic feeds enhance gut motility (306), modify intestinal disaccharidase enzymes, alter microflora, and stimulate gut hormone secretions, which is essential in the postnatal adaptation of the GIT (307). It has been linked with improvement in feeding tolerance, earlier attainment of full enteral feeds, shorter hospital stay and better postnatal weight gain (305, 306). Early trophic feeding is also associated with a reduction in the incidence of infection (286) and did not increase the risk for NEC (308). However, due to the concern that early introduction of enteral feeds may increase the risk of NEC, some clinician delays the initiation of enteral feeding to preterm infants (147). The nil per oral state and use of antibiotics can lead to intestinal mucosal atrophy and inflammation, decrease the digestive enzymes and mucosal IgA, and promotes colonisation by a pathogenic microorganism, which worsen by the use of antibiotics as part of the care of preterm infants increasing the risk for sepsis, NEC and feeding intolerance (309).

Furthermore, delayed commencement of enteral feeding is accompanied by prolonged use of PN that might prolong hospital stay and increase the risk of PN-associated complications (85, 86). Therefore, early trophic feeding is the most common approach to start enteral feeding in preterm infants. However, in certain clinical situations, trophic feeding could not be given, and nil per mouth status is unavoidable.

1.11.1.3 Progression of enteral feeding

Debate continues about the best strategies for progressing the feeds and the rate of advancement due to the concern that early and rapid progress of enteral feeding might increase the risk of NEC (310, 311). A Cochrane review reported that delay in the introduction of progressive enteral feeding more than four days has no significant effects on the incidence of NEC, mortality, and other morbidities in very preterm infants, however, the delay resulted in more days to attain full enteral feeding (312). Another Cochrane review evaluated the effects of slow advancement of enteral feeds (less than 24ml/kg/day daily increment) versus fast speeds (30 to 40 ml/kg/day) found that slow rates did not decrease the risks of feeding intolerance, NEC or mortality, however, the faster speeds group achieved full enteral feeds and regained birth weight earlier (313). Thus, feeding practice should be balanced between the risk and benefits, considering different health outcomes.

1.11.1.4 Method of feeding

Preterm infants have poor sucking and swallowing/breathing coordination that increase the risk of aspiration. Organised oesophageal function develops after 32 weeks of gestation and coordination at 33 to 34 weeks (314, 315). Therefore, tube feeding, either nasogastric or orogastric tube, is commonly used as an initial method to feed preterm infants.

Nasal resistance accounts to about 40% of airway resistance, thus, the nasogastric tube may increase the work of breathing and may lead to respiratory problems such as apnoea

and desaturation (316). An orogastric tube is preferred in feeding preterm infants especially those who have respiratory distress or at increased risk of apnoea. (316, 317). However, orogastric and nasogastric feeding tubes are both used in neonatal units due to limited evidence (318).

There is also uncertainty whether continuous or enteral bolus feed is the optimal method (319). Some studies reported that bolus enteral feeding associated with increased incidence of apnoea, respiratory compromise and fewer weight gains and continuous feeds are more tolerated by preterm infants (320) while others found no differences in the incidence of apnoea or growth (321, 322). Therefore, the method of feeding should be balanced; continuous feeding may be more advantageous initially while bolus can be used when the infants developed sucking and swallowing/coordination and in stable infants. Table 1.4 summaries strategies used in the practice of enteral feeding of preterm infants (323).

Table 1.4 Strategies of enteral feeding in preterm infants

Type of milk	Mother's own milk is the best and first option Donor breast milk only if the mother's own milk is not available or insufficient. Formula an alternative if a mother's own milk is not available or insufficient or paternal choice. Breast milk fortifiers, when milk feeding reached 100ml/kg/day especially for extremely preterm infants.
Method of feeding	Nasogastric or orogastric tube, and intermittent intra-gastric (bolus) feeding or continuous enteral feeding. No evidence to support significant differences. Cup feeding, bottle and breast or all are usually started later when infants reach full enteral feeds
Time to start	As early as the infant's clinical status permits but mostly within the first 3 to four days after birth.
Trophic or minimal enteral feedings	Highly recommended than complete fasting. Trophic feedings 1ml/kg/hr is usually to start with
Progression of feeding	Early provision of progressive enteral feeds and fast advancement of milk feeds (30–35ml/kg/day) is safe and does not increase the incidence of NEC. Daily increment 10 to 30 ml/kg/day until reaching 150ml/kg/day. Demand feeding when the infant reaches full enteral feeds
Frequency of feeding	One or two hourly feedings are common to commence. If tolerated and full enteral feeds reached, lengthened to 3 to 4 hourly feeding.
Supplementation	Vitamins are commenced when the infant achieved full enteral feeds. Zinc, calcium and phosphorus. Iron usually started at 6 to 8 weeks postnatal age.

Although the early introduction of enteral feeds and faster advancement are an acceptable approach for enteral feeds (324, 325), feeding intolerance and NEC are still threatening factors that restrain enteral feeding. Preterm infants especially those born before 28 weeks of gestation and lowest birthweight infants are at high risk for growth restriction during the neonatal period and hospital stay (303). Therefore, interventions, which might improve feeding tolerance for preterm infants are likely, improve their short and long-term health outcomes. For instance, in the1960s, before the establishment of NICU, the UK reduced deaths of preterm infants during hospitalisation after modifying the feeding practice for those infants (326). Therefore, optimising feeding practice and nutritional support for preterm infants have been emphasised as one of the UK research priorities for preterm infants (327).

1.12 Response of gastrointestinal tract to enteral feeding

During intrauterine life, the foetus is nourished from the mother through the placenta. After birth, with the interruption of the placental circulation, the newborn should adapt to the new nutritional mode through the GIT to obtain the nutrient necessary for the growth and development. Maturation of the GIT occurs during the last trimester of pregnancy (328); therefore, preterm infants have immature GIT with poor digestion and absorption. Various aspects of intestinal dysfunctions appear to be critical challenges causing feeding intolerance in preterm infants, such as intestinal dysmotility and delayed gastric emptying that may lead to stasis and overgrowth of pathogenic bacteria (158). Digestive functions, such as gastric acid and enzymes secretions are also impaired (158).

Additionally, immature immunity and fragile mucosal barriers are thought to render preterm infants particularly susceptible to intestinal inflammation and injury; consequently, the GIT could not fulfil its functions. Therefore, during the neonatal period, feeding intolerance is commonly recorded in the preterm infant that reflects the anatomical and functional immaturity of the GIT. Nevertheless, in some cases, the signs of feeding intolerance, such as high gastric residuals, vomiting and abdominal distension, could not

be differentiated from NEC (82). Therefore, interruption of the feeding is needed that lead to the prolonged use of PN and delayed full enteral feeds with unfavourable effects on length of hospital stays, infant growth and other health outcomes.

Suboptimal nutrition during this critical period of life might alter the structures and functions of organs and systems of the body, which is described as “nutritional programming”, (329) for example, the promotion of neurodevelopmental outcomes of preterm infants by human milk (285, 330). Early life nutrition has not only short-term impacts on growth and functional development of the body but also has long-term influences on neurodevelopment, morbidities and mortality in adulthood (331, 332).

The GIT also has major endocrine and immune functions. Many growth factors and hormones, which regulate gastrointestinal growth and development in newborn infants, have been identified. Gut hormones such as gastrin, peptide tyrosine-tyrosine, pancreatic polypeptide, incretins and ghrelin, are peptides produced by the neuroendocrine cells of the gut and play a vital role in many gastrointestinal functions such as digestion (enzyme secretion), mucosal growth, blood flow and motility (328).

Evidence suggested that blood levels of gut hormones were increased after trophic and enteral feeding in term and preterm infants (306) and feeding intolerance may lead to disturbance in gut hormones secretions increasing the risk of NEC (333). As described in section 1.11.2, human milk and colostrum are abundant in many growth factors, which have direct influences on the functions of GIT (234, 237, 238) and they might also exert an indirect trophic effect by increasing the secretion of certain gut hormones (334, 335). Gut hormone responses in preterm infants is further discussed in section 5.1.2.

1.13 Oropharyngeal administration of colostrum (OPC)

Given the potential benefits of colostrum, it is essential to consider the method for administering it in the first few days of life. In preterm infants, colostrum is commonly administrated by orogastric or nasogastric tubes, as trophic or enteral feeds, when the

infant's clinical status permits. However, by this route colostrum will bypass the oral and pharyngeal cavities so that the oropharyngeal mucosa will not be exposed to the protective factors of colostrum. Consequently, pathological microorganisms may take the opportunity to colonise the mouth while the potential immunomodulation of the colostrum biofactors via contact with oropharyngeal mucosa are bypassed. Hence, a different approach for providing colostrum to preterm infants is required.

Moreover, the oral mucosa is one of the main routes for entry of pathogens; thus, oral care appears to be essential in certain preterm infants particularly ventilated infants and ill infants who cannot receive oral feeds. Most of the current oral care marketed products are not approved for use in neonates. Therefore, normal saline or sterile water have been used for oral care in neonatal intensive care units (336). Regular use of normal saline alters the innate antimicrobial properties of the upper airway secretions increasing infection risk (337). Although sterile water appears to be more safe than saline, may not protect against infection. Colostrum is natural; probably safe product might protect these compromised infants against colonisation by pathological organisms and promote the growth of beneficial bacteria.

1.13.1 Proposed mechanisms of OPC as a protective intervention

As described in Section 1.11, there are similarities between the constituents of colostrum and the amniotic fluid (277, 338). Fetal intestinal growth occurs mainly during the last trimester, promoted by fetal swallowing of amniotic fluid which is loaded with antibodies, cytokines and growth factors (158). Interruption of the continuous influx of amniotic fluid to the intestinal lumen by preterm birth may have a negative impact on the healthy growth and development of the GIT (158, 278). Coating the oropharynx with a small volume of colostrum could continue the effects of the amniotic fluid in utero. Rodriguez et al. (339) have suggested that cytokines in colostrum may stimulate the oropharyngeal associated lymphoid tissues (OFALT) and gut-associated lymphoid tissues (GALT) to modulate the immune system of ELBW infants (339). OFALT and GALT are well-known components of

the Mucosal-associated lymphoid tissues (MALT) (340), which represents an immunological system that function independently from the systemic lymphoid tissues. MALT function as the principal mucosal location where immune responses are initiated and activated lymphocytes can then reach more effector sites (341). MALT contains approximately 40% to 60% of lymphocytes of the body. MALT comprised of anatomically distinguished lymphoid tissues including; GALT such as the Peyer patches, the appendix and isolated follicles in the intestine, the bronchial associated lymphoid tissues (BLAT) and the OFALT(Waldeyer's ring) at the entrance of the respiratory and GIT (342).

OFALT, the lymphatic tissue of the oropharyngeal cavity, includes adenoids (pharyngeal tonsil), tubal tonsils, Tonsils (palatine tonsil) and the lingual nodules (lingual tonsil) interspersed with microscopic lymphoid tissues throughout the oropharyngeal mucosa to make the Waldeyer's ring (Figure1.3). The Waldeyer's ring acts as a promising frontline of defence against the entry of microorganisms (343). The OFALT lymphocytes and monocytes have more direct contact with cytokines found in breast milk or pharmaceutical compounds compared to GALT where peristalsis and the mucus may intervene between the lymphocytes and the immune factors (344). Furthermore, OFALT has almost a neutral pH and minimum peptidases that diminishes proteolysis of cytokines; however, the cyclical swallowing of saliva may reduce contact time with OFALT. Cytokines administrated by the oropharyngeal route, activate the OFALT immune cells leading to secretion of cytokines that in turn activate other cells. Additionally, some of the activated cells from the OFALT migrate through the lymphatic ducts to reach local lymph nodes of the neck and enter the bloodstream to be disseminated to distant sites such as other lymphoid tissues, lung, liver and bone marrows (344) (Figure 1.3).

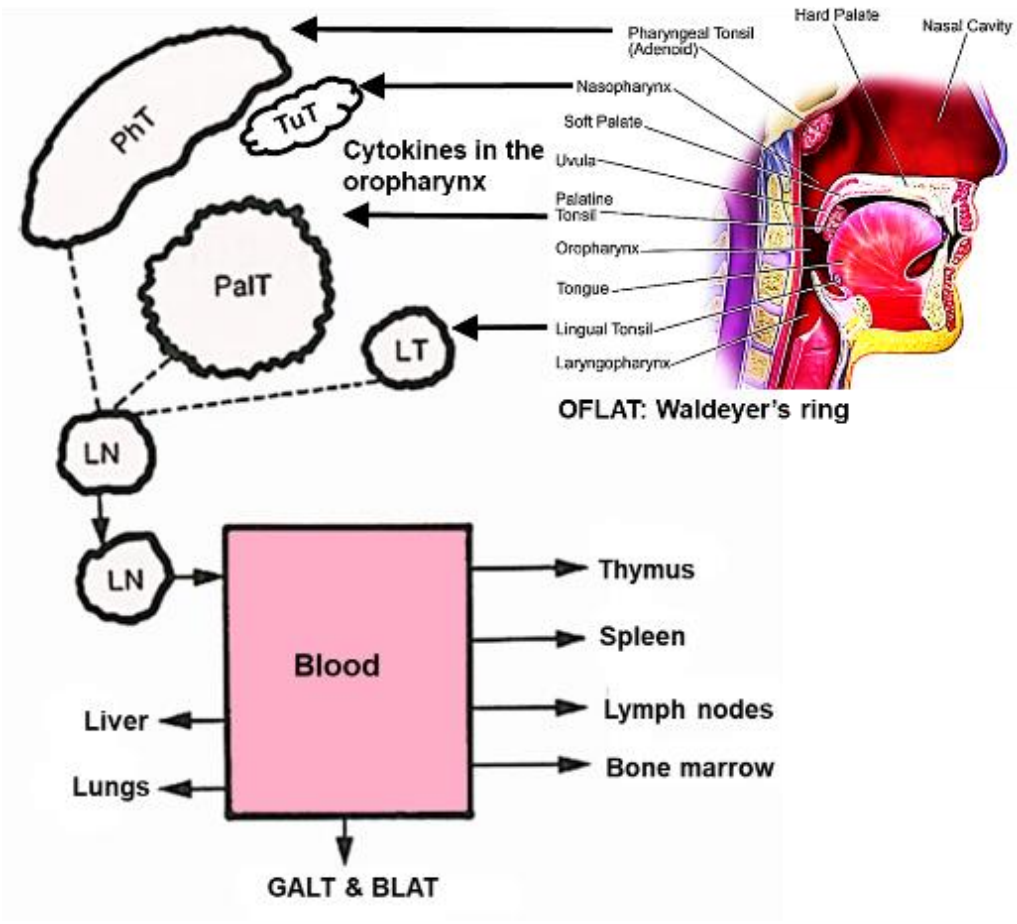


Figure 1.3 Delivery of cytokines by the oropharyngeal route

A schematic diagram presents activation of the immune system by oropharyngeal administration of cytokines. PhT: pharyngeal tonsil; TuT: tubal tonsil; PalT: Platine tonsil; LT: lingual tonsil; LN: lymph node; OFALT: oropharyngeal-associated lymphoid tissues; GALT: gut-associated lymphoid tissues; BALT: bronchial-associated lymphoid tissues. Diagrams adapted from Bocci 1991 (344).

The potential interaction of drugs or milk cytokines had been arising long time ago by Bocci (344) after reporting of a positive effect of low dose of interferon-α in HIV positive patients in 1987 (345). The administration of cytokines like interferon by the oromucosal route and its benefits have been reported in experimental animal and human research. Oromucosal administration of low dose Interferon-α/β has been provided to humans in different clinical situations including influenza outbreaks, acquired immune deficiencies, and chronic hepatitis (346-348). Currently, the Oromucosal route is increasingly considered as a desirable route for vaccination due to its potential ability to induce local and systemic immune responses (349, 350). Therefore, OPC was proposed as an immune modulator for preterm infants.

It was hypothesised that administration of colostrum by the oropharyngeal route allows the bioactive components of colostrum to modulate the immune system and protect against infection by the following potential mechanisms (351-353):

- inhibition of the attachment of microorganisms to the oropharyngeal mucosa by colostrum factors such as IgA, lactoferrin and oligosaccharides
- absorption of bioactive components of colostrum by the oropharyngeal mucosa into the circulation
- interaction and stimulation of the MALT by colostrum cytokines
- prebiotic effect of colostrum factor such as oligosaccharides
- anti-inflammatory effects of colostrum cytokines, PUFA and other factors
- antioxidant effects of the various component of the mother's colostrum such as lactoferrin, peroxidase and catalase.

Administration of colostrum by the oropharyngeal route does not involve swallowing by the infant but allows colostrum instilled in the oral cavity to act locally and be absorbed by the buccal mucosa to modulate the infant's immune system. Different terms have been used to describe the procedure like oral swabbing, colostrum oral care, buccal care, oropharyngeal colostrum, oral priming or swabbing, oral immune therapy and oromucosal but the principal hypothesis is identical.

Recently emerging evidence has suggested the potential benefits of OPC on health outcomes for preterm infants (354-358). Research studies to date contain some significant drawbacks that limit generalisability, such as small sample sizes, inconsistent methods of application and the data are from retrospective or pilot studies. However, there are some studies with relevant results; in a retrospective study conducted after OPC was included in the care of preterm infants in the neonatal unit (353). A sample of 369 ELBW infants was evaluated for clinical outcomes before and after introducing OPC practice. The colostrum group commenced enteral feeding earlier than the pre-colostrum group and weighed more at 36 weeks. The authors speculated that exposure of the infants to growth factors in

colostrum might have promoted growth and function of the GIT, but there were no differences in day to full enteral feeds, the incidence of NEC and mortality between the two groups. However, this study included only ELBW infants, limited to gastrointestinal complications and potential confounders were not considered. Another observational study reported a trend towards reduction of positive tracheal aspirate and blood cultures after the implementation of colostrum for oral care in ventilated preterm infants (355).

Lee et al. (356), in a RCT, reported an increase in urinary (SIgA, lactoferrin and IL-1β) and salivary (TGF-β and IL-8) immune factors within two weeks and a decline in the incidence of clinical sepsis with OPC versus sterile water. They suggested that OPC use in EXP infants (< 28 weeks of gestation) may have the potential to enhance the immune system of the sick preterm and prevent infection and mucosal inflammations; however, it was a very small RCT included only 48 infants. In 2016, another RCT (358) demonstrated that OPC was associated with a shorter hospital stay and a higher rate of breast milk feedings at discharge home with no significant difference in salivary immune peptides.

1.13.2 Procedure for OPC administration

OPC involves placing a small amount (0.2-0.5ml) of preferably freshly expressed colostrum over the buccal mucosa by syringing and gentle swabbing. Alternatively, a swab, soaked in colostrum can be used to coat the oral mucosa with colostrum. Previous studies have followed the procedure for OPC, which initially described by Rodriguez et al. (354). The procedure comprises placing the tip of a syringe filled with 0.2 ml of colostrum along the right side of the infant’s mouth and slowly delivering 0.1 ml of colostrum into the infant’s mouth directing posteriorly towards the oropharynx. While the syringe inside the infant’s mouth redirected to the left side, another 0.1 ml is delivered guided in the same manner (Figure 1.4), the oral mucosa then swabbed gently for a few seconds. OPC administration was started within the first 48 hours after birth and provided two hourly for 48 hours (354).

In 2015, the protocol for OPC was updated that OPC to be started within the first 96 hours of life and administered two hourly for 48 hours followed by three hourly administration for a more extended period depending on the infant's gestational age (359). Neonatal units (360-363) increasingly use OPC in the care of preterm, and ill neonates. However, there is no standard protocol for OPC administration and different methods have been used regarding the frequency, the volume of colostrum (fresh or frozen) per dose, duration of treatment, and the technique (syringe or swab) (356-358).

Figure 1.4 Administration of colostrum by the oropharyngeal route
Source: A: Lee et al. (356); B: Page et al (364).

1.14 Rationale of the thesis

Although there have been massive advances in neonatal medicine and improvements in survival rates; preterm infants still carry a considerable burden of short and long-term morbidities including a high risk of invasive infections, and NEC (133, 134). Both of these conditions are particularly important because of their high prevalence and association with other long-term morbidities such as poor growth and adverse neurodevelopmental outcome (139, 365). They are also associated with prolonged hospital stays and significant rises in the cost of care, to hospitals, families and societies (47, 68) that rationalises more research into preventive measures and potentially cost-effective aimed at reducing the incidence of infection and NEC. The WHO reported that approximately

75% of prematurity-related complications could be overcome with cost-effective interventions, such as Kangaroo mother care for thermal care and support for breastfeeding and maternal antenatal steroids therapy in the preventions of respiratory distress syndrome (69).

Premature infants have immature immune responses. Mother's own colostrum contains many immunological and trophic factors that promote immunological and gastrointestinal maturity (185, 186). Many of these factors are present in higher concentration in colostrum than mature milk (264). Moreover, studies postulated that colostrum from mothers who delivered preterm infants has higher levels of immunological factors than colostrum from mothers with term infants (192, 201). Therefore, mother's colostrum could be an appropriate immune therapy for infection control and improving outcomes for the preterm infants.

OPC use in the first few days of life might stimulate the infant's immune system and protect from infections and other conditions such as NEC (339), and improve the rate of breastfeeding (366). There are limited studies that addressed the effects of early OPC on health outcomes of preterm infants. Previous studies mostly included VLBW, ELBW or/and EXP infants (353). This thesis considered all preterm categories including moderate and late-preterm infants, who contribute to approximately 72% to 85% of preterm births (19, 62) and have a higher risk compared to full-term infants especially in long-term developmental outcomes (367, 368). To the best of my knowledge, no previous study has evaluated OPC in the UK, although, some neonatal units have adopted OPC in the care of preterm infants (360, 362, 369).

OPC is a simple, easy and low-cost procedure, can be performed by nurses, doctors, caregiver, and parents. It does not require high technology equipment and no significant additional resources will be needed to implement OPC administration, which, if its efficacy and safety proved, could be adopted rapidly across neonatal services at a low cost that could be used in low and moderate-income sittings. OPC practice may also encourage the

parents of preterm infants to have an active role in the care of their infants, which may have a positive effect on the feeling of helplessness experienced by the parents.

Based on incidents occurring during early life, especially during the critical period of brain growth, may permanently influence health and wellbeing of later stages of life (331, 332, 370); early action and preventive strategies are essential to impact on the burdens of diseases (68, 371). The WHO reported in 2014 that worldwide there were disappointing reductions in neonatal mortalities, and prematurity-related complications are the leading cause of neonatal deaths (71). WHO also highlighted that caring for preterm, LBW and sick newborn infants are crucial for reducing deaths, disability, and long-term complications, and considered neonatal deaths as a sensitive marker for effective health system (71). The ongoing challenges of preterm births emphasise the need for better understanding of this preventive intervention and its effects.

1.15 Generic view of the thesis

As described in this chapter, the broad benefits of mother's milk are well known and the unique composition of mother's colostrum indicates that its primary functions are protective and trophic. I am particularly interested in the protective benefits of colostrum and the potential impacts of providing mother's colostrum during the early life on health outcomes of preterm infants.

This thesis was conducted according to the University of Nottingham (UoN) Code of Research Conduct and Research Ethics (372) and the UK policy framework for health and social care research (373). All the studies included in this thesis were conducted at the UoN at the Division of Child Health, Obstetrics & Gynaecology (COG) and ethically approved by the Medical School Ethics Committee at the UoN. Where the study involves National Health Services (NHS), ethical approval was granted from the UK Research Ethics Service (RES). Human tissue samples were dealt with under the Human Tissue Act 2004 (374). Ethical considerations for each study will be described in the individual chapter.

1.16 Hypothesis and aims

1.16.1 Hypothesis

Based on the protective properties of mother colostrum as potential immunotherapy, I hypothesised the following:

- oropharyngeal administration is a useful route to deliver colostrum to preterm and sick infants. This route allows the bioactive components of colostrum to contact directly with the oropharyngeal lymphoid tissues to stimulate the infant's immune system
- OPC protects against infection and NEC, promotes infant growth and improves health outcomes of preterm infants
- OPC could be implemented efficiently in neonatal practice.

1.16.2 Aims of the thesis

The aims of this PhD research were as follows:

- to explore the current practice of OPC in the care of preterm infants in the UK
- to collate the evidence regarding oropharyngeal administration of mother's own colostrum in preventing mortality and morbidities of preterm infants
- to evaluate the feasibility of providing colostrum by the oropharyngeal route to preterm infants in the UK
- to assess the effects of OPC on the health outcomes of preterm infants.

The subsequent studies were designed to achieve these aims as follows:

Chapter 2: Oropharyngeal administration of mother's own colostrum to preterm infants: a survey of practice

Some neonatal units adopted the practice of OPC administration as a part of the standard care for preterm infants as a potential preventive measure to reduce complications and improve growth and outcomes of the infants. This chapter describes the current use of OPC in the UK neonatal units by surveying neonatal professionals in the UK regarding

OPC administration in the care of preterm infants. Such surveys may provide reference standards and help health professionals to evaluate their practices. Surveys also can highlight gaps in health practices; therefore, may guide further research.

Chapter 3: Oropharyngeal colostrum in preventing mortality and morbidity in preterm infants: a Cochrane systematic review

Based on the findings of a survey of the UK neonatal professionals (Chapter 2), OPC is increasingly adopted by the UK neonatal units and has been recommended by some neonatal professionals. To bridge the gap, I systematically reviewed currently available evidence on the use of OPC in the care of preterm infants**.** This chapter presents the results of a Cochrane systematic review and meta-analysis assessed RCTs investigating the effects of using OPC on health outcomes of preterm infants.

Chapter 4: The impact of oropharyngeal administration of mother's colostrum on the clinical outcomes of preterm infants: a case-control study

A matched case-controlled study was conducted to evaluate the effects of OPC on short-term health outcomes of preterm infants, in Nottingham neonatal units after the implementation of OPC for the care of preterm infants. Cases were preterm infants born before 32 weeks of gestation and received OPC during the early neonatal period. Controls were matched for; infant's sex, gestational age and the closet birth weight, and selected from those infants who were admitted to Nottingham neonatal units before the adoption of OPC administration.

Chapter 5: Gut hormone responses to oropharyngeal administration of mother's colostrum to infants in neonatal intensive care

This study was conducted to investigate the response of a set of gut hormones to OPC in preterm and sick infants requiring neonatal intensive care. Plasma samples from participant infants were analysed using Multiplex magnetic-beads immunoassay. This study was an observational, non-randomised comparison; compared infants who received mother's colostrum by the oropharyngeal route (OPC group) during the early neonatal period with those infants who did not receive OPC (No-OPC group).

Chapter 6 Conclusion

This chapter will summarise the key findings and consider the strengths and limitations of this PhD project. The implications for clinical practice and future research will be highlighted.

Chapter 2. Oropharyngeal administration of mother's own colostrum to preterm infants: a survey of practice

2.1 Chapter overview

This chapter aimed to explore the current practice of oropharyngeal administration of colostrum (OPC) in the UK. Neonatal professionals make a good target population for gathering information about the care of newborn babies (375). It is within this context that the current study was designed as a survey to evaluate the practice of OPC and assess the knowledge, attitudes of the UK neonatal professionals towards the use of OPC in the care of preterm infants.

2.2 Background

OPC is a new additional route introduced to deliver colostrum to preterm infants and those who are critically ill and cannot tolerate enteral feeds during the early neonatal period. More details about OPC administration are described in Section 1.14. The use of OPC is progressively increasing among neonatal units in different countries; some neonatal units have introduced the use of colostrum for oral care, and OPC (360, 361, 376, 377) with the aim of promoting infant's immune function and protecting against infection and necrotising enterocolitis (NEC) (351, 352). However, neonatal units vary in their guidelines and strategies for using OPC.

2.2.1 Surveys in research

Surveys are commonly used in epidemiological studies, for service evaluations in health and to provide information for policy-makers (378, 379); they can also evaluate the attitude and beliefs of health care providers and may identify the knowledge gap (380).

Surveys are a valuable tool for collecting information from a target population regarding their knowledge, perceptions, practices and expectations of a specific topic for research.

In surveys, a sample is selected to be representative of the population of interest to collect data for a particular study. Surveys can be conducted in multiple modes as, face-to-face interviews, via post, telephone or as an internet-based survey and they are classified into interviewer-completed and self-completed surveys (381, 382). Face-to-face and mail questionnaires have been the most widely used format in survey-based research (383, 384).

Each method of conducting a survey has advantages and limitations; the study topic, the targeted participants and the aims of collected information could guide the selection of the best method (382). For example, face-to-face interview surveys are flexible and might increase cooperation and response rates. However, this type of survey might be limited by interviewer bias, sample size and coverage of the target population and may induce anxiety, especially with sensitive questions (384). Additionally, they are less cost-effective and more time-consuming than postal and online surveys (385). Self-completed surveys are independently completed by the respondents; have the advantages of ease, less cost, quicker and wider distribution. Generally, surveys may contain questions which are considered unnecessary or misunderstood by the participants; therefore, the questions might be ignored by the respondents.

Recently with the extensive use of the internet, there has been an expansion for the use of an online web-based survey especially in large sample research (386-389). Online surveys have the advantages of lower cost, faster distribute, more accessible to a specific and large population across a large geographic area (nationally and internationally) (390). Respondents could also complete the questionnaire at times convenient for them, as well as divide their responses over multiple sessions (391). Performing surveys by online self-completion reduce the risk of reporting bias by decreasing the possibility of researchers' influencing on the responses (384).

However, online surveys may have lower response rates in comparison to paper questionnaires and interviews (392, 393). Through, the low cost and ease of sending

emails, online surveys offer the researchers a promising way to follow up non-respondents by sending reminder emails (394). Criticisms of online surveys that are such emails could be perceived as junk mails and that they may also be influenced by technological variabilities such as the use of networks connections, browsers and computers' configurations (391, 392).

As this study was a survey of a clinical practice across the UK, an online web-based survey was chosen to achieve efficient distribution and response (381). Firstly, our targeted participants were neonatal professionals could be reached by emails (395). Secondly, this method offered the benefits of saving time, being cost-efficiency, practicality and that the responders directly completed their responses into the software, facilitating later data analysis. Furthermore, directing the responders to questions according to the previous answer given by the participant decreased the load of non-applicable questions presented to the respondents. The online survey also enabled the use of compulsory questions helping to reduce the risk of missing data and neonatal professionals could complete the online survey when it was convenient for them and across multiple sessions.

2.2.2 Rationale of the study

OPC administration is increasingly adopted by the UK neonatal units (360, 362, 369, 396, 397) though it is not yet known whether using OPC in the care of preterm infants, will improve health outcomes and promote the growth of these vulnerable infants. A few studies have been conducted to assess this question with variable results.

Considering the uncertainty about OPC use, and to the best of my knowledge following a comprehensive literature search, there are no published studies that focus on the practice of OPC in the UK. A survey of neonatal professionals provides an initial step to assess OPC use in UK neonatal units. The results may provide important information to health services and decision makers and a step toward obtaining the required evidence for ongoing practices, informing future practices and providing a base for future research.

2.2.3 Hypothesis and aims

The hypothesis was that most of the neonatal units in the UK did not use OPC for the care of preterm infants. I also hypothesised that those who are practising OPC in their units did not have a standard policy.

This survey aimed to:

- ascertain the use of OPC in UK neonatal units
- determine the current practice of OPC such as patient characteristics and indications
- determine the variation in OPC administration
- describe the knowledge and attitude of neonatal professionals in the UK regarding the use of OPC.

2.3 Methods

The study was approved by the Medical School Ethics Committee at the University of Nottingham: R16042015 SoM CHOG (Appendix1).

2.3.1 Study design

It was a prospective, cross-sectional study consisting of an online internet-based survey.

A web-based structured questionnaire was designed and conducted using the Bristol online survey (BOS) software (www.survey.bris.ac.uk). Bristol online survey (currently Online surveys: www.onlinesurveys.ac.uk) is a useful tool designed for academic research, education and public sector organisations for creating online surveys of unlimited numbers for unlimited respondents. BOS is managed by the Joint Information Systems Committee (Jisc) (398), which is an organisation providing digital services for UK education and research. BOS is a practical, easy to use tool, entirely compliant with UK data protection and meets UK accessibility requirements. It was thought to meet the needs of this study; furthermore, the University of Nottingham offers free access to BOS to postgraduate research students within the university.

The questionnaire was designed to allow the potential respondents to complete the questionnaire online; therefore, a web-based design was chosen instead of an email-survey (a questionnaire attached to an email).

2.3.2 The questionnaire

There was no previous survey focused on OPC administration; therefore, the questionnaire was developed based on a previous similar survey study that aimed to determine the practice of heated humidified high-flow nasal cannula oxygen in UK neonatal units (399). The previous survey was also a web-based survey, and it has been successful in collating data on clinical practice and perceptions from neonatal professionals.

The survey was comprised of four sections (Appendix 2):

- Section One; this section provided the participants with a summary of OPC administration and the objective of the study. It explained the nature of participation and that confidentiality would be maintained. The participants were directed to the next relevant question or section according to a designed route. Routing allows respondents to be directed through an online survey based on their answers. Routing can also help to branch the survey into sections that designed to specified groups of participants.
- Section Two; completed by OPC users answering questions exploring the practice of OPC.
- Section Three; was completed by those participants who were not using OPC.
- Section Four; professionals' information section, included questions such as job description and work experience.

The questions were mainly closed-ended questions in a multiple choice format or single answers; this form of questioning facilitates completion of the survey and reduce variability between the respondents enhancing comparisons (400). Though, closed-ended questions may miss options that would be limited to the respondents; to minimise this possibility, a

response option of ‘Other’ was included. Additionally, some questions were open-ended that allows the participants to comment on what they wanted in response to the question that had not been asked, or wanted to make it known. This type of question may prevent any potential understanding issues (400). However, open-ended questions are likely to take a longer time, particularly with such clinical professionals; therefore, they may lower the response rate. Furthermore, the unstructured format may introduce variability in coding the answers, that could affect the validity of the study (400).

To ensure the effectiveness of the survey and to estimate how long it took to complete, colleagues (including; consultant neonatologist, research fellow, paediatrician, dietician and PhD students) in the Division of Child Heath, Obstetrics & Gynaecology (COG), at the UoN, piloted the questionnaire. Comments from this piloting process were used to revise the survey tool further. The adjusted questionnaire was then submitted to the ethical committee for approval. The approved questionnaire was sent to the neonatal professionals within the UK.

2.3.3 Participants

The targeted population was neonatal professionals from neonatal units in the UK, regardless of the level of the neonatal unit. Doctors and nurses were included in the study. The participants were identified via the British Association of Perinatal Medicine (BAPM) (401). BAPM is a non-governmental, professional association. It was founded in 1976 with the aim to improve and optimise the standard of perinatal care within the UK by providing support and advocacy for perinatal professionals, and babies and their families (402). BAPM also manages the UK neonatal networks web page, as well, it promotes and supports research and innovation in perinatal and neonatal medicine (402).

Neonatal networks are clinically managed Operational Delivery Networks (ODNs) for neonatal services within the UK. Neonatal networks were established in 2004 to provide neonatal care by clinically managed networks with the aim to reduce perinatal mortality and improve the quality of care (403). ODNs enable communication, share knowledge and

collaboration between neonatal units. Each network consists of a group of neonatal units according to the geographical area covered.

The BAPM classifies neonatal units into three categories according to the level of care they provide (404).

- Neonatal Intensive Care Units (NICUs), provide care for ill and unstable babies who need mechanical respiratory support (mechanical ventilation, continuous positive airway pressure (CPAP), low birth weight, preterm < 28 weeks of gestation, surgical care and any additional support such as central line insertion, and exchange transfusion. NICUs also provide high dependency and special care.
- Local Neonatal Units (LNU), provide high dependency and special care for babies who need CPAP, parenteral nutrition, tube feeding and babies needing short-term intensive care (e.g. following apnoeic episodes).
- Special Care Units (SCU), provide care for babies who require respiratory and cardiac monitoring, oxygen therapy, phototherapy, tube feeding and babies recovering from other levels of care.

Neonatal networks in the UK were identified from a list available at the BAPM website that accessed April 2015 (www.bapm.org/neonatal-networks). The contact details of the neonatal network staff were ascertained from a list on the website of BAPM that provided an avenue to approach the potential study participants.

2.3.4 The survey process

Invitation emails explaining the purpose of the study were sent to the neonatal network staff (lead doctor, lead nurse, administrator or manager) to provide the contact details of the lead doctors and lead nurses of their corresponding neonatal units (Appendix 3). The invitation emails were followed by two reminder emails to those networks who did not provide contact details of their units.

A list was prepared including lead doctors and nurses of neonatal units whose contact details have been provided by their neonatal network staff.

Emails with the survey link were sent to the lead doctor and nurse of each neonatal unit (Appendix 4). To improve the response rate, following the initial email launching the survey, three reminder emails (Appendix 4) were sent to potential participants at two-week intervals. Reminder emails can increase a survey response rate by about 33% (405). Telephone follow up was planned to approach those who did not respond to these approaches by completing the survey. However, trials of the phone calls were conducted, and it was found not feasible. To find those units who did not respond, the respondent units, which were identified through BOS software, were compared to the list of the neonatal units that have been identified via BAPM website (401). Phone numbers were found from the hospital website of each nonresponding unit. Those who responded to the telephone call (only 3 neonatal units) preferred the survey link resent rather than to complete it over the phone. The survey was launched on May 18th, 2015 and closed on October 30th, 2015.

2.3.5 Data management

- Participants completed their responses directly into the BOS software. The data were exported as anonymous Microsoft Excel and SPSS files for analysis.
- The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 22 for Windows was used for the analysis.

Descriptive statistics (frequencies and percentage) were used to analyse the responses of the survey.

2.3.5.1 Ethical consideration

2.3.5.2 Consent

Participation in the survey was voluntary, and consents were not obtained. Completion of the online survey was taken as implied consent. No payments or incentives were offered to the participants.

2.3.5.3 Safety considerations

This survey did not include patients nor families. It included neonatal health professionals, was estimated to take the participants a maximum of approximately ten minutes to complete.

2.3.5.4 Confidentiality

The questionnaire was identified by the neonatal unit and not by individual respondents except for those who had provided their emails in response to an optional question (Q 27.a “If you would like to join study research to assess buccal colostrum administration, please could you provide your contact details, if possible”). Responses were not identified by individual and compiled together, and analysed as a group, this has been highlighted in the first section of the questionnaire.

2.3.5.5 Data protection

The survey administrator collected the data, which were stored by the research team securely in the Division of COG at the UoN during the data collection period, or their nominated replacement, for seven years or longer if needed. The anonymised electronic data were saved on a password-protected computer, provided by the UoN. Any personal data was dealt with according to the UK new General Data Protection Regulation (406) and Data Protection Act 2018 (407).

2.4 Results

Almost all of the contacted neonatal networks had responded by providing the contact details of the lead doctors and nurses of their corresponding neonatal units. Of the 21 neonatal networks who were contacted, 12/21 (57.1%) networks responded to the invitation email. Six networks (6/21 (28.5%)) responded to the first reminder email. Two networks (2/21 (9.5%)) responded to a second reminder email while one network did not provide the contact details of the lead doctors and nurses and requested that they send the survey link to their local units on my behalf. Figure 2.1 demonstrates the survey process.

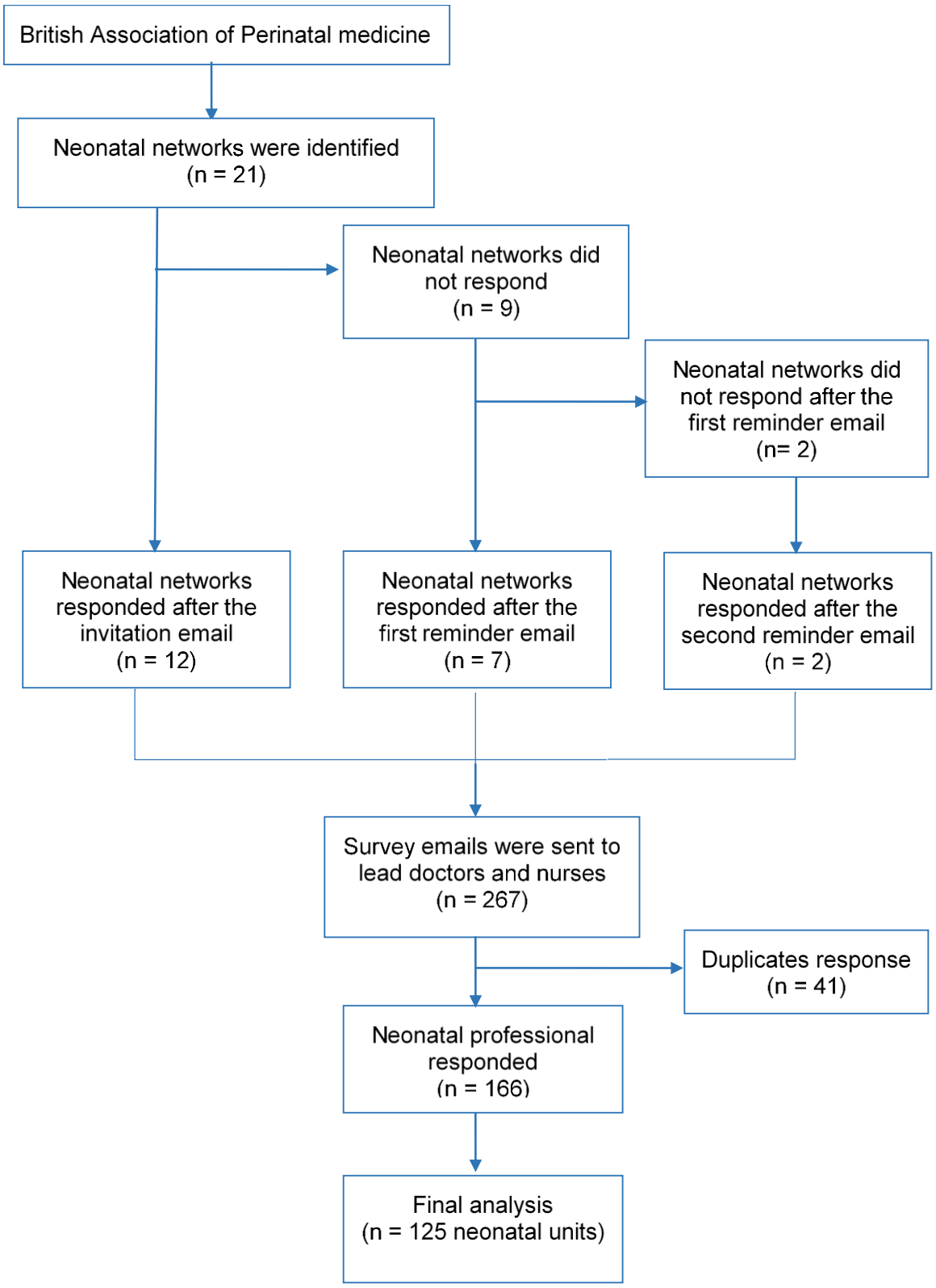


Figure 2.1 The study flow chart

Selection of included questionnaires in the final analysis

2.4.1 Survey response and sample characteristics

A total of 267 neonatal professionals working in the UK neonatal units were sent the survey link. Responses were received from 166/267 (62%) of the neonatal professionals who were surveyed (doctors: 80/166 (48%); nurses: 86/166 (52%), and 52% of them have been working in neonatal care for more than 20 years (Figure 2.2). It was not possible to precisely assess how many potential respondents viewed the survey link and did not complete the questionnaire.

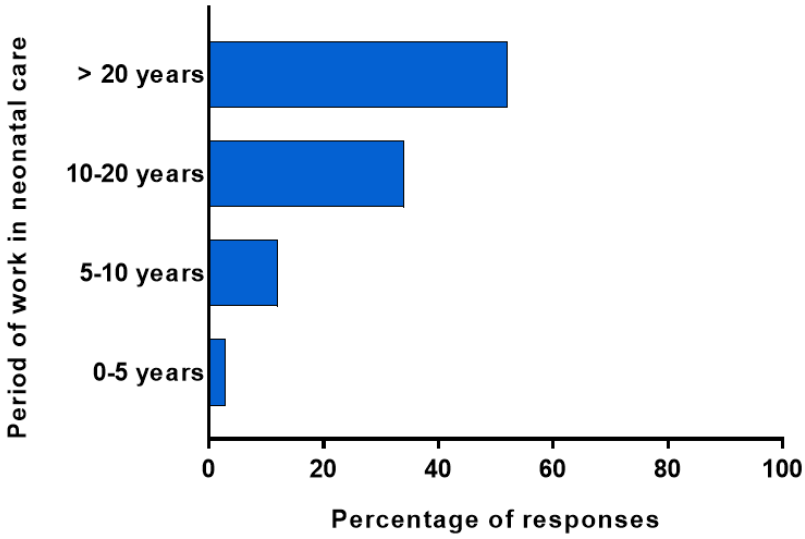


Figure 2.2 Respondents' length of work in neonatal care
Response to the survey question: “Please state approximately how many years you have been working in neonatal care?”. Bar: percentage of responding units

There were 41 duplicate responses (two responses from the same unit, one from the nurse and one from the doctor). Considering the duplicates; 166 responses represented 125 neonatal units which accounted for 60% of 206 neonatal units in the UK according to data from the BAPM website, April 2015. Responses were received from almost all neonatal networks within the UK with variable rates (Table 2.1). Designation levels of the responding units compared to the UK neonatal units are presented in Table 2.2.

Table 2.1 Distribution of respondents units by neonatal network

Neonatal network (BAPM, 2015)	Responding units (n = 125)	Network units (n = 206)	Percentage (%)
Central*	6	8	75
Trent*	4	6	67
East of England	11	17	65
London: North Central & East	7	12	58
London: North West London	3	7	43
London: South	7	10	70
Cheshire & Merseyside ≠	5	9	56
Greater Manchester ≠	7	8	88
Lancashire & South Cumbria ≠	3	5	60
South East Coast	6	13	46
South West	10	12	83
Southern West Midlands	6	10	60
Staffordshire, Shropshire, & Black County	5	7	71
Thames Valley & Wessex	11	16	69
Yorkshire & Humber	12	19	63
Wales	5	13	38
North of Scotland	0	3	0
South East Scotland & Tayside	3	6	50
West of Scotland	5	8	63
Northern	6	11	55
Northern Ireland	2	7	29

BAPM**:** British Association of Perinatal Medicine; *East Midlands Neonatal operational delivery network (ODN); ≠ North West Neonatal ODN

Table 2.2 Level of respondent units versus UK neonatal units

Unit level	UK NUs* (n = 206)	Respondent units (n = 125)	Percentage (%)
Neonatal Intensive Care Unit	61	51	84
Local Neonatal Unit	88	57	65
Special Care Unit	57	17	30

NU: neonatal unit; *: data according to British association of Perinatal medicine, 2015); n: number

Duplicate responses from the same unit were further reviewed to determine consistency among the respondents. There was a perfect agreement between duplicate responses received from the same unit (Cohen's Kappa coefficient ranged from 0.71 to 1). Discrepancies were found only in two questions namely, "Do you have written guidelines on the use of oropharyngeal colostrum?" and "Do you document oropharyngeal colostrum on the infant's record charts?" (Section 2.4.3.4).

Exclusion of one response from each duplicate was done randomly by the statistical software (SPSS). The data analyses that follow were carried out on a sample of 125 units except for questions considering individual perceptions that the analysis was conducted using the total responses (166 responses) as a dominator, Figure 2.1.

2.4.2 Use of colostrum in the UK neonatal units

According to responding neonatal unit lead doctors and nurses, almost all neonatal units used colostrum 120/125 (96%) when have been asked, "Do you administer colostrum to preterm infants in your unit?"

Colostrum was administered to preterm infants by different routes. With the multiple options available for answering this question, many respondents gave more than one answer that reflecting the use of several routes by neonatal units (nasogastric tube (NGT): 116/349 (33%); orogastric tube (OGT): 100/349 (29%); in the mouth: 96/349 (28%); bottle: 37/349 (10%) responding units.

Human colostrum was the only type of colostrum used in the UK neonatal units and the mother's own milk was the most used type of milk (Figure 2.3).

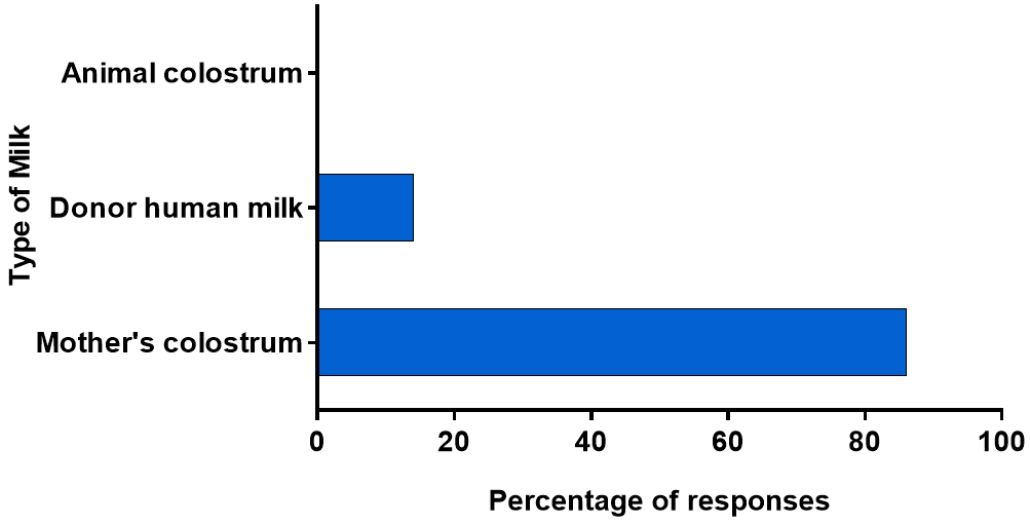


Figure 2.3 Type of Colostrum used
Response to the survey question: “Which type of colostrum do you use?”
Bar: percentage of responding units

2.4.3 OPC administration in the UK neonatal units

2.4.3.1 Use of OPC by the neonatal units

Of the responding units that use colostrum 86/120 (71%) administer OPC to preterm infants. This accounts to 40% of the UK neonatal units. Out of the 86 units that use OPC; 31/86 (36%) were NICU, 42/86 (49%) LNU and 13/86 (15%) SCU. OPC use has been introduced for more than four years by approximately one third of responding units (Figure 2.4).

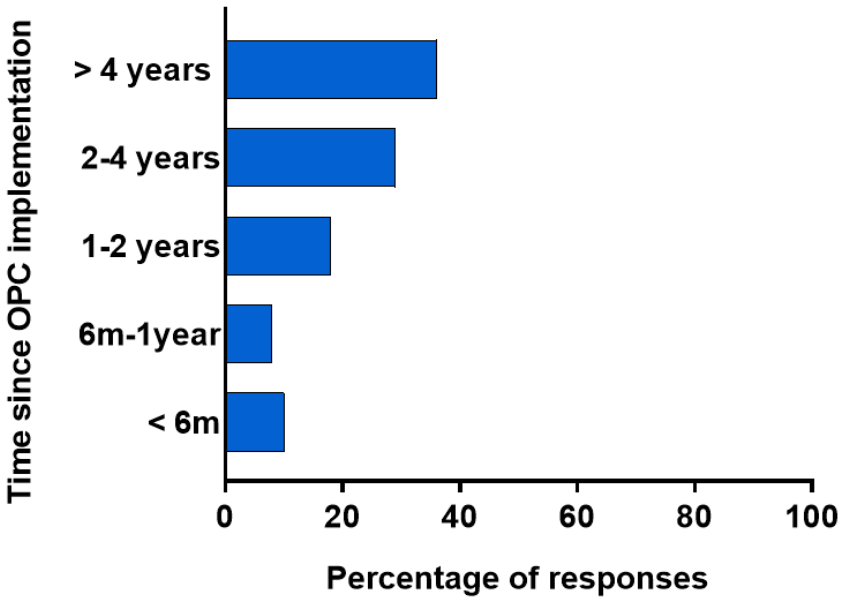


Figure 2.4 Duration of OPC use by the neonatal units
Responses to the survey question: "How long has it been since OPC administration was introduced in your unit?"
Bar: percentage of responding units; OPC: oropharyngeal colostrum; m: month

2.4.3.2 Patients characteristics and OPC administration

Data about the use of OPC in preterm infants was analysed using the number of the units using OPC (86 units) as a dominator.

2.4.3.2.1 Infant's gestational age

OPC was administered to preterm infants at different gestational ages, and many respondents gave more than one answer, which is probably because some units use more than one gestational age group. Approximately 50 % of the respondent units use OPC at any gestational age of the infants (Table 2.3). 'Other' criteria were answered by only five units (4%), and free texts were:

- "It depends on the condition of the baby."
- "No specific policy but generally they are above 27 weeks."
- "No specific guideline but try to administer colostrum oropharyngeally to all under 28 weeks or otherwise 'high risk for NEC' infants, e.g. <1000g, Absent /Reversed EDF."
- "31 weeks onwards."
- "Term babies."

Table 2.3 Infant's gestational age for OPC administration
"Based on gestation in which group of preterm infants do you use oropharyngeal colostrum?"

Gestational age (GA)	Responses (n=124)	Respondent units using OPC (n = 86) (%)
< 28 weeks	14	11
28- <32 weeks	24	19
32-<37 weeks	21	17
Any GA	60	49
Other	5	4

n= number of responses; OPC: oropharyngeal colostrum; Any GA: all gestational age

2.4.3.2.2 Infant's birth weight

Most of the responded neonatal units administered at any birth weight range (Table 2.4).

Multiple answers reflect units who use several birth weight ranges for OPC administration.

Table 2.4 Infant's birth weight for OPC administration
Responses to the survey question: "Based on birth weight, for which range do you use oropharyngeal colostrum?"

Birth weight (g)	Responses (n= 114)	Respondent units using OPC (n = 86) (%)
< 1000	10	9
1000-1500	13	11
1500-2000	10	9
2000-2500 g	7	6
Any weight	74	65

n= number of responses; OPC: oropharyngeal colostrum

2.4.3.2.3 Infant's postnatal age

Administration of OPC to preterm infants was commenced regardless of the infant's postnatal age by about half of the respondent units. Once more, the multiple answers were probably reflecting units who administer OPC in several postnatal ages (Table 2.5).

Table 2.5 Infant's postnatal age for OPC administration

Responses to the survey question: "At what age of the baby do you commence administration of oropharyngeal colostrum?"

Age of infant (hours since birth)	Responses (n=119)	Respondent units using OPC (n = 86) (%)
Within 24 hours	37	31
24 to 48 hours	19	16
48 to 72 hours	4	4
72 to 96 hours	3	2
Any postnatal age	56	47

n= number; OPC: oropharyngeal colostrum

2.4.3.2.4 Infant's clinical status to give OPC

OPC administration had been used whatever the infant's clinical status by 32/86 (37%) of the respondent units (Figure 2.5). 'Other' reasons for OPC not being given were reported by 12/86 (14%) units, and free texts were:

- "No colostrum available (mother or donor)".
- "Maternal HIV".
- "Known Upper GIT- anatomical anomalies".
- "No guidelines/policy, it was personal practice".
- "NEC or surgical interventions".
- "Critically ill infants".

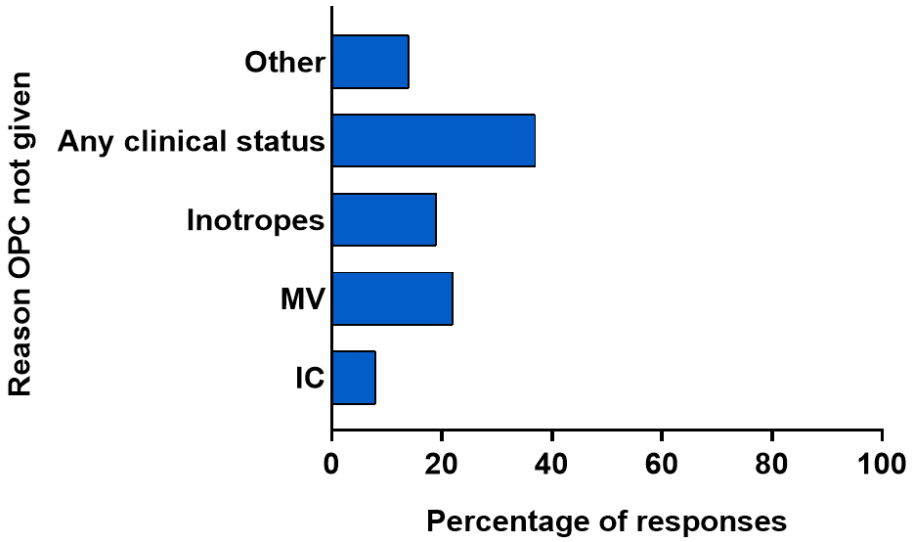


Figure 2.5 Infant's clinical status to give OPC
Response to the survey question; "In which situation would oropharyngeal colostrum not be given?" Bar: percentage of responses; OPC: oropharyngeal colostrum; inotropes: intravenous infusion of inotropes; IC: intensive care; MV: mechanical ventilation; any clinical status: regardless of infant's clinical condition.

2.4.3.2.5 Infant's feeding regimen and milk type

Participants were asked about giving OPC with other feeding regimens, OPC administration had been used with variable feeding regimens i.e. trophic feeds (<1ml/kg/hr of milk): 24/86 (28%) units; enteral feeding (>1ml/Kg/hr of milk): 20/86 (23%); parenteral nutrition: 19/86 (22%); nil per oral stat: 12/86 (14%); nil via OGT/NGT: 11/86 (13%).

OPC had been given to preterm infants receiving different types of milk; all milk options were used by 48/86 (56%), mother's milk only 9/86 (11%), mother's own milk combined with formula 21/86 (24%), and mother's own along with donor milk 8/86 (9%). No units used donor human milk along with formula milk. Mother's milk was, therefore, a requirement in all units for this feeding approach.

2.4.3.3 Adverse effects associated with OPC administration

Almost all the units that administer OPC (82/86 (95%) reported no significant adverse effects related to OPC administration whereas 4/86 (5%) reported adverse effects; one, a decrease in oxygen saturation (Spo_2) to below 80%, and another 3 units reported both a decrease in SpO_2 and bradycardia (heart rate below 100 beats/min), Figure 2.6.

2.4.3.4 OPC guidelines and documentation

OPC administration had been mostly used without written guidelines or policies to follow 69/86 (80%) units, Figure 2.6.

In answering the survey question: “Do you have written guidelines on the use of oropharyngeal colostrum?”, discrepancies (where the nurse and the doctor responded differently) were found from only four units (4/86 (5%)) among duplicate responses. A reliability analysis using Cohen’s kappa statistics was conducted to determine consistency among duplicate respondents (408, 409). There was a moderate agreement between doctors and nurses, Kappa coefficient (κ) = 0.53 ($p = 0.02$).

OPC administration was often not documented on the infant’s clinical record charts. 54/86 (63%) of the neonatal units who were using OPC responded that they do not document it and 32/86 (37%) documented the OPC use on the clinical chart (Figure 2.6). Discrepancies were found between the duplicate responses from eight units (Kappa coefficient (κ) = 0.15; $p = 0.4$) which is considered a slight agreement (408, 409). The eight units with discrepant responses were excluded from the analysis for the variable; “documentation of OPC on the infant’s record chart”. Sensitivity analysis was also conducted by considering both responses of nurses and doctors. Based on the nurses’ responses, 49/86 (57%) of the units did not document OPC and 29/86 (33%) documented the use of OPC on the infant’s record chart. Considering the doctors’ responses; 51/86 (59%) did not document OPC use and 27/86 (31%) documented the use of OPC on the infant’s record chart.

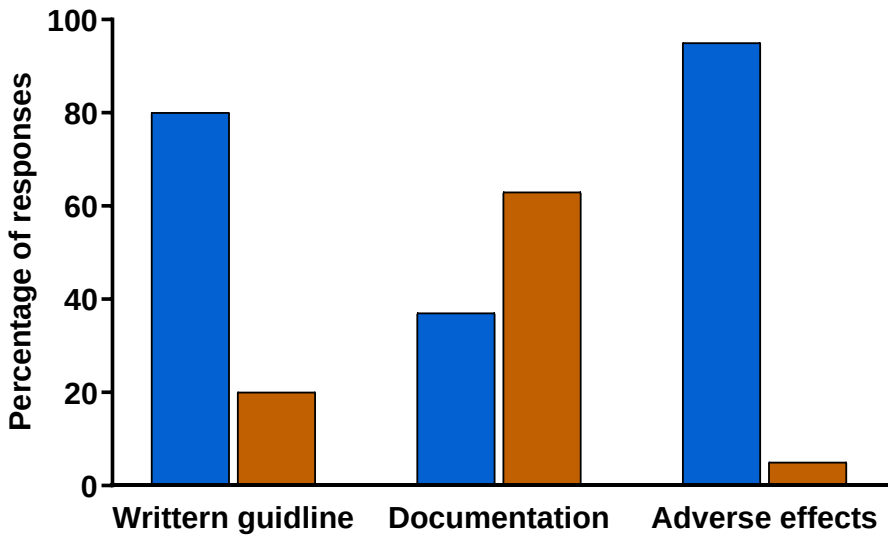


Figure 2.6 OPC adverse effect, documentation and guidelines

Responses to the survey questions:

"Have you experienced any adverse effects with the use of oropharyngeal colostrum?" (n = 86)

"Do you have written guidelines on the use of oropharyngeal colostrum? (n = 82)

"Do you document oropharyngeal on the infant's record charts?" (n = 78)

Documentation: recording OPC administration on infants' charts; Blue bar: No; Brown bar: Yes; Bar: percentage of responses

2.4.3.5 Individual perception towards OPC administration

This section describes individual perceptions of neonatal professionals who administer OPC; these questions were analysed using the total respondents.

2.4.3.5.1 The procedure of OPC administration

Almost all OPC users (107/166 respondents) felt that it was easy to administer colostrum by the oropharyngeal route. However, they responded with different individual perceptions (Figure 2.7).

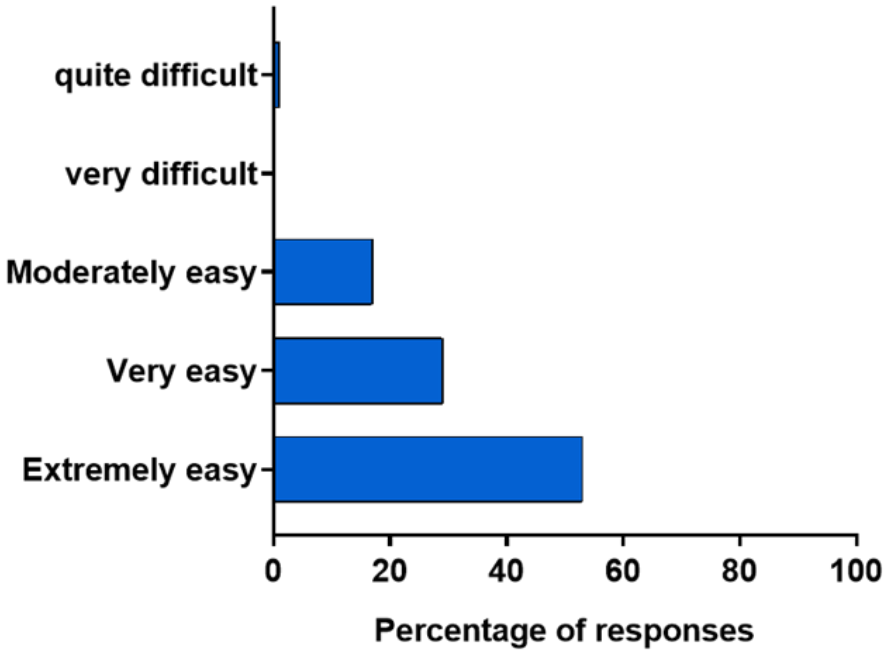


Figure 2.7 Ease of OPC administration
Response to the survey question; "How easy is it to administer colostrum by the oropharyngeal route?" Bar: percentage of cases; OPC: oropharyngeal colostrum

2.4.3.5.2 Recommendation of OPC use to other sites

All the OPC users, 107/166 respondents (64%) recommended oropharyngeal colostrum as part of the standard care of preterm infants with variable levels of recommendation (Figure 2.8).

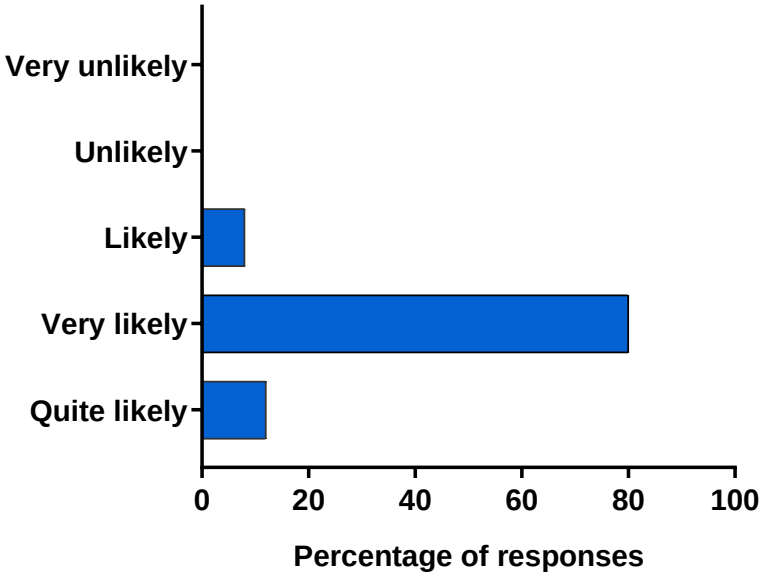


Figure 2.8 Recommendation of OPC administration to other sites
Response to the survey question: "Would you recommend OPC as part of the standard care of preterm infants?" Bar: percentage of responses; OPC: oropharyngeal colostrum

2.4.4 Units not currently using OPC

Of the 120 responding neonatal units that use colostrum, 34/120 (28%) units did not practise OPC administration (OPC non-users). Of these 34 units, 18/34 (53%) were NICUs, 12/34 (35%) LNUs and 4/34 (12%) SCUs. Data from the units not currently using OPC was analysed using the number of these units (34 units) as a dominator.

2.4.4.1 Reasons for not using OPC

Not being knowledgeable about OPC administration was the most common reason for not using OPC by those units who were not currently using OPC administration (Figure 2.9). "Other" was answered by 3/34 (9%) units, and they commented that "their units were SCUs and mostly admitted babies who can tolerate enteral feeding and were able to swallow"

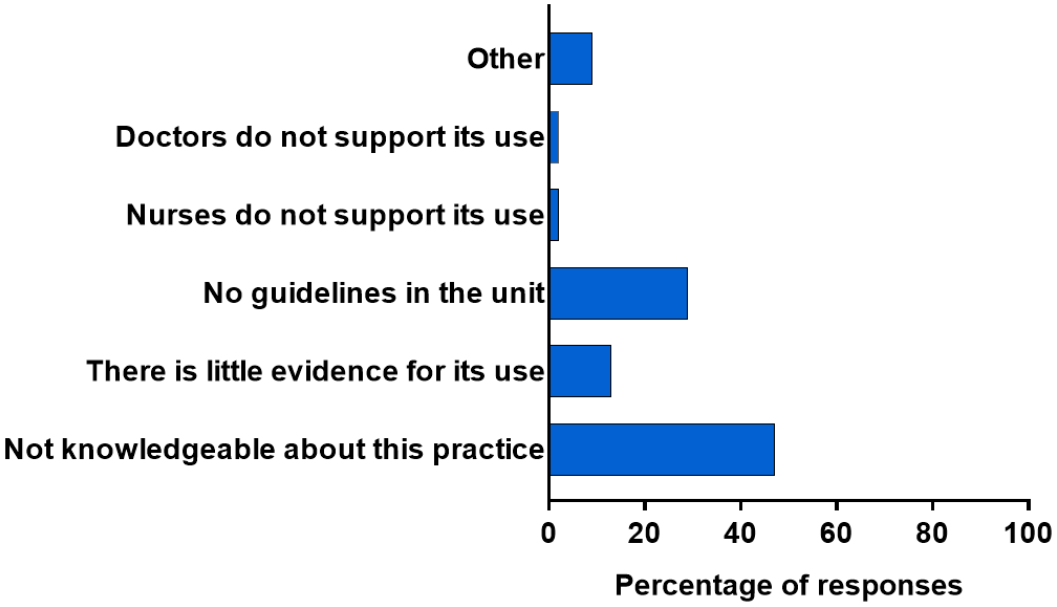


Figure 2.9 Reasons for not using OPC
Response to the survey question: "Why are you currently not using oropharyngeal colostrum (OPC) in your unit?" Bar: percentage of responses.

2.4.4.2 Use of colostrum by the units currently not using OPC

Although the units who did not practise the administration of OPC, most of these units were giving colostrum to preterm infants down a gastric tube when asked "Do you give

colostrum down a gastric tube?"; 19/34 (56%) responded "mostly" and 11/34 (32%) "always" while 3/34 (9%) answered "sometimes" and 1/34 (3%) "occasionally".

Colostrum was given to preterm infants down a gastric tube regardless of the infant's feeding regimen; 14/34 (41%) units give colostrum with trophic feeding, 12/34 (36%) enteral feeding, 7/38 (21%) parenteral nutrition and 1/34 (2%) units give it with nil by mouth or nil per oral status. These units were mostly administering mother's own milk in the order that it is expressed when asked "Do you administer mother's own milk in the order that it is expressed ?"; they answered; 29/34 (85%) "mostly": 22/34 (65%); "always": 7/34 (20%); "sometimes": 4/34 (12%); "occasionally": (3%).

2.4.4.3 Introduction of OPC by neonatal units currently not using it

Of the 166 respondent neonatal professionals, 52/166 (31%) did not use the oropharyngeal route in their neonatal units, many indicated the intention to introduce it in the future (Figure 2.10).

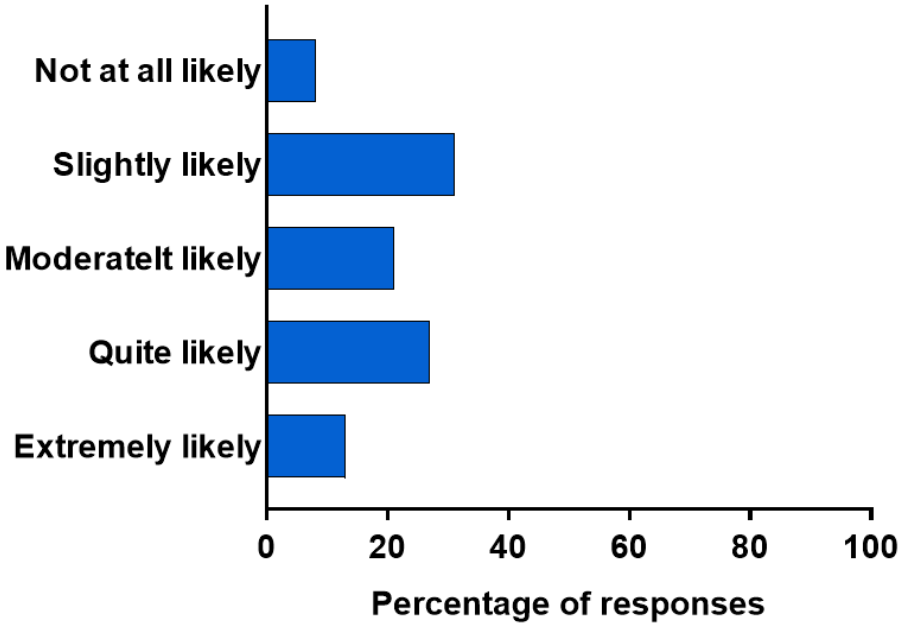


Figure 2.10 Introduction of OPC administration by units not using OPC
Responses to the survey question: "If you are not currently giving oropharyngeal colostrum, how likely are you to introduce it in the future?" Bar: percentage of responses; OPC: oropharyngeal colostrum

2.4.5 Interest in a research study

Most respondents 106/166 (64%) indicated that they would be interested in joining a research study to evaluate oropharyngeal administration of mother's own colostrum to preterm infants and provided their contact details. Table 2.6 presents the characteristics of the respondents.

Table 2.6 Characteristics of the respondent professionals who were interested in research evaluating OPC administration

Criteria	Description
Job title	Doctors: 64/166 (39%); Nurses: 42/166 (25%)
Years of work	> 20 years: 52/166 (31%); 10-20 years: 41/166 (25%); 5-10 years: 12/166 (7%)
OPC use	Using OPC: 65/166 (39%); not using OPC: 41/166 (25%)

OPC: oropharyngeal colostrum.

2.5 Discussion

2.5.1 Key findings

Of the 267 neonatal professionals surveyed, 166 submitted completed questionnaire giving a 62% response rate, represented 125 neonatal units. The respondent units represented almost all neonatal networks within the UK (Table 2.1); this could enhance the generalisability of the survey findings. Responses were mainly received from NICU and LNUs, whilst, SCUs were poorly represented. In this type of survey, a response rate of 62% is considered a good response (395). It is important to attain the highest achievable response rate to minimise non-response bias in health care surveys, and web-based questionnaires (392, 410). Therefore, this survey was designed to avoid the reported problems of internet surveys. The target population was neonatal professionals who are expected to have easy access to email and are comfortable with this tool (411). To enhance the survey response rate three reminder emails were sent to the targeted

population, and almost all the neonatal units across the UK were approached to participate.

Administration of OPC was used by approximately 40% of UK neonatal units and, this is consistent with the international progressive increase in the use of OPC for the care of preterm infants (353, 355). OPC administration was mostly used by NICUs and LNUs whilst it was less used by the SCUs. This finding was expected as SCUs usually admits babies who can tolerate enteral/oral feeding (404), in contrast to the NICUs and LNUs, that admit more sick infants who cannot tolerate enteral feeding or infants who may tolerate enteral feeding by gastric tube and could have OPC as well. It could also be attributed to less responses from SCUs versus NICUs and LNUs.

2.5.2 Practice of OPC administration in the UK

Three-quarters of the respondent units practising OPC administration used it without written guideline and policy so that 76% of the OPC users had no pre-defined criteria for its use. This finding highlighted the need for the creation of guidelines and continuous evaluation and education.

Based on the responding neonatal units that use OPC, OPC was administered for all gestational age, any birth weight, and regardless of the infant's postnatal age, contrary to previous studies where OPC has mainly been used in extremely preterm and very low birth weight infants within the early postnatal period (353, 356, 412). The previous studies were observational and randomised controlled trials that focused mainly on the impacts of OPC on the health outcomes of preterm infants. However, these studies had small sample sizes and some drawbacks in their methodology, in particular, some were trials not blinded (356, 357). OPC was provided to preterm infants regardless of the clinical status and the infant's feeding regimen which is comparable with other studies as OPC was not given as a part of feeding protocols (355, 357, 413).

There was very likely to be marked variations in which babies were given OPC and in administration methods, as approximately two-thirds of the OPC users had no pre-defined

criteria or guideline for its use. This variability in the practice of OPC administration could result from OPC as a novel intervention recently implemented in UK neonatal units. As reported by the responding neonatal units, only one-third of the respondent units introduced OPC for more than four years whilst the others had introduced if from six months to less than two years. However, variation in neonatal practice is a well-known challenge in perinatal and neonatal care among units, regions and countries (414, 415).

Interestingly, ninety-five per cent of the units using OPC reported that they were not aware of any significant adverse effects with the procedure. This finding is comparable with the results of previous studies that have explored the administration of OPC to preterm infants (< 32 weeks gestation) (353-355, 416-418). Despite, variation in the procedure of OPC administration between these studies (some using the syringe technique described by Rodriguez et al. (352, 417, 419) and others used a swab for OPC application (413)), they have consistently reported no adverse effects related to the OPC procedure. This finding suggests that OPC is potentially a safe intervention, which could be used in the care of preterm infants. However, this study and the previous studies were not designed nor powered to assess the safety of this new intervention. Another important finding in this survey was that 83% of neonatal professionals surveyed reported that administering colostrum by the oropharyngeal route was easy. This clinical survey demonstrated uncertainty about OPC use in UK neonatal units and highlighted the knowledge gap in this specific intervention.

2.5.3 Perception of neonatal professionals towards OPC

The neonatal professionals responding to the survey would highly recommend OPC use as part of the standard care of preterm infants as reported by those surveyed (92% of OPC users highly recommended OPC use for other sites). Similarly, 62% of those who do not use OPC were planning to introduce it in their units. Moreover, they reported that lack of knowledge (46%) and lack of guidelines (28%) were the reasons for not administering colostrum by the oropharyngeal route. These findings highlighted the need for education

and written guidelines and also that who respond are more likely to be engaged with the OPC use.

Despite the lack of high-quality evidence to support the use of OPC in the care of preterm infants, there is a progressive increase in the use of OPC within neonatal units. The progressive increase in the use of OPC, in neonatal units, might be driven by engagement of the neonatal professionals with the protective effects of mother's colostrum and its potential benefits for preterm infants (137, 193, 420, 421) and the suggested feasibility of OPC use in preterm infants especially in those infants who are not able to tolerate enteral feeds (353-355, 358, 422). The focus on improving the health outcomes of preterm infants may also encourage the use of a potentially safe and cost-effective intervention, which does not require any advanced technology.

2.5.4 Strengths and Limitations

To the best of my knowledge, there was no previous study have explored the practice of OPC administration within the UK neonatal units. Besides, no studies have focused on the perceptions, attitudes of neonatal professionals or parents/caregivers towards the use of OPC in preterm infants. Some previous surveys focused on the knowledge, attitude and practice of mothers towards colostrum and breastfeeding (423-426). This survey provided data about the use and perception of OPC by obtaining information from neonatal professionals within the UK neonatal units.

The study covered several aspects of using OPC in the UK, including practices of OPC and the professional knowledge and perceptions toward it, enabling the evaluation of many fundamentals related to the OPC use within neonatal units. Moreover, opinions were sought from both doctors and nurses. In neonatal practice, feeding and OPC administration, and documentation in the infant clinical charts, are often a nursing prerogative, and hence, their opinions about the use of such new intervention would be valuable.

This survey had some limitations, which include that as a survey, the results may indicate self-reported experience and not describe actual practice. The findings of this study reflect the knowledge and attitude of neonatal professionals who participated in the survey and could not necessarily be generalised to all neonatal units in the UK due to a possible nonresponse bias that is expected in any survey study (427). No differences between the respondents and non-respondents were predicted assuming nonresponse was at random as all the surveyed professionals were lead doctors and lead nurses of neonatal units (428). However, the non-respondents may have different practice and perceptions towards OPC administration to preterm infants.

Reporting bias could have emerged as those neonatal units who do not use OPC were more represented among the non-respondents. However, to enhance the response rate, every effort was made in the form of three reminder emails that followed by telephone follow-up. Contrary to interview surveys, as a self-completed questionnaire, potentially biased questions may have been included; however, this should have been minimised by piloting the questionnaire.

Another limitation of the study may have been the identification and verification of the participants' contact details such as error messages were received saying "invalid email address" or the participants are no longer working in the National Health Services (NHS). Moreover, emails may not have reached target participants from either automatic blocking or the survey email received as a junk email or not precedence for some clinicians working to assist patients in very busy neonatal units (390).

Additionally, duplicate responses were received from some of the responded neonatal units with discrepancies between doctors and nurses in answering certain questions that raises concern about the ability of the survey to capture the actual use of OPC within the unit. However, units with discrepancies in specific questions were excluded from the analysis of those questions and reliability analysis was conducted, which showed,

generally, there was a good consensus relating to OPC administration among doctors and nurses from the same neonatal unit.

2.6 Conclusion

This study has shown that:

- OPC has been introduced into UK neonatal practice despite a lack of high-quality evidence regarding its use.
- OPC administration varies among UK neonatal units. OPC was frequently used without written guidelines or policy. The variation in the use of OPC by the UK neonatal units, reported in this survey indicates a need for the development of clinical guidelines and policies to practice OPC administration.
- Administering colostrum by the oropharyngeal route appears to be an easy and practical procedure that is well tolerated by preterm infants. This observation might be reassuring to those neonatal units considering using OPC.
- More research is needed to assess the safety, and efficacy of OPC administration in the care of preterm infants.

Chapter 3. Oropharyngeal colostrum in preventing mortality and morbidity in preterm infants: Cochrane systematic review

3.1 Chapter overview

Based on the findings of a survey of the UK neonatal professionals (Chapter 2), oropharyngeal administration of mother's colostrum (OPC) is increasingly adopted by neonatal units and recommended by neonatal professionals. To bridge the knowledge gap, I systematically reviewed currently available evidence on the use of OPC in the care of preterm infants. This chapter presents a Cochrane systematic review and meta-analysis assessing the available randomised controlled trials (RCTs), which have evaluated the effects of OPC in preventing mortality and other morbidities in preterm infants. The review was published in the Cochrane Database of Systematic Reviews (CDSR) (429).

3.2 Background

3.2.1 Evidence-Based Medicine (EB-Medicine)

Evidence is the information used in making conclusions and can be strong or weak depending on the quantity and quality of the source. EB-Medicine involves the explicit integration of reliable, objective, critically evaluated, high-quality evidence with clinical experience and patient's preferences, by systematically searching the best existing medical and clinical research (430). EB-Medicine aims to improve the quality of healthcare by ensuring health care decisions are taken by incorporating the clinical practices, patients' preferences and their clinical circumstances, societal expectations and the best available evidence from research relevant to the clinical problems (431).

Furthermore, utilising an EB-Medicine approach keeps healthcare professionals up to date with the growing medical research. The practice of EB-Medicine follows basic steps (430, 432):

- translating clinical issues into answerable questions,
- finding the best evidence,
- critically appraising the available evidence for internal and external validities,
- assessing the applicability of the results, making decisions and incorporating into practice,
- evaluating the performance of the applied strategies regularly (433).

3.2.2 Systematic reviews

Health-care professionals commonly use review articles as a summary of evidence for a specific medical topic (434). There are two types of literature reviews; narrative and systematic. Narrative reviews are generally subjective, deal with an overview of a particular topic, and frequently have no predefined inclusion criteria for studies selection, and they typically do not explicitly describe their methodology. Therefore, they have a high risk of bias (435).

Systematic reviews are a form of secondary analysis that focuses on a specific question, use predefined inclusion criteria, and precise, structured methods for searching and critically appraising primary studies for the review question, resulting in a synthesised summary of the available literature (435). They, therefore, produce findings that are more objective by evaluating the consistency and generalisability of the studies, which could not be apparent in individual research or narrative literature reviews.

Moreover, systematic reviews often include quantitative meta-analysis, which limits bias and may improve the power and precision of conclusions (436). Systematic reviews can be conducted to answer questions related to healthcare issues, such as interventions, prevention, diagnosis, adverse effects and diagnostic tests, and may also identify a new hypothesis, suggest further research and resolve conflicting bodies of evidence (437). The

primary objective of the systematic review is to help people decide about a specific issue (438); therefore, reviewers should consider who will use the results of the intervention studied. There are essential steps for conducting systematic reviews including the followings (439):

- A focused review question should be defined.
- A comprehensive search of available databases.
- Selection of primary studies.
- Data extraction and analysis.
- Assessment of studies' quality.
- Synthesis of the results of included studies.
- Interpretation of the results and reporting.

Systematic reviews have been criticised because of potential publication bias (publication of research with positive results and non-publication of trials with negative or null results), reporting bias (selective reporting of outcomes with favourable results), validity of the studies and knowing the findings of potential studies (440). Conclusions of systematic reviews thus need to be cautiously interpreted and integrated with practice (441).

3.2.3 Randomised controlled trials

There are many designs used to conduct clinical research, such as RCTs, non-RCTs, quasi-control, cross-sectional and longitudinal observational studies. These designs differ in their power to investigate the effectiveness of interventions. A principle of EB-Medicine, therefore, is to rank studies at different strengths according to their design in a "hierarchy of evidence" (442). Since the introduction of EB-Medicine, several versions of the hierarchy of evidence have been described (443, 444), and most of them indicate which study designs should be more potent in answering a research question but there is no universal standard hierarchy (445). RCTs and systematic reviews and meta-analysis were placed at the top of the pyramid (445, 446). Although hierarchy of evidence, for instance, the Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence (447), presents

a useful tool to find the most rigorous evidence for many clinical questions, the definitive assessments of the quality of the evidence are not provided. It has been argued that using hierarchy overlooked the potential risk of bias in RCTs and systematic reviews (448). It might also reduce the use of judgment by systematic review authors (449). Therefore, critical appraisal of the evidence is essential to evaluate the validity and strength of recommendation of evidence when making decisions. Evidence should not be thought valid because it is a systematic review of RCTs but appraising the systematic review can be considered specifically for issues related to the validity of the review studies, the size and precision of the effect of the assessed intervention and the applicability of the findings (435).

RCTs are considered the gold standard design for healthcare interventions (435). However, RCTs are also prone to bias in the methodology, during analysis and reporting of the trials (450), such as failure to conceal allocation and blind, loss to follow-up, inappropriate consideration of the intention-to-treat principle, stopping early for benefit and selective reporting of outcomes according to the results. Nevertheless, observational studies were placed down in the hierarchy of evidence; these study designs can be more appropriate for evaluating potential adverse effects, long-term outcomes and diagnostic tests (451)

3.2.4 Meta-analysis

Meta-analysis is a statistical method used to combine data (considered to be combinable) from different studies for synthesising estimates of outcomes. The data are pooled quantitatively and reanalysed using a specific statistical method with the aim of producing results that provide more consistent evidence from across numerous studies (452). Meta-analysis has the potential for increasing the statistical power, improving precision, answering questions not proposed by individual studies and resolving disagreements from conflicting statements (453). In meta-analysis, the effect of an intervention is reported as a point estimate and its 95% confidence intervals (CI) along with the exact P value for each

study included (453), as they are helpful for assessing the clinical usefulness of intervention (454). Meta-analysis estimates the magnitude of the effect of an intervention, establishes the direction of the effect and investigates the consistency of the effect across studies but does not provide information concerning the strength of evidence (435, 453). The Forest plot provides a visual presentation of the data that being pooled into the analysis, an overall summary estimate of the results, the degree of variability across studies and the risk of bias in each study (455). Whilst, meta-analysis is increasingly used to present health care evidence, meta-analyses of separate studies can be misleading, especially if within study bias and reporting biases are not carefully considered (456). Therefore, reviewers should be transparent about the main question that the trials are addressing.

3.2.5 Cochrane systematic reviews (CSRs)

The Cochrane Collaboration was founded in 1993 as a collaborative centre with the aims of creating and maintaining a database of up-to-date systematic reviews of RCTs of health care interventions that can be accessed through electronic media (457, 458). It was titled in honour of the British epidemiologist Archibald Cochrane who endorsed the importance of RCTs as evidence for medicine collaboration in 1979: “It is surely a great criticism of our profession that we have not organised a critical summary, by speciality and subspecialty, adapted periodically, of all relevant randomised controlled trials” (459).

CSRs are acknowledged as one of the best sources for healthcare professionals to obtain evidence in an accessible and robust format for practice in medicine (460, 461). Therefore, they primarily focus on the search of RCTs of the effects of interventions (or diagnostic test accuracy) (462), as RCTs are more likely to yield unbiased results about the effects of interventions than other review methods (463). Non-randomised studies can be included especially if RCTs are not available and if evidence could not be obtained from RCTs, such as rare and long-term outcomes, or in consideration of the safety of intervention (464).

CSRs use a standard, rigorous method to reduce bias to provide the best and most current evidence to guide decision-making (463, 465). Cochrane adopted the approach developed by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group (443, 466) to transparently assess and classify the quality of evidence (467). GRADE is an organised and transparent process for creating and reporting summaries of evidence for systematic reviews and recommendations in healthcare and is perceived as the most effective approach that links assessments of the quality of evidence to clinical recommendations (467). It differentiates between the quality of evidence and the strength of a recommendation in practice. The GRADE approach has been adopted by many organisations such as the World Health Organization (WHO), American College of Physicians, British Medical Journal (BMJ) Clinical Evidence and the UK National Institutes of Health and Care Excellence (443, 468, 469). The evidence is then combined and analysed to provide robust, explicit recommendations that can be appropriately used to inform clinical practice.

Moreover, CSRs are maintained in the CDSR within the Cochrane Library and regularly updated to monitor the emergence of new evidence (470). This electronic publication facilitates search and contribution across the world. CSRs can also assist with patient care in places which are resource poor and cannot undertake full, robust evidence reviews because of lack of expertise and access to medical journals. Free access is available for users from countries classified as low-or middle-income by the World Bank (460).

CSRs have been criticised for often being inconclusive (471); however, such reviews can still be beneficial by highlighting areas of research where further studies are required. Others argue that CSRs can under-estimate reporting bias, which is influenced by their conclusion (472). However, Cochrane regularly updates its methodology to ensure that CSRs are a high-quality source of evidence (473). Another criticism is that review titles are added according to the preference of the reviewers not based on the needs of public

health. Nevertheless, review authors are divers in term of being worldwide volunteers from different backgrounds including health professionals, researchers and consumers (460).

3.2.5.1 Process for conducting CSRs

The process of conducting CSR starts at registration of the title to prevent duplication and ensure relevance and practicability of the proposed question to health care. A well-formulated review question will also direct other stages of the review process, such as specifying eligibility criteria, planning the search strategy to search for studies, defining and collecting data (474). Registration is followed by the preparation of the protocol, which is submitted for peer review and publication. Authors then start searching and analysing the results. Next authors are draft review and submit it for peer review and publication. The published review should be periodically updated.

3.2.5.2 Methodology of CSRs

Cochrane has developed a standard method for conducting systematic reviews (463). CSRs have a uniform structured format to help readers to find the results of research rapidly and to evaluate the validity, applicability and implications of the finding, ensure explicit and concise reporting of the reviews and minimise reviewers' effort, facilitates electronic publication and regular update of reviews and allows the conductions of overviews reviews (475). Review Manager (RevMan) (476) is a required software to use when preparing protocols and conducting reviews (475). In 2016, Cochrane introduced updated standards for conducting and reporting CSRs (Methodological Expectations of Cochrane Intervention Review (MECIR)) (473) to ensure transparency in interpretation and representation of the reviews at the highest possible quality, which is crucial to inform clinical practice and health policy decisions. The CSRs are conducted following fundamental steps; further details of the steps are given in Section 3.3 and Table 3.2

3.2.6 Rationale of the review

OPC in the first few days of life is a new intervention that it has been proposed as a route to deliver the benefits of colostrum to preterm infants (339, 413). OPC being widely introduced as it may offer potential benefits which may, or may not, outweigh the extra work that OPC administration requires (360, 361, 396, 397). Further discussion of OPC is given in Section 1.14.

A systematic review of the evidence, to identify benefits and harms, might be useful evidence before recommendations can be made for, or against, OPC. This review was the first Cochrane review evaluating OPC use in preterm infants. The proposed review question was assessed by the Cochrane Neonatal Group (CNG) to identify duplication and overlap with other systematic reviews. The importance and priorities of the review question were based on the global burden of diseases. There is one systematic review (413) that reported the safety and feasibility of OPC with unclear effects on the health outcomes of preterm infants. However, the previous review included studies with different study designs; RCTs, observational, cross-sectional and longitudinal studies and clinical audit. Additionally it was a qualitative analysis and did not involve a quantitative meta-analysis (413). The review presented in this chapter included only RCTs, the gold standard for clinical research and in E-B-M (477) with recognition of the limitations and disadvantages of RCTs (442, 448).

This Cochrane review was conducted to collate the existing evidence to assess whether early OPC safely prevents mortality and morbidity in preterm infants. It was anticipated that in the presence of sufficient evidence, an evidence-based recommendation could be made for the use of OPC in preterm infants during the neonatal period.

The review question was, therefore “is OPC compared to controls, effective in preventing mortality and morbidity and improving outcomes for preterm infants?”

3.2.7 Objectives of the review

3.2.7.1 Primary objective

To evaluate the effect of early (during the first 48 hours of life) oropharyngeal administration of mother's own colostrum on morbidities, including NEC, late-onset invasive infection and mortality in preterm infants compared to control.

3.2.7.2 Secondary objectives

To assess studies for evidence of safety and harm such as aspiration pneumonia.

To compare the effects of early OPC versus no OPC, placebo, late OPC (after 48 hours of life), and nasogastric colostrum.

3.3 Methods

This review followed the MECIR and the guidance of the CNG (478). Dr Amna Widad Nasuf (AN, author of this thesis) was the principal reviewer; Dr Shalini Ojha (SO) and Dr Jon Dorling (JD) were co-reviewers.

Before conducting the systematic review, a review protocol was written by AN and edited by SO and JD. The review protocol was published in the CDSR (479). Publication of the protocol before undertaking the review reduces the effect of authors' biases and the potential for duplication. Additionally, an electronic publication of the protocol in the CDSR (480), enables users to forward their comments, permits peer review of the planned methods and enhances transparency (481).

3.3.1 Eligibility criteria

Studies have only been included if they met the pre-set criteria and measured at least one of the pre-specified outcomes.

3.3.1.1 Types of studies

Published RCTs where the unit of randomisation was the infant or cluster randomised trials where the neonatal unit was the unit of randomisation were considered for this review. Quasi-randomised or non-randomised trials such as controlled before and after studies were excluded. The review was not limited to any particular region or language. This review also included unpublished data (as recommended by Cochrane) to reduce publication bias which has an important influence on the validity of the review (440). Whilst, the inclusion of unpublished data may introduce bias (data are not peer reviewed), it has been widely supported by many of review authors (482) and journal editors (483).

3.3.1.2 Participants

Trials were only considered if they enrolled preterm infants (less than 37 weeks' gestation) receiving care in any neonatal unit.

3.3.1.3 Interventions

Studies were included if they involved OPC to preterm infants in the first 48 hours of life. OPC usually involves the instillation of a small amount of colostrum (0.1 to 0.5 ml) inside the cheeks of the infant by oral syringe or using a sterile swab soaked with colostrum (352). The procedure was usually given every two to three hours within the first 48 hours of life. This review considered trials that used OPC by any regimen and technique such as, instillation by a syringe, direct application to the oral mucosa by swab or any other ways such that the fluid is absorbed by the oral mucosa. OPC procedures could also be described by different terms such as oral care, oral swabbing oral colostrum, oromucosal route, oropharyngeal or/and oral immune therapy.

The following interventions were included:

- Administration of fresh or frozen/thawed OPC to preterm infants in the first 48 hours of life, irrespective of when enteral feeding is introduced, type of milk or feed advancement regimen is used for enteral feeding.

- Colostrum instillation inside the infant' s cheeks by oral syringe or by gentle application over the tongue, around the gums, and along the lips using a swab or sponge soaked with a small amount of colostrum (0.1 to 0.5 mL), at least once and usually repeatedly in the first 48 hours of life.
- Any procedure for OPC administration by which colostrum could be absorbed by the oral mucosa.

3.3.1.4 Comparison

This review considered trials, comparing early OPC versus sham administration of water, oral formula, or donor breastmilk, or no intervention. Trials comparing OPC versus nasogastric or nasojejunal administration of colostrum were also considered.

The following comparisons were planned:

- Early OPC, defined as OPC commenced before 48 hours of age, versus sham administration of water, oral formula, donor breast milk, or no intervention.
- Early OPC versus early colostrum administration by nasogastric (NGT) or nasojejunal (NJT).
- Early OPC versus late OPC, defined as OPC commenced after 48 hours of age.

However, the review search did not retrieve any study that compared early OPC versus colostrum administration by NGT/NJT, nor versus late OPC, therefore this review only compared early OPC versus sham administration of water, normal saline, oral formula, donor breast milk, or no intervention.

3.3.2 Outcome measures

3.3.2.1 Primary outcomes

- Incidence of NEC (Bell's stage 2 or 3 (167)) until discharge to home.

- Incidence of microbiologically confirmed LOI until discharge to home with LOI defined as positive blood or cerebrospinal fluid culture for microbial infection after 72 hours of life (174).
- Death before discharge to home.

3.3.2.2 Secondary outcomes

- Days to full enteral feeds.
- Length of hospital stay (days) from birth to discharge home.
- Pneumonia (defined as chest X-ray changes/treated with at least five days of antibiotics) before discharge to home.
- Formally reported adverse effects (e.g. aspiration, gagging/choking on administration, bradycardia, desaturation, increase in oxygen requirement, disturbances in vital signs) between the start of the intervention and discharge home.
- Chronic lung disease (defined as the need for oxygen supplementation at 36 weeks' postmenstrual age).
- Retinopathy of prematurity (all stages and severe stage > 2).
- Weight gain from birth to discharge home (using weight percentiles or Z-scores) and time to regain birth weight.
- Days of parenteral nutrition before discharge to home.
- Days of antibiotic therapy before discharge to home.
- Rate of receiving any breast milk at discharge to home.
- Rate of receiving only breast milk (and not formula) at discharge to home.
- Death in the first year of life.
- Neurodevelopmental outcome at 18 to 24 months assessed by a clinician or parent-reported questionnaire.

3.3.3 Search methods for identification of studies

3.3.3.1 Electronic searches

The criteria and standard methods of the CNG were used for the search strategy (484). The first search was conducted in March 2015, repeated in September 2015 and April 2016. NA updated the search in August 2017.

A comprehensive search was conducted using bibliographic databases, which are related to health care: Cochrane Controlled Register of Trials (CENTRAL) 2017, Issue 8 in the Cochrane Library; Medical Literature Analysis and Retrieval System Online (MEDLINE) via PubMed (1966 to August, 2017); Excerpta Medica Database (EMBASE) (1980 to August, 2017); and Cumulative Index to Nursing and Allied Health Literature (CINAHL) (1982 to August, 2017) using the following search terms: (colostrum, oropharyngeal*colostrum, oral*care), plus database-specific limiters for RCTs and neonates (Appendix 5). Clinical trials registries were also searched for ongoing or recently completed trials (clinicaltrials.gov, the World Health Organization's International Trials Registry Platform and the International Standard Randomised Controlled Trial Number).

3.3.3.2 Searching other resources

An additional search was conducted using the proceedings of the annual meetings of the Paediatric Academic Societies (1993 to 2017), the European Society for Paediatric Research (1995 to 2017), the Royal College of Paediatrics and Child Health (2000 to 2017), the Perinatal Society of Australia and New Zealand (2000 to 2017) and the National Association of Neonatal Nurses. The reference lists of the included studies and published reviews, which are usually appropriate sources (485), were also used for finding relevant studies.

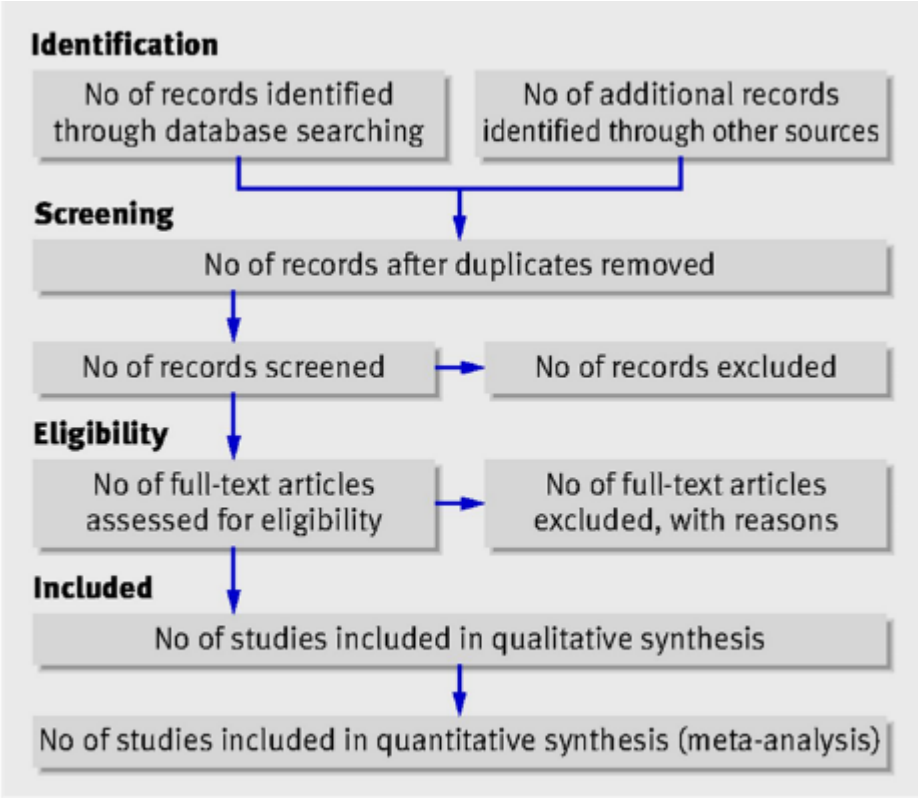
Trials reported as abstracts only were eligible if sufficient information to fulfil the inclusion criteria was available from the abstracts, or their authors. Authors of completed and unpublished trials were contacted to provide additional information.

3.3.4 Data collection and analysis

3.3.4.1 Selection of studies

The standard process recommended by the Cochrane Handbook was followed (Chapter 7 (486)). To ensure transparency and enhance detection of errors, two reviewers (AN and SO) independently screened the title and abstract of all articles retrieved through the above search. Studies, which did not consider early OPC and those not described as RCTs were excluded (Table 4.1).

AN and SO independently assessed the full text of potential articles selected by the principal reviewer (AN) to determine which studies were eligible for inclusion and consideration of duplicate reporting of the same trial. As studies might be reported in different articles or abstracts, a review search may retrieve several reports for potentially relevant studies. Therefore, identification of duplicate publications of the same study is a vital step in selecting studies for inclusion in the review; as inadvertent multiple inclusion of studies can introduce significant bias in the meta-analysis (487). Duplicate reports were identified using the name of the authors, numbers of participants, date, duration and setting of the study, intervention details and baseline characteristics of participants. Any disagreements were settled by discussion until consensus was reached and with adjudication as needed by the third author (JD). The process of selecting eligible studies was presented using the Preferred Reporting Items for Systematic reviews and Meta-analysis (PRISMA) flow diagram (488), Figure 3.1.



David Moher et al. BMJ 2009;339:bmj.b2535

Figure 3.1 Preferred Reporting Items for Systematic reviews and Meta-analysis (488)

3.3.4.2 Data extraction and management

Data were extracted independently by AN and SO, and compared. Discrepancies were resolved through discussion and by involving JD. NA modified the Cochrane data collection form template based on the review eligibility criteria and outcome measures.

The following data were extracted from each study:

- Study ID, trial authors and their contact details.
- Method of the research (design, duration of the trial, the setting of the trial, sequence generation, allocation concealment, blinding).
- Participants (total number, gestational age, sex, country, socioeconomic & ethnic group, diagnosis and status).

- Intervention (number, time, technique, dose and duration, any additional interventions).
- Outcomes (time of outcome, reporting method, effect size).

The study authors were contacted for clarification of unclear data and any additional information when necessary.

AN entered the collected data into the RevMan software version 5.3 (476) which is the software mandated by the Cochrane Collaboration for reviews under its protocols. The included studies were presented as a 'characteristics of included studies' table.

3.3.5 Assessment of risk of bias in included studies

As data from studies vitally influence the finding of a systematic review, assessment of the validity of included studies is a fundamental component of a CSR. There are two types of validity, external and internal. External validity refers to generalisability and applicability of the study results in other populations. Internal validity refers to the confidence in any causal associations between the variables and is determined by how the study minimises systematic bias (489). Risk of bias (ROB) assessment strengthens the relationship between the features of the study design and their potential impact on the results of the trial. The Cochrane 'Risk of Bias Assessment Tool' (490) was used to evaluate the methodology and ROB of the included studies; the tool has been implemented in RevMan software. AN and SO separately assessed the ROB for all included studies. Any disagreement was resolved by discussion or by consultation with JD.

For each study, the following sources of bias and their related domains were evaluated and presented in the 'Characteristics of included studies' table:

- Selection bias (Random sequence generation and Allocation concealment).
- Performance bias (Blinding of participants and care providers).
- Detection bias (Blinding of outcome assessors); the methods used to blind outcome assessors from knowledge of which intervention a participant received.

- Attrition bias (Incomplete outcome data assessment through withdrawals, dropouts, or protocol deviations). Completeness was classified according to the percentage of missed data.
- Reporting bias (Selective outcome reporting).
- Other biases, any important concerns about other possible sources of bias that could put it at high risk of bias were defined (for example, whether there was a potential source of bias related to the specific study design or whether the trial was stopped early due to some data-dependent process).

Within a study, each domain was assessed as low (bias unlikely to modify the results), high (a bias that reduces confidence in the results), or unclear risk of bias (a bias that makes some concern on the results due to lack of information or uncertainty). This was achieved by precise judgment depending on what has been described in the study report as detailed in Table 3.1. The possible extent and direction of the bias and its potential impact on the results were considered. Sensitivity analysis has been planned to explore the impact of the level of bias if needed.

Table 3.1 Cochrane's Risk of Bias tool (adapted from Higgins 2017 (490))

Domain	Low risk	High risk	Unclear risk
Selection bias - Random sequence generation - Allocation concealment	any truly random process, e.g. random number table; computer random number generator telephone or central randomisation; consecutively numbered sealed opaque envelopes	any non-random process, e.g. odd or even date of birth; hospital or clinic record number using open random allocation; unsealed or non-opaque envelopes, alternation; date of birth	No sufficient description to judge
Performance bias - Blinding of participants and personnel	effective blinding	not blinded trials	No sufficient description to judge
Detection bias - Blinding of outcome assessors	effective blinding	not blinded trials	No sufficient description to judge
Attrition bias - Incomplete outcome data	<10% missing data	>10% missing data	No sufficient information reported nor provided by the author
Reporting bias	All of the study's pre-specified outcomes and all expected outcomes of interest to the review were reported	not all the study's pre-specified outcomes were reported. The study fails to include results of a key outcome that would have been expected to be reported	No sufficient information to make a judgment
Other bias	any concerns possible sources of bias not covered above	no other bias	No sufficient information to make a judgement

3.3.6 Data analysis and management

RevMan 5.3 software (491) was used for data analysis. Meta-analysis, which is an essential step in CSRs, was conducted using the fixed-effect model that assumes a common, true effect in a set of studies, and estimates the best effect for an intervention (492). While, random effects modelling was used when there was moderate or high heterogeneity (I^2>50%) between the included studies, this model assumes that the true effect is variable between the studies and estimated the average effect (453).

3.3.6.1 Measures of treatment effect

Effect estimates were calculated using risk ratios (RR) for dichotomous data and mean differences (MD) for continuous data, with respective 95% CI along with the exact p values. When continuous data were reported as median and range or interquartile range, trial' authors were contacted to provide the mean and standard deviation (SD), and if not provided, the mean and SDs were estimated using a formula (interquartile range (IQR) = approximately 1.35 of the SD) recommended by the Cochrane Handbook (Chapter 7.7.3.5 (486)). When it was considered applicable to combine two arms of a trial, treatment effects were obtained from the combined data using the RevMan calculator (493). Forest plots were used for graphical presentation of meta-analysis results and the area to the left of the line of no effect was in favour of OPC.

3.3.6.2 Unit of analysis issues

The unit of analysis was the participating infant in each of the included trials. An infant was considered only once in an analysis. For cluster RCTs, it was planned that the participating neonatal unit or a section of the neonatal unit would be the unit of analysis. However, no cluster randomised trial was identified for this review.

3.3.6.3 Dealing with missing data

The principle reviewer (AN) contacted trial investigators to request essential missing data in the outcomes or unclear data. Intention-to-treat analyses were conducted.

3.3.6.4 Assessment of heterogeneity

Heterogeneity between effect sizes of the included studies was determined by inspecting the forest plot (overlapping of the studies CI), the Chi^2 test (with a P value of < 0.1) and the I^2 for heterogeneity. The percentage of the variability in effect estimates was used to describe inconsistency between trials that was due to heterogeneity rather than due to chance in accordance with the guidelines recommended by the CNG for interpreting the I^2 statistic: < 25% = none, 25% to 49% = low, 50% to 74% = moderate, and > 75% = high heterogeneity. If moderate or high heterogeneity was detected (I^2 > 50%), potential causes (for example, differences in study design, participants, interventions and definitions and measurement of outcome assessments) were explored in subgroup and sensitivity analyses.

3.3.6.5 Assessment of reporting biases

Assessment of potential reporting bias using funnel plotting was planned. However this was not conducted as only six trials were included in the review as a minimum of 10 studies are required for the funnel plot to be valid and could detect asymmetry (494, 495).

3.3.6.6 Subgroup analysis

If sufficient data were available, the following subgroup analyses were planned to assess the intervention in specific participant groups:

- Infants born < 30 weeks' gestation.
- Infants born < 1500 grams.
- Infants who were small for gestational age at birth (birth weight less than 10th centile).

However, subgroup analysis was not performed as the gestational age, and birth weights of the participants were matched between the included studies. Moreover, the outcomes were not reported in sufficient detail, and they were not available on request.

3.3.6.7 Sensitivity analysis

Sensitivity analyses were planned to determine if findings were affected by including only studies using adequate methods (low risk of bias).

3.3.7 Assessing the Quality of evidence

The quality of evidence for the main comparison at the outcome level was evaluated according to the GRADE approach (467) using the online version (GRADEpro GDT) software (www.gradepro.org). GRADEpro software has the advantages to import data directly from RevMan, assisting in calculating relative and absolute risks related to the examined intervention and producing a table in a format which can directly be imported into RevMan as a Summary of finding (SOF) table (496), this software can save time and ensure consistency.

RCTs were considered as high quality that can be downgraded based on five categories: risk of bias within and across studies (type of evidence), inconsistency (heterogeneity), indirectness (applicability and generalisability), imprecision of the estimates of effect (sample size, number of events and 95% CI) and publication bias (positive studies, profit interest).

For each outcome, every category was assessed as not serious, serious and very serious depending on the characteristics of the studies reported that outcome. The quality of evidence was downgraded by one level for serious concern and two level for very serious and graded according to the GRADE approach as one of the following four grades (496, 497):

- “High: We are very confident that the true effect lies close to that of the estimate of the effect”.
- “Moderate: We are moderately confident in the effect estimate. The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different”.

- “Low: Confidence in the effect estimate is limited. The true effect may be substantially different from the estimate of the effect”.
- “Very Low: We have very little confidence in the effect estimate. The true effect is likely to be substantially different from the estimate of effect”.

AN and SO independently assessed the quality of the evidence and AN created the final SoF table for outcomes rated as critical or important for clinical decision making. Any disagreement was resolved by discussion with JD. According to the policies of CNG, a maximum of seven outcomes to be included in the SoF table, therefore, the following clinically critical and important outcomes were included in the SoF table (Table 3.3)

- Incidence of NEC (Bell stage 2 or 3) until discharge to home.
- Incidence of LOI until discharge to home.
- Death before discharge to home.
- Time to full enteral feed.
- Length of hospital stay (days) from birth to discharge to home.
- Pneumonia.
- Reported adverse effects.

If the ROB was arising from inadequacies in allocation concealment, assignment randomisation, completeness of follow-up or outcome assessment blinding such that confidence in the effect estimates was reduced, the quality of evidence was downgraded accordingly (498). The directness of evidence was judged by the applicability of the evidence to the review question not to the generalisability of the evidence (499). Consistency was assessed by the similarity of point estimates, the extent of overlap of confidence intervals of the studies and statistical measurement of heterogeneity (I^2). The quality of evidence was downgraded when inconsistency across study results was large (I^2 >50%) and unexplained (i.e. some studies suggest important benefit and others no

effect without a clinical explanation; (500). Precision was assessed by the sample size, number of events and with the 95% CI around the pooled estimation (501).

3.4 Results

3.4.1 Search results

The search strategy retrieved 287 records from the database and 29 additional records from the clinical trials registers (Figure 3.2). After duplicates were excluded, screening of the titles and abstracts of 294 articles and after exclusion of clearly irrelevant titles, 14 articles using OPC in preterm infants for potential inclusion were retrieved.

On further reviewing of full reports of the potential 14 trials, two papers were excluded as they were duplicate publications of trials included from other publications with the most data (McFadden 2012 (502) and Rodriguez 2011 (417)). Two studies were excluded; Lee 2015 (356) and Zhang 2017 (419) (Table 3.2) because the intervention (OPC) was started after 48 hours of life which is not consistent with the pre-defined inclusion criteria of the review (Section, 3.5.1). One study, Rodriguez 2015 (352) was a published protocol of an ongoing trial, and one record was an uncompleted clinical trial (503), both were classified under ongoing studies. Another study was published in Spanish and after translation to English, was excluded because it was a non-RCT (504) and one was a feasibility study for OPC (422) (Figure 3.2). Six trials fulfilled the inclusion criteria of the review protocol.

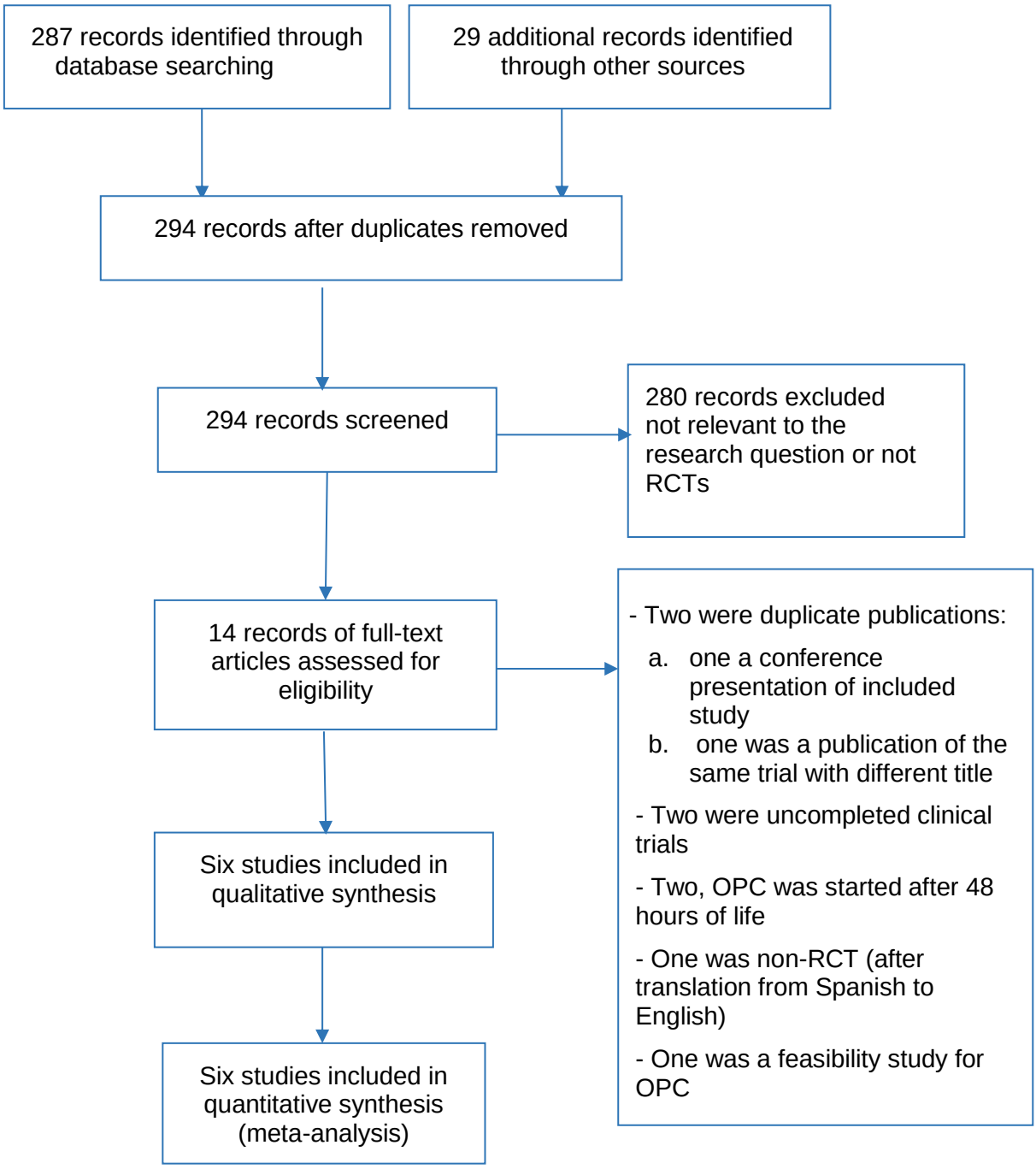


Figure 3.2 Study flow chart

Flow chart illustrating selection of studies included in this review.
RCTs: randomised controlled trials; OPC: oropharyngeal colostrum.

3.4.2 Included studies

Six original RCTs were eligible for inclusion in this review and data extraction (Rodriguez 2011(417); McFadden 2012 (502); Sohn 2015 (357); Romano-Keeler 2016 (358); Mota-Ferreira 2016 (505) (referred in the published review: NCT02912585); Glass 2017 (506)).

All included studies compared the administration of early oropharyngeal colostrum versus sham administration of water, placebo, or donor breast milk, or no intervention. Five studies were published, and Mota Ferreira 2016 (505) was only described in an unpublished report that we obtained from the study author. Five of the included studies took place in the USA and one in Brazil. Individual preterm infants were the unit of randomisation in all of the included studies as no cluster-randomised trials were identified. Four of the studies; McFadden 2012 (502), Sohn 2015 (357), Romano-Keeler 2016 (358), and Glass 2017 (506) were designated as not blinded and only two trials; Rodriguez 2011 (417) and Mota-Ferreira 2016 (505), were described as blinded RCTs. Table 3.2 presents the features of the included studies.

Table 3.2 Characteristics of included studies

Study ID	**Rodriguez 2011 (417)**
Methods	A blinded, placebo-controlled, randomised controlled trial. Setting: Level III neonatal unit, NorthShore University Hospital, Chicago, USA. January 2006 to August 2007
Participants	Sixteen infants (9 intervention, 7 control). Inclusion criteria: birth weight <1000 gm and/or gestation < 28 weeks; appropriate weight for gestational age Exclusion criteria: presence of congenital anomalies, gastrointestinal or renal disorders, receipt of vasopressor medications at a dosage >10 mcg/kg/min, maternal chorioamnionitis, history of substance abuse, positive HIV.
Interventions	0.2 ml of Own Mother Colostrum (OMC) or sterile water (placebo) according to the infant's group assignment. Using a syringe 0.1 ml was administrated by placing the tip of the syringe into the infant's mouth, alongside the right buccal cavity, and directing it posteriorly towards the oropharynx over a period of at least two minutes, then on the left side. The procedure was started within 48 hours of life, every two hours over 48 consecutive hours.
Outcomes	Primary outcomes: level secretory immunoglobulin A, Lactoferrin, and interleukin-10. Secondary outcomes: NEC*, days to full enteral feeds, days to full per oral feeds, length of hospital stay, bacteraemia, pneumonia, CLD*, Retinopathy of prematurity (ROP)**, Corrected gestational age at discharge and death.
Notes	The study protocol is not available. * Diagnostic criteria not specified; ** Data provided by the author.

Study ID	McFadden 2012 (502)
Methods	A prospective randomised trial. Not blinded Setting: NICU, The Woman's Hospital of Texas, USA. August 2011 to January 2012
Participants	Twenty-nine infants (11 intervention and 18 control). Inclusion criteria: gestational age 26 to 34 weeks, Intubation and mechanical ventilation, or support with nasal continuous positive pressure (CPAP). Exclusion criteria: age > 24 hours, major congenital anomalies, infants diagnosed with an infection in the first 24 hours of life or born to mothers with active infection, parental refusal, mothers not speaking English and mothers not wishing to breastfeed.
Interventions	Oral care: moisten a swab with sterile water (control A), normal saline (control B) or colostrum/human milk (intervention). Gently swirl swab along inside of mouth - wiping cheeks, tongue, palate and lips. Oral care was administrated every 3 to 6 hours or more often as indicated.
Outcomes	Primary outcomes: oral colonisation (oral culture) and time to oral colonisation. Secondary outcomes: ventilator-associated pneumonia (VAP), NEC*; days of antibiotics; days to reach full enteral feeds; length of hospital stay; length of time on ventilation, NCPAP and CLD*
Notes	No protocol was available. * diagnostic criteria not specified
Study ID	**Sohn 2015 (357)**
Methods	A randomised controlled clinical trial. Not blinded. Setting: NICU, University of California Davis Children's Hospital in Sacramento, California, USA. November 2013 to October 2014
Participants	Twelve infants (6 intervention and 6 control). Inclusion criteria; birth weight < 1500g, aged under seven days, intubated within 48h of birth and maternal colostrum available. Exclusion criteria; a lethal medical condition.
Interventions	0.2 ml of the mother's colostrum via sterile syringe into the baby's oral cavity (0.1 ml into each buccal pouch) every two hours for 46 hours. The comparison group received routine care.
Outcomes	Primary outcomes: oral microbiota and VAP Secondary outcomes: ventilator days, days of antibiotics, age at first feeding (days), days to full feeds; NEC (stage 2, 3), early and late-onset sepsis, other pneumonia; CLD and death.
Notes	Registered at clinicaltrials.gov: (NCT02306980).

Study ID	Romano-Keeler 2106 (358)
Methods	An open-label, prospective randomised clinical trial. Setting: NICU, Monroe Carell Jr. Children's Hospital at Vanderbilt, USA. February 2013 to July 2014.
Participants	Ninety-nine infants (48 intervention and 51 control). Inclusion criteria: gestational age <32 weeks Exclusion criteria: refusal to participate, enrolment in competing studies or Spanish-speaking only.
Interventions	Oral priming with mother's colostrum that involved administration of 0.1 mL colostrum to each cheek every 6 hours for five days started in the first 48 hours of life. Comparison group; no oral priming with mother's colostrum.
Outcomes	Primary outcomes; salivary immuno-peptides before/after oral colostrum priming. Oral microbiota in a subgroup. Secondary outcomes; length of hospital stay, total days intubated, age at feeding initiation, days to 100ml/kg/day of enteral feeds, days of antimicrobial exposure, incidence of NEC and late-onset bacteraemia; type of feed at hospital discharge.
Notes	Registered at clinicaltrials.gov: NCT01776268
Study ID	**Mota Ferreira (NCT02912585)* (505)**
Methods	A double-blinded, randomised, placebo-controlled trial. Setting: NICU, Clinics Hospital of Federal University of Uberlandia, Brazil. From 15 July 2013 to 15 July 2015.
Participants	One hundred forty-nine infants (81 intervention and 68 control). Inclusion criteria: birth weight < 1500 g; gestational age < 34 weeks. Exclusion criteria: congenital anomalies; gastrointestinal disorders; maternal history of substance abuse; positive HIV status.
Interventions	OPC (interaction) and placebo (sterile water). They followed the same protocol used by Rodriguez et al. that is; "0.2 ml of Own Mother Colostrum (OMC) or sterile water (placebo) according to the infant's group assignment. Using a syringe 0.1 ml was administrated by placing the tip of the syringe into the infant's mouth, alongside the right buccal cavity, and directing it posteriorly towards the oropharynx over a period of at least two minutes this was repeated on the left side and carried out every two hours over for 48 consecutive hours". The procedure was started within 48-72 hours of life.
Outcomes	Primary outcomes: Incidence of late-onset sepsis and serum and urinary IgA levels.

	Secondary outcomes: NEC (Bell's stage 2 or 3); Bronchopulmonary dysplasia (diagnostic criteria not specified); ROP (grade 3); length of hospital stay; death before discharge
Notes	Registered at clinicaltrials.gov: NCT02912585 Unpublished data. The investigator has provided the results of the study at request. ***** study ID in the published review
Study ID	**Glass 2017 (506)**
Methods	Open-label, placebo-controlled, randomised study. Setting: NICU, Penn State Milton S. Hershey Medical Center, Hershey, Pennsylvania, USA. January 2011 to January 2016.
Participants	Thirty infants (17 interventions and 13 control). Inclusion criteria: birth weight <1500gm, mothers planning to provide colostrum. Exclusion criteria: major congenital anomalies or chromosomal syndromes incompatible with life, mothers not willing to provide colostrum for their infant in the first week of life, or infants of mothers with known HIV, hepatitis B, or hepatitis C
Interventions	“Oral care with either mother’s own colostrum (intervention) or sterile water (control) every 3 hours from day of life two until 7. For the oral care procedure, 0.2 mL of mother’s colostrum or sterile water was applied to the oral mucosa by an intensive care nurse using a cotton-tipped applicator every 3 hours during care times”.
Outcomes	Primary outcomes: change in salivary secretory Ig-A concentration from baseline to 2 weeks of age Secondary outcomes: Incidence and severity of NEC; culture-positive sepsis; feeding tolerance; days of the first enteral feeding; time to full enteral feedings (defined as 140 mL/kg/d).
Notes	Registered at Clinicaltrials.gov: NCT01443091 Results were initially available as a conference abstract. The trial author provided additional information at request. Review data included information from the subsequent publication (506) and information provided by the author.

NICU: neonatal intensive care unit; NEC: necrotising enterocolitis; CLD: chronic lung disease; VAP: ventilator-associated pneumonia

All the studies were small and from single centres. They enrolled 335 infants with sample sizes between 12 and 149 participants. Four studies; Rodriguez 2011, Sohn 2015, Mota Ferreira 2016 and Glass 2017, prespecified prematurity with birth weight < 1500 grams as an inclusion criterion. Two studies; McFadden 2012 and Sohn 2015, included only infants

who were mechanically ventilated. Overall, the infants had a gestational age ranging from 25 to 32 weeks of gestation and birth weights from 410 to 2500 grams. Table 3.3 details the participants' criteria by a study, which varied between studies.

Table 3.3 Characteristics of participants in the included studies

Study	Participants (number)	Gestational age (weeks)	Birth weight (grams)
Rodriguez 2011(417)	16	25-28	410-1250
McFadden 2012 (502)	27	27-32	590-2530
Sohn 2015 (357)	12	25-30	490-1300
Romano-Keeler 2016 (358)	99	28-31	905-1602
Mota Ferreira 2016* (505)	149	26-31	787-1217
Glass 2017 (506)	30	27-29	1020-1169

* Unpublished data provided by the author

3.4.3 Interventions and comparisons

The included studies randomised infants to receive mother's own colostrum by the oropharyngeal route and the time of starting the OPC was within the first 48 hours of life. Four trials; Rodriguez 2011 (417), Sohn 2015 (357), Romano-Keeler 2016 (358) and Mota Ferreira 2016 (505), followed the protocol for OPC described by Rodriguez et al. (administration of 0.2 mL colostrum/control by syringe: 0.1 mL on each side of the oropharynx) (354). The other two used different protocols; McFadden 2012 (502) used 0.2 mL colostrum for oral care administered via "gentle swab along the inside of the mouth"; Glass 2017 (506) also administrated 0.2 ml colostrum using a cotton-tipped applicator.

All trials reported early OPC administration in preterm infants compared to a control (sham water, normal saline, a placebo, or no intervention). Therefore only one comparison was evaluated; that is early oropharyngeal colostrum versus sham water, normal saline, placebo, or no intervention. Two studies; Sohn 2015 (357) and Romano-Keeler 2016

(358), did not use a placebo and provided no additional intervention to participants randomised to the control group. Three studies; Rodriguez 2011 (417), Mota Ferreira 2016 (505), and Glass 2017 (506), administered sterile water to infants in the control group, and McFadden 2012 (502) included two control groups; one receiving sterile water and the other normal saline. Investigators similarly administered control interventions to colostrum's administration to the intervention group. However, Mota Ferreira 2016 gave human donor milk in the absence of the mother's colostrum. Therefore, infants who received donor milk have been included in the OPC group (as they were randomised to receive OPC) to maintain the intention to treat analyses.

3.4.4 Reported outcomes

All the included trials reported short-term outcomes (participants have been followed up until hospital discharge). No study reported long-term follow up.

3.4.4.1 Primary outcomes

All the included studies reported the pre-specified primary outcomes of the review (section 4.5.2); 'incidence of NEC', 'incidence LOI' and 'death before discharge home'. Four trials; Sohn 2015 (357), Romano-Keeler 2016 (358), Mota Ferreira 2016 (505) and Glass 2017 (506), defined NEC as Bell's stage 2 or 3, whereas two trials; Rodriguez 2011 (417) and McFadden 2012 (502), provided no specific diagnostic criteria. Three studies; Sohn 2015 (357), Mota Ferreira 2016 (505) and Glass 2017 (506), defined LOI as clinical signs and a positive blood culture. Glass 2017 provided additional criteria for defining LOI (onset after day three of life and antibiotic therapy for at least five days), and three studies; Rodriguez 2011 (417), McFadden 2012, Romano-Keeler 2016 (358), did not provide a pre-specified definition.

3.4.4.2 Secondary outcomes

Secondary outcomes were variably reported by the included trials; 'time to full enteral feeds' was reported by all the included trials; 'length of hospital stay' was reported by four

trials; Rodriguez 2011 (417), McFadden 2012 (502), Romano-Keeler 2016 (358) and Mota Ferreira 2016 (505). 'Pneumonia' and 'chronic lung disease' were reported by three studies; Rodriguez 2011 (417), McFadden 2012 (502), Sohn 2015 (357). Sohn 2015 defined CLD (oxygen required at 36 weeks' corrected gestational age, or at discharge, if sooner). Mota Ferreira 2016 reported bronchopulmonary dysplasia as an outcome but did not define the diagnostic criteria used. 'Days of parenteral nutrition' was reported by two trials; Mota Ferreira 2016 (505) and Glass 2017 (506); 'days of antibiotic therapy' was reported by three trials; McFadden 2012 (502), Sohn 2015 (357) and Romano-Keeler 2016 (358). 'Retinopathy of prematurity' was reported by Rodriguez 2011 and Mota Ferreira 2016. Three outcomes were reported by only one trial; 'weight gain from birth to discharge home' was reported by Mota Ferreira 2016 (505); 'receiving only or any breast milk at discharge home' were reported by Romano-Keeler 2016 (358) and 'ventilator-associated pneumonia' by Sohn 2015 (357).

Adverse events associated with OPC were reported on by all the included trials. However, there were no clear definitions for adverse events were described, and adverse events were narratively reported. As no numerical data were provided, this outcome was presented as a narrative summary. None of the included trials reported 'death in the first year of life' and 'neurodevelopmental outcomes at 18 to 24 months'.

3.4.5 Excluded studies

Two studies were excluded from this review because OPC was provided after 48 hours of life. Lee 2015 (356), was a double-blind, placebo-controlled RCT that included 48 infants born at < 28 weeks' gestation who were randomised to receive 0.2 mL of their mother's colostrum or sterile water (control) via the oropharyngeal route every three hours for three days. However, most of the infants included in this study received colostrum after 48 hours of life; therefore, this study was excluded from the analysis. Similarly, Zhang 2017 (419) was a double-blind, placebo-controlled trial, including 64 with birth weight < 1500 grams, compared administration of mother's colostrum (0.1ml) to each side of the cheek

versus similar administration of normal saline. Mean age at the first dose of colostrum or normal saline was > 48 hours in both groups; hence this study was not included in the review.

3.4.6 Risk of bias in included studies

In general, the included studies had a variable risk of bias across the domains. Most of the included studies were not blinded, and there were concerns about allocation concealment. One study consisted of unpublished data (Mota Ferreira 2016 (505)). The risk of bias of the six included trials was considered as high to unclear. Table 3.4 summaries studies criteria for judging the risk of bias for each study.

3.4.6.1 Selection bias

All trials indicated that treatment was allocated randomly; however, two trials; McFadden 2012 (502) and Sohn 2015 (357), did not specify the process used to generate the random sequence. Similarly, two studies (Romano-Keeler 2016 and Mota Ferreira 2016) did not state the methods of allocation concealment, and Glass 2017 (506) reported that the allocation method was “not applicable”.

3.4.6.2 Performance and detection bias

Only two studies; Rodriguez 2011 (417) and Mota Ferreira 2016 (505) , were blinded and described these aspects appropriately (used opaque syringes to deliver treatment); therefore they were judged as having a low risk. Four trials were not blinded; McFadden 2012 (502), Shon 2015 (357), Romano-Keeler 2016 (358) and Glass 2017 (506), these trials were judged as being at high risk for performance and detection bias. However, in this review detection bias for the outcome of death before discharge home was considered as having a low risk as death is unlikely to be influenced by blinding.

3.4.6.3 Attrition bias

Four trials; Rodriguez 2011, Sohn 2015, Romano-Keeler 2016 and Mota Ferreira 2016, were assessed to be at low risk of attrition bias. Mota Ferreira 2016 reported that 32

infants from the colostrum group were excluded and received human donor milk. The trial (Mota Ferreira 2016) investigators provided information for the 32 infants, and this was added to the OPC group. Therefore, this trial was categorised as low risk for attrition bias. Two trials; McFadden 2012 and Glass 2017, were judged as having a high risk of attrition bias. McFadden 2012 reported that three participants were not included in the final analysis for the outcome; 'length of hospital stay'. Those three infants were still in the hospital when the analysis was conducted, and intention-to-treat was not applied. Glass 2017 excluded 13 participants due to trial constraints and data from those infants were not included in the analysis and intention-to-treat was not conducted in the final analysis of the trial.

3.4.6.4 Reporting bias

All the pre-specified expected outcomes of interest for the review were reported except for two outcomes namely, 'death in the first year of life' and 'neurodevelopmental outcomes at 18 to 24 months'. Four of the included studies; Sohn 2015, Mota Ferreira 2016, Romano-Keeler 2016 and Glass 2017, were registered in a trial register (clinicalTrials.gov). Two studies; Rodriguez 2011 and McFadden 2012 (502) did not publish a protocol; however, all the outcomes described in the methods section were reported in their results.

3.4.6.5 Other bias

All included studies were at low risk of other bias except for one trial (Glass 2017), which was considered as having a potential source of other bias because there were data from 13 participants which were not analysed; this was determined from additional information provided by the study author explaining this. Furthermore, the estimated sample size in the protocol, as published at ClinicalTrials.gov (NCT01443091), was 60 infants while in the published report, it was 30 infants and no explanation is stated in the published report (506).

The overall risk of bias was high across all included studies as four out of six studies were not blinded and due to concerns about allocation concealment and incomplete outcome

data. Judgements on the risk of bias in the studies are presented in “Risk of bias” summary (Figure 3.3) and “Risk of bias” graph (Figure 3.4).

Table 3.4 Criteria of the risk of bias for included studies

Study	Selection bias				Performance bias		Detection bias		Attrition bias		Reporting bias	
	Risk	**Sequence generation**	**Risk**	**Allocation concealment**	**Risk**	**Blinding of participants/ personnel**	**Risk**	**Blinding of outcome assessors**	**Risk**	**Incomplete outcome data**	**Risk**	**Selective reporting**
Rodriguez 2011 (417)	Low	Details of randomisation were provided by the author.	Low	Adequate concealment before enrolment	Low	The blinding procedure was described	Low	Sufficient information	Low	Additional information provided by the author and intention-to-treat applied	Unclear	Outcomes described in the methods were reported in the results. The study protocol was not available
McFadden 2012 (502)	Unclear	No sufficient details of the randomisation methods were given	Low	Adequate concealment prior to enrolment	High	Not blinded	High	Not blinded	Low	Three infants were still in the hospital when the study was completed. intention-to-treat applied	Unclear	No protocol was available
Sohn 2015 (357)	Unclear	No details were given on how randomisation was done	Low	"Neonates were randomly assigned to the colostrum group using sealed opaque envelopes."	High	Not blinded	High	Not blinded	Low	All randomised infants were included in the final analysis	Low	The study protocol was available at clinicaltrials.gov (NCT02306980)

Study	Selection bias				Performance bias		Detection bias		Attrition bias		Reporting bias	
	Risk	**Sequence generation**	**Risk**	**Allocation concealment**	**Risk**	**Blinding of participants/ personnel**	**Risk**	**Blinding of outcome assessors**	**Risk**	**Incomplete outcome data**	**Risk**	**Selective reporting**
Romano-Keeler 2016 (358)	Low	“a numeric list generated a priori to receive an intervention”	Unclear	No details were provided	High	Not blinded	High	Not blinded	Low	Randomised infants were included in the final analysis	Low	Outcomes described were reported. Clinicaltrials.gov (NCT01776268)
Mota - Ferreira 2016 (505)	Low	Computer random number generation.	Unclear	No details were provided regarding allocation concealment.	Low	Adequate blinding procedure was described.	Low	Adequate blinding procedure was described.	Low	"32 randomized to colostrum group were excluded because colostrum was not available". The author provided additional information regarding this group. Intention-to-treat applied	Unclear	the protocol was available at clinicaltrials.gov: NCT01776268). Unpublished data

Study	Selection bias				Performance bias		Detection bias		Attrition bias		Reporting bias	
	Risk	**Sequence generation**	**Risk**	**Allocation concealment**	**Risk**	**Blinding of participants/ personnel**	**Risk**	**Blinding of outcome assessors**	**Risk**	**Incomplete outcome data**	**Risk**	**Selective reporting**
Glass 2017 (506)	Low	"Random number generation"; additional information provided by the author	High	"Not applicable"; additional information provided by the author	High	Not blinded	High	Not blinded	High	13 participants were excluded and not analysed	High	Sample size in the protocol (clinicaltrials.gov NCT01443091) was 60 infants. The report included only 30 (no explanation was provided)

Unclear: lack of information or uncertainty

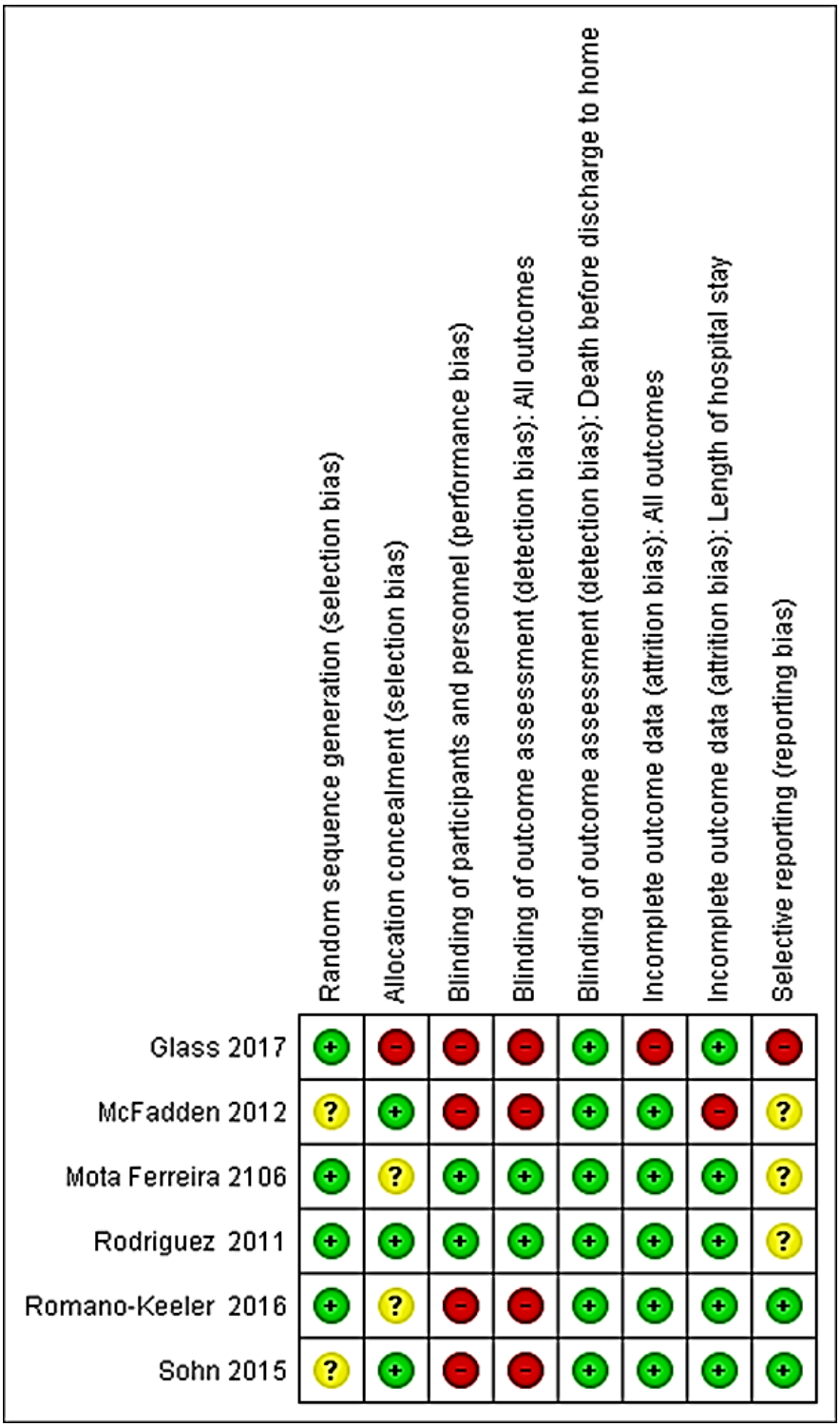


Figure 3.3 Risk of bias summary for the included studies

Individual assessment of risk of bias items for each included study.
Green ball: low risk; Red ball: high risk; Yellow; unclear (uncertainty or lack of information). Rodriguez 2011(417); McFadden 2012 (502); Sohn 2015 (357); Romano-Keeler 2016 (358); Mota-Ferreira 2016 (505); Glass 2017 (506).

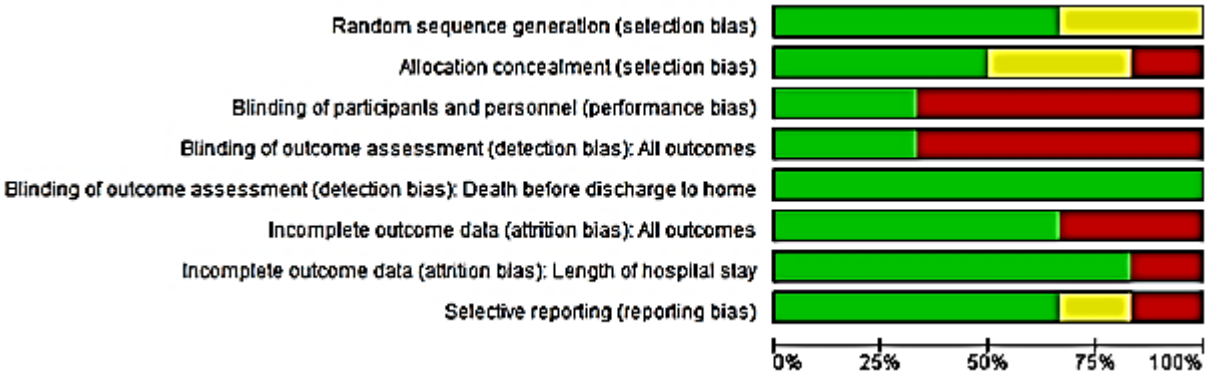


Figure 3.4 Overall risk of bias for each domain in the included studies
Assessment of risk of bias for each item presented across all included studies. Each bar presents a risk of bias item. Green bar: low risk; Red: high risk; Yellow: unclear risk of bias.

3.4.7 Effects of the intervention

3.4.7.1 Main review comparison

This review included only one comparison as no available data for the other planned comparisons. Early OPC versus control (water, normal saline, placebo, or no intervention) was the main comparison included in the final data analysis of this review.

3.4.7.2 Primary outcomes

3.4.7.2.1 Incidence of NEC until hospital discharge

All included trials; Rodriguez 2011(417), McFadden 2012 (502), Sohn 2015 (357), Romano-Keeler 2016 (358), Mota-Ferreira 2016 (505) and Glass 2017 (506), reported on the incidence of NEC in 335 enrolled preterm infants (OPC: 172; control: 163 infants). Meta-analysis did not show an effect on the risk of NEC (Figure 3.5). The estimate is based on four studies including 290 participants, as two studies had no cases of NEC (Rodriguez 2011 and McFadden 2012). The typical risk difference was 0.01 (95% CI -0.03 to 0.06). There was no evidence of heterogeneity between the studies for this outcome (I^2 = 0%). The quality of evidence was very low due to imprecision (small sample size and wide CI) and high to unclear risk of bias.

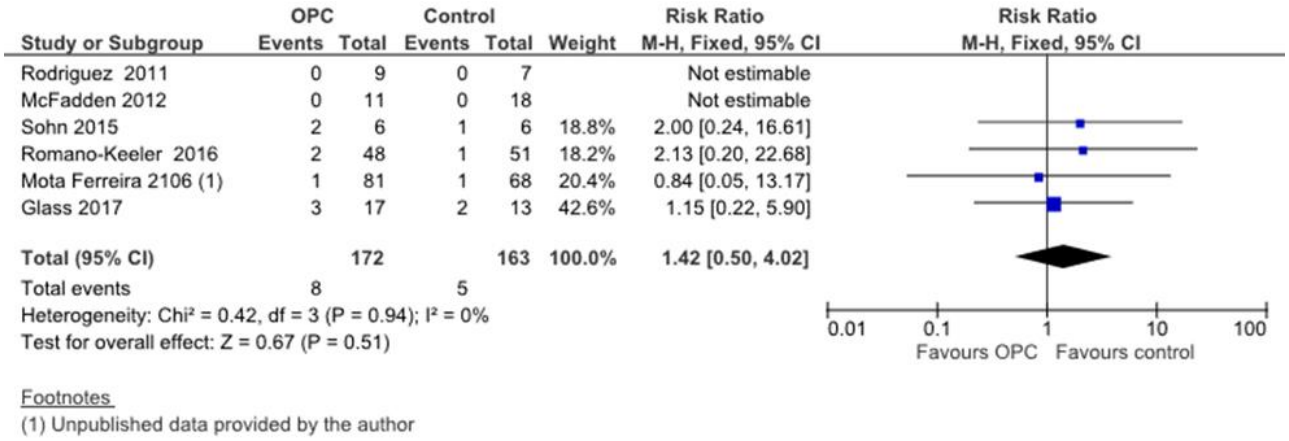


Figure 3.5 Forest plot comparing the incidence of NEC for infants receiving OPC or control

Each trial is represented by a horizontal line; width of the line: 95% CI. Blue box: mean effect estimate and the weight assigned to the trial. Middle vertical line (1): no effect. Black diamond: overall effect of estimate (upper and lower angles: effect size; width: 95% CI); MH: Mantel-Haenszel; Fixed: analysis model; CI: confidence interval; Chi^2: Chi-square; df: degrees of freedom; P: p-value; I^2: I-square: statistical heterogeneity; Z: test for statistical significance. NEC: necrotising enterocolitis; OPC: oropharyngeal colostrum.

3.4.7.2.2 Incidence of LOI until hospital discharge

All included trials; Rodriguez 2011(417), McFadden 2012 (502), Sohn 2015 (357), Romano-Keeler 2016 (358), Mota-Ferreira 2016 (505) and Glass 2017 (506), reported on the incidence of late-onset infection in 335 enrolled preterm infants (OPC: 172; control: 163 infants). There was no statistically significant difference in the incidence of LOI; meta-analysis did not show an effect (Figure 3.6). There was no evidence of heterogeneity between the studies for this outcome (I^2 = 0%). The quality of evidence was very low due to imprecision (small sample size and wide CI) and high to unclear risk of bias.

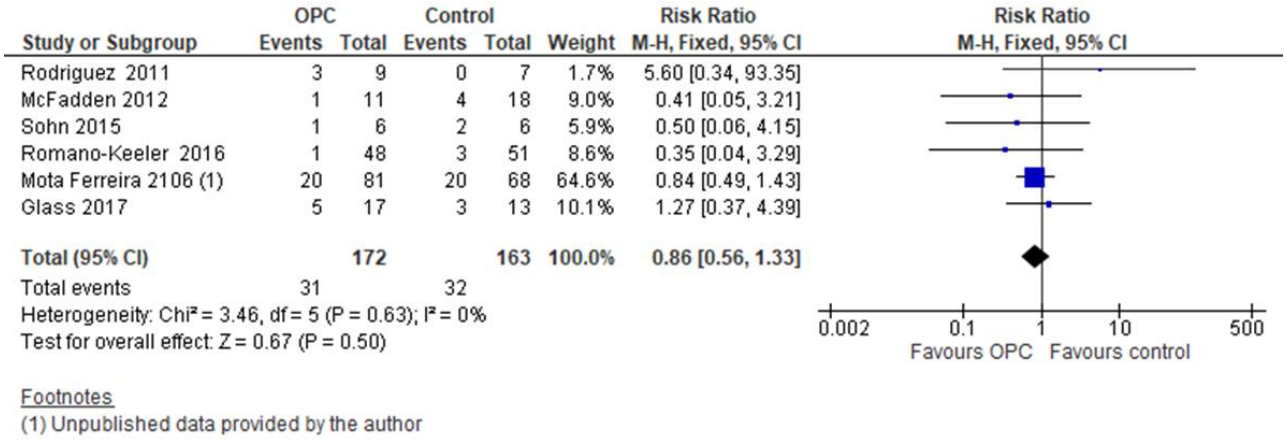


Figure 3.6 Forest plot comparing the incidence of LOI for preterm infants receiving OPC or control

Each trial is represented by a horizontal line; width of the line: 95% CI. Blue box: mean effect estimate and the weight assigned to the trial. Middle vertical line (1): no effect. Black diamond: overall effect of estimate (upper and lower angles: effect size; width: 95% CI); MH: Mantel-Haenszel; Fixed: analysis model; CI: confidence interval; Chi^2: Chi-square; df: degrees of freedom; P: p-value; I^2: I-square: statistical heterogeneity; Z: test for statistical significance; LOI: late-onset infection; OPC: oropharyngeal colostrum

3.4.7.2.3 Death before discharge to home

All the trials; Rodriguez 2011(417), McFadden 2012 (502), Sohn 2015 (357), Romano-Keeler 2016 (358), Mota-Ferreira 2016 (505) and Glass 2017 (506), reported on death before discharge home in 335 enrolled preterm infants (OPC: 172; control: 163 infants). There was no statistically significant difference in mortality between the two groups; meta-analysis showed no effect (Figure 3.7). One study (Glass 2017) had no death in the enrolled infants. Therefore, the estimate is based on five studies in 305 infants. No evidence indicates heterogeneity between studies for this outcome (I^2 = 0%). The quality of evidence was very low due to imprecision (small sample size and wide CI) and high to unclear risk of bias.

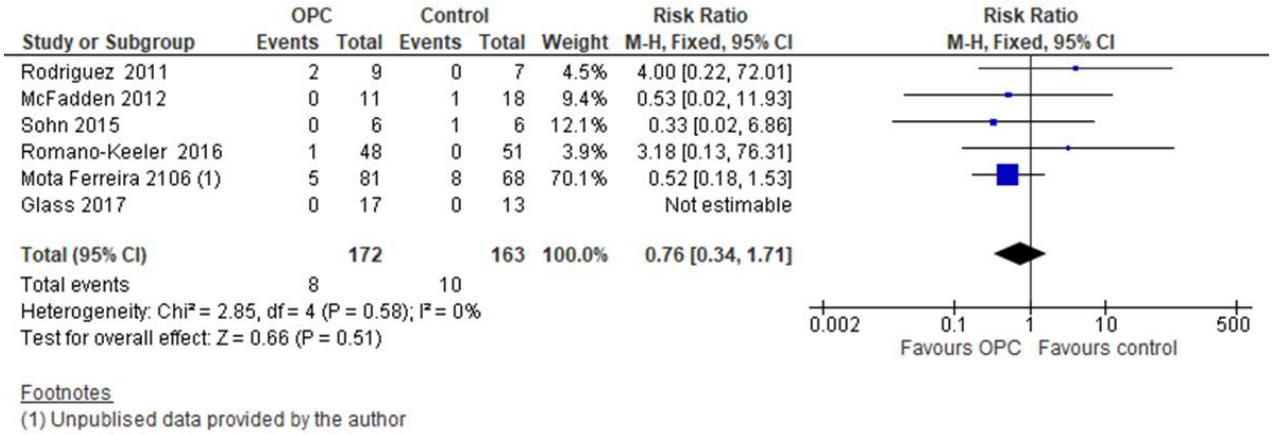


Figure 3.7 Forest plot comparing death before discharge home for preterm infants receiving OPC or control

Each trial is represented by a horizontal line; width of the line: 95% CI. Blue box: mean effect estimate and the weight assigned to the trial. Middle vertical line (1): no effect. Black diamond: overall effect of estimate (upper and lower angles: effect size; width: 95% CI); MH: Mantel-Haenszel; Fixed: analysis model; CI: confidence interval; Chi^2: Chi-square; df: degrees of freedom; P: p-value; $I^{2:}$ I-square: statistical heterogeneity; Z: test for statistical significance; OPC: oropharyngeal colostrum.

3.4.7.3 Secondary outcomes

3.4.7.3.1 Days to full enteral feed

The six included trials; Rodriguez 2011(417), McFadden 2012 (502), Sohn 2015 (357), Romano-Keeler 2016 (358), Mota-Ferreira 2016 (505) and Glass 2017 (506), reported on time to full enteral feed in 335 enrolled preterm infants (OPC: 172; control: 163 infants). Meta-analysis demonstrated that infants who received early OPC attained full enteral feeds earlier compared with controls (Figure 3.8). At the study level, only two trials reported an effect in favour of OPC; Rodriguez 2011 and Mota Ferreira 2016. There was moderate heterogeneity (I^2 = 53 %) across the studies which could be due to variability between trials in the definition of time to reach full enteral feeds (100 to 150 mL/kg/d). Additionally, two studies reported the data as median and interquartile range; hence, the means and SD were estimated. As the heterogeneity could be explained, a fixed-effect model was used for analysing this outcome because it is more powerful and estimates the best effect for an intervention. Further exploration of heterogeneity demonstrated that excluding Sohn 2015's study reduced the heterogeneity from I^2 = 53% to 29%, Figure 3.8.

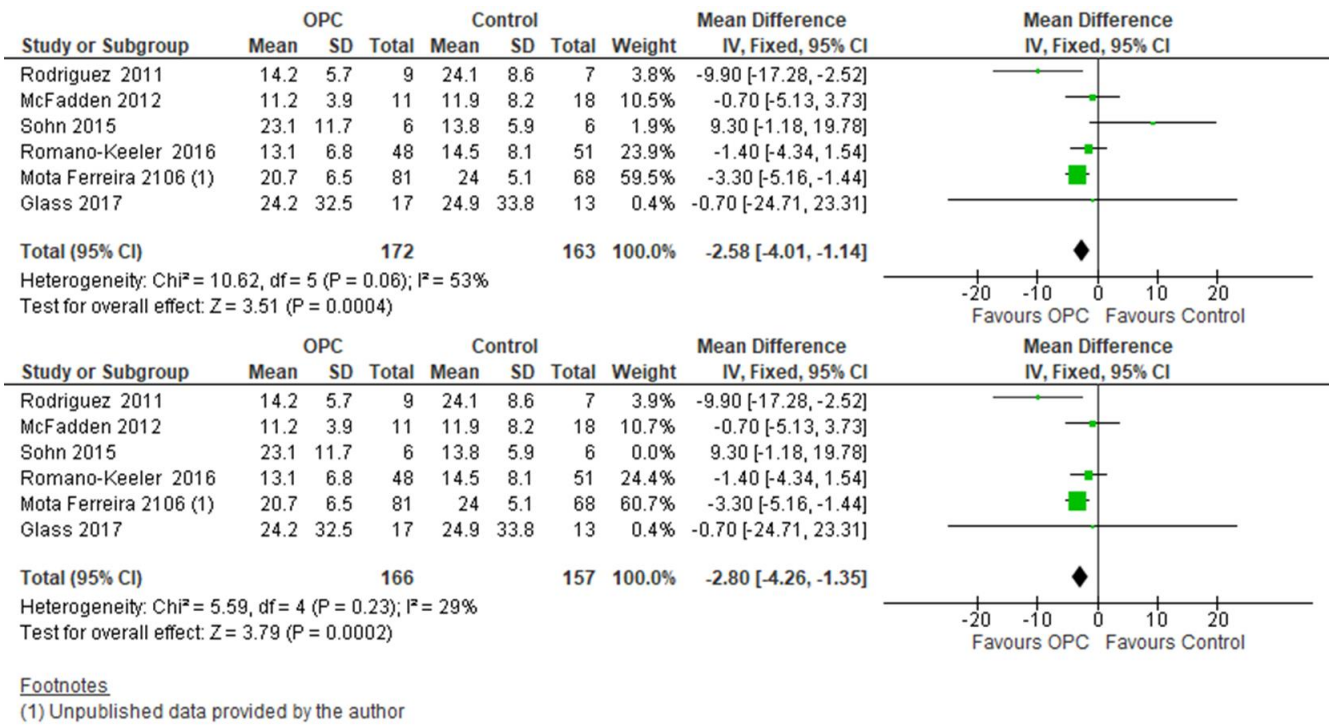


Figure 3.8 Forest plot comparing days to full feeds for preterm infants receiving OPC or control

Each trial is represented by a horizontal line; width of the line: 95% CI. Green box: mean effect estimate and the weight assigned to the trial. Middle vertical line (0): no effect. Black diamond: overall effect of estimate (upper and lower angles: effect size; width: 95% CI); SD: standard deviation; IV: Inverse Variance; Fixed: analysis model; CI: confidence interval; Chi^2: Chi-square; df: degrees of freedom; P: p-value; I^2: I-square: statistical heterogeneity; Z: test for statistical significance; OPC: oropharyngeal colostrum.

Using random-effects model, meta-analysis showed that infants who received OPC attained full enteral feeds earlier compared with controls with minimal reduction in the effect estimate. However, the 95%CI widened that could explain the statistical non-significant difference.

Similarly heterogeneity decreased by excluding Sohn's trial, Figure 3.9

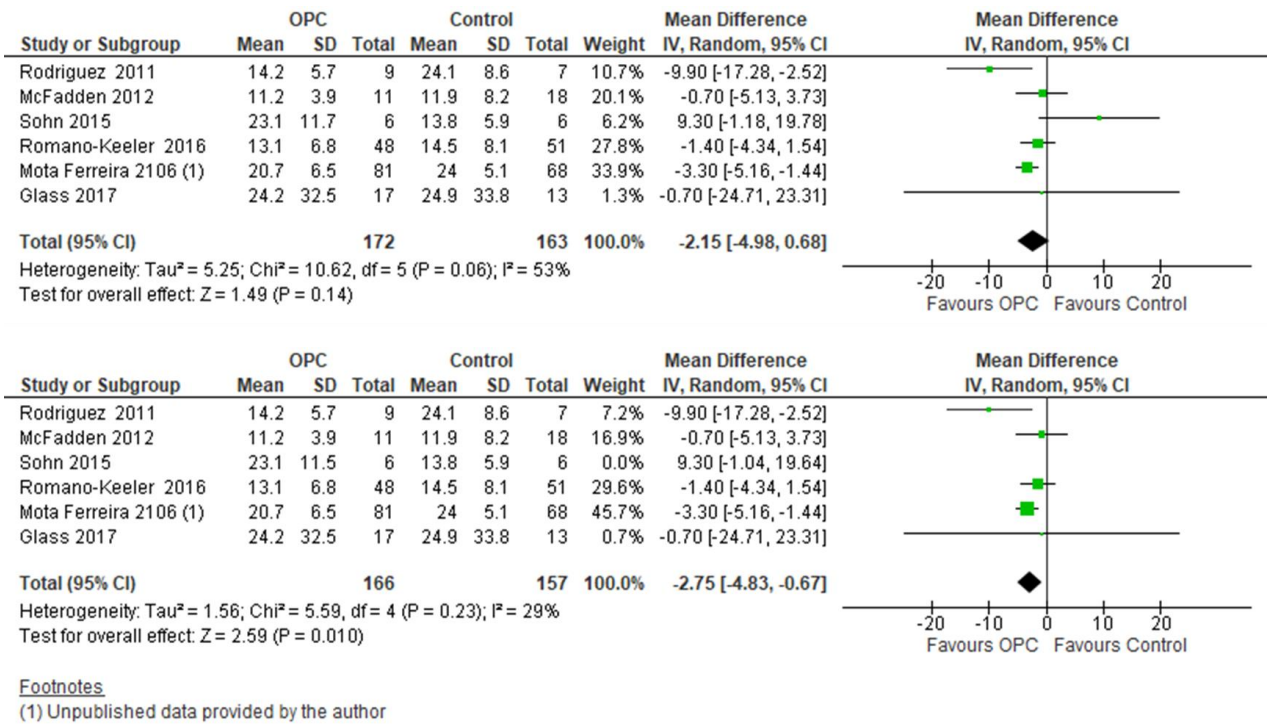


Figure 3.9 Forest plot comparing days to full feeds for preterm infants receiving OPC or control (Random-effects model)

Each trial is represented by a horizontal line; width of the line: 95% CI. Green box: mean effect estimate and the weight assigned to the trial. Middle vertical line (0): no effect. Black diamond: overall effect of estimate (upper and lower angles: effect size; width: 95% CI); SD: standard deviation; IV: Inverse Variance; Fixed: analysis model; CI: confidence interval; Chi^2: Chi-square; df: degrees of freedom; P: p-value; I^2: I-square: statistical heterogeneity; Z: test for statistical significance; OPC: oropharyngeal colostrum

3.4.7.3.2 Length of hospital stay

Four trials; Rodriguez 2011(417), McFadden 2012 (502), Romano-Keeler 2016 (358) and Mota-Ferreira 2016 (505), reported on the length of hospital stay in 293 enrolled preterm infants (OPC:149; control: 144 infants). There were no significant differences in the length of hospital stay between the two groups in individual studies; meta-analysis did not show an effect (Figure 3.10). There was low to moderate heterogeneity (I^2 = 49%) across the trials suggesting variability between the studies. This Heterogeneity was retrospectively explored; one study included infants with a larger birth weight (McFadden 2012). Exclusion of this study reduced heterogeneity to I^2 = 12% and did not alter the estimated effect in the meta-analysis for the length of hospital stay. The quality of evidence was very low due to imprecision (small sample size and wide CI), high to unclear risk of bias, and moderate heterogeneity.

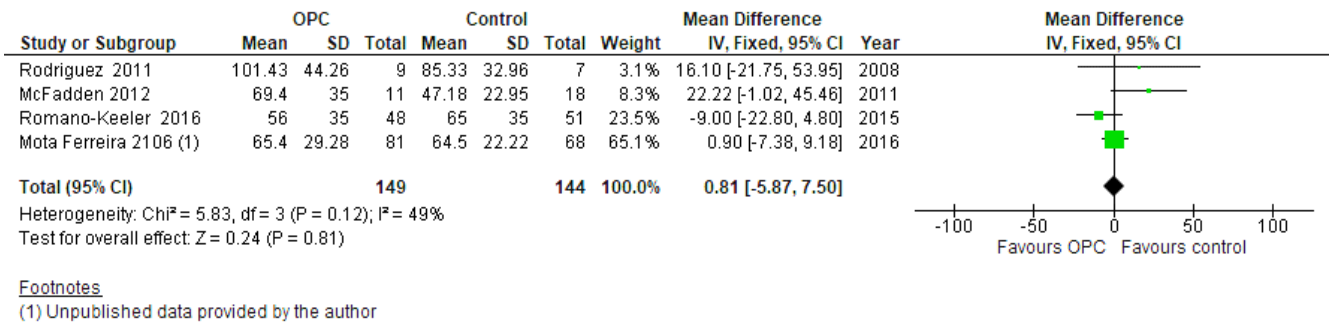


Figure 3.10 Forest plot comparing length of hospital stay for preterm infants receiving OPC or control

Each trial is represented by a horizontal line; width of the line: 95% CI. Green box: mean effect estimate and the weight assigned to the trial. Middle vertical line (0): no effect. Black diamond: overall effect of estimate (upper and lower angles: effect size; width: 95% CI); SD: standard deviation; IV: Inverse Variance (statistical method); Fixed: analysis model; CI: confidence interval; Chi^2: Chi-square; df: degrees of freedom; P: p-value; I^2: I-square: statistical heterogeneity; Z: test used for statistical significance; OPC: oropharyngeal colostrum.

3.4.7.3.3 Pneumonia

Three trials; Rodriguez 2011, McFadden 2012, and Sohn 2015, reported the occurrence of pneumonia in 57 enrolled preterm infants (OPC: 26; control:31 infants). There was no significant difference between the two groups; meta-analysis did not show an effect on rate of pneumonia before discharge home (Figure 3.11). There was no evidence of heterogeneity (I^2 = 17%). The quality of evidence was very low due to imprecision (small sample size, very wide CI), performance bias (one trial was not blinded) and selection bias.

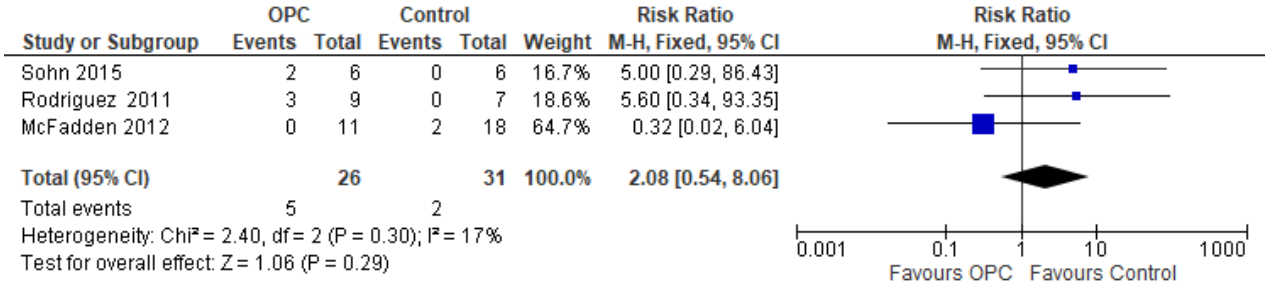


Figure 3.11 Forest plot comparing the incidence of pneumonia for preterm infants receiving OPC or control

Each trial is represented by a horizontal line; width of the line: 95% CI. Blue box: mean effect estimate and the weight assigned to the trial. Middle vertical line (1): no effect. Black diamond: overall effect of estimate (estimate (upper and lower angles: effect size; width: 95% CI); M-H: Mantel-Haenszel; Fixed: analysis model; CI: confidence interval; Chi^2: Chi-square; df: degrees of freedom; P: p-value; I^2: I-square: statistical heterogeneity; Z; test used for statistical significance; OPC: oropharyngeal colostrum.

3.4.7.3.4 Chronic lung disease (CLD)

Three trials (Rodriguez 2011, McFadden 2012, and Sohn 2015) reported on the incidence of CLD in 57 enrolled preterm infants (OPC: 26; control:31 infants). There was no significant difference in the incidence of CLD between the two groups; meta-analysis did not show an effect. There was no evidence of heterogeneity between the studies (I^2 = 0%). There was another trial (Mota Ferreira 2016) reported bronchopulmonary dysplasia as an outcome but did not provide pre-defined criteria. However, including this study did not alter the result of the meta-analysis significantly (Figure 3.12). The quality of evidence was very low due to imprecision (small sample size and wide CI) and performance (one study unblinded) and reporting bias.

Study or Subgroup	OPC Events	OPC Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
1.8.1 CLD						
Rodriguez 2011	2	9	1	7	2.7%	1.56 [0.17, 13.87]
McFadden 2012 (1)	1	11	3	18	5.5%	0.55 [0.06, 4.61]
Sohn 2015	3	6	2	6	4.8%	1.50 [0.38, 6.00]
Subtotal (95% CI)		**26**		**31**	**13.1%**	**1.11 [0.40, 3.08]**
Total events	6		6			
Heterogeneity: Chi² = 0.70, df = 2 (P = 0.71); I² = 0%						
Test for overall effect: Z = 0.20 (P = 0.84)						
1.8.2 CLD and BPD						
Mota Ferreira 2106	32	81	33	68	86.9%	0.81 [0.57, 1.17]
Subtotal (95% CI)		**81**		**68**	**86.9%**	**0.81 [0.57, 1.17]**
Total events	32		33			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.11 (P = 0.27)						
Total (95% CI)		**107**		**99**	**100.0%**	**0.85 [0.60, 1.20]**
Total events	38		39			
Heterogeneity: Chi² = 1.16, df = 3 (P = 0.76); I² = 0%						
Test for overall effect: Z = 0.91 (P = 0.37)						
Test for subgroup differences: Chi² = 0.31, df = 1 (P = 0.58), I² = 0%						

Footnotes
(1) 4 infants have CLD not reported to which group

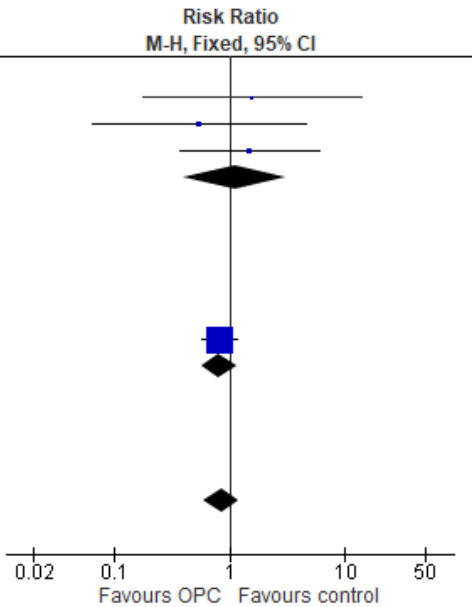


Figure 3.12 Forest plot comparing the incidence of CLD for preterm infants receiving OPC or control

Each trial is represented by a horizontal line; width of the line: 95% CI. Blue box: mean effect estimate and the weight assigned to the trial. Middle vertical line (1): no effect. Black diamond: overall effect of estimate (upper and lower angles: effect size; width: 95% CI); CLD: chronic lung disease; M-H: Mantel-Haenszel; Fixed: analysis model; CI: confidence interval; Chi^2: Chi-square; df: degrees of freedom; P: p value; I^2: I-square: statistical heterogeneity; Z; test used for statistical significance; OPC: oropharyngeal colostrum.

3.4.7.3.5 Days of antibiotic therapy

Three trials; McFadden 2012, Sohn 2015 and Romano-Keeler 2016, reported on the 'days of antibiotic therapy' in 140 enrolled preterm infants (OPC: 65; control: 75 infants). There was no a significant difference between the two groups for the outcome 'days of antibiotic therapy'; meta-analysis did not show an effect (Figure 3.13). There was a very high heterogeneity across the studies (I^2 = 91%).

At the study level, one trial (McFadden 2012) found that infants who received oral care with colostrum required more days of antibiotic therapy when compared with those who received oral care with saline or sterile water. This heterogeneity was retrospectively explored and identified that McFadden 2012 enrolled infants with larger birth weight. Excluding data from this study eliminated the heterogeneity but did not change the effect estimate. The quality of evidence was very low owing to imprecision (very small sample size and very wide CI), performance (the included trials were not blinded) and reporting bias, and very high heterogeneity.

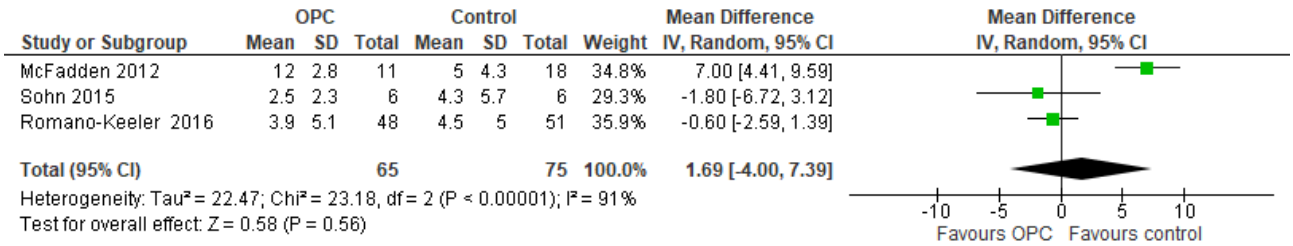


Figure 3.13 Forest plot comparing days of antibiotics therapy for preterm infants receiving OPC or control

Each study is represented by a horizontal line; width of the line: 95% CI. Blue box: mean effect of estimate and weight of the study. Middle vertical line (1): no effect. Black diamond: overall effect of estimate (upper and lower angles: effect size; width: 95% CI); IV: Inverse Variance; Random: analysis model; CI: confidence interval; Chi^2: Chi-square; df: degrees of freedom; P: p value; I^2: I-square: statistical heterogeneity; Z: test used for statistical significance; OPC: oropharyngeal colostrum.

3.4.7.3.6 Days of parenteral nutrition

Two trials; Mota Ferreira 2016 and Glass 2017, reported 'days of parenteral nutrition' in 179 preterm infants; (OPC: 98; control: 81 infants). There was no significant difference in the

days of parenteral nutrition use between the OPC and control groups; meta-analysis did not show an effect (Figure 3.14). No evidence suggested heterogeneity across the studies (I^2 = 0%). The quality of evidence was very low due to imprecision (very small sample size, very wide CI) and performance and reporting bias detected in these studies.

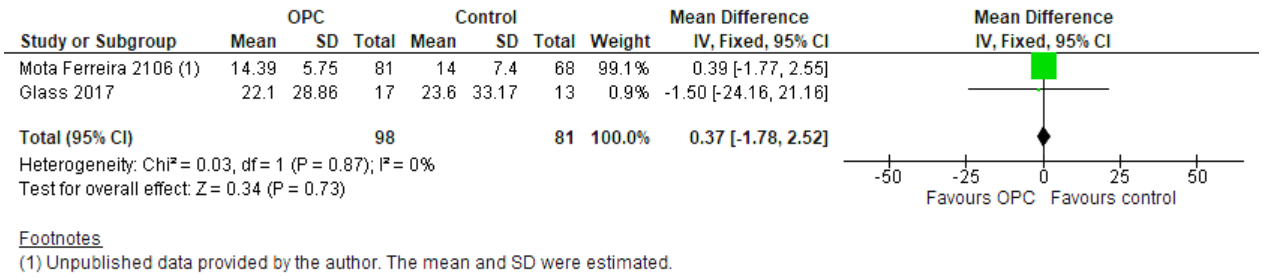


Figure 3.14 Forest plot comparing days of parenteral nutrition for preterm infants receiving OPC or control

Each study is represented by a horizontal line; width of the line: 95% CI. Blue box: mean effect of estimate and weight of the study. Middle vertical line (1): no effect. The black diamond: overall effect of estimate (upper and lower angles: effect size; width: 95% CI); SD: standard deviation; IV: Inverse Variance; Fixed: analysis model; CI: confidence interval; Chi^2: Chi-square; df: degrees of freedom; P: p value; I^2: I-square: statistical heterogeneity; Z: test used for statistical significance; OPC: oropharyngeal colostrum.

3.4.7.3.7 Weight gain from birth to discharge home

One unpublished trial, Mota Ferreira 2016, reported weight gain from birth to discharge home in 149 enrolled preterm infants (OPC: 81; control: 68 infants). There was no significant difference in the weight gain from birth to discharge home between the two groups (MD -15.00 (95% CI - 50.83 to 20.83); participants: 149; P = 0.60). The quality of evidence was very low due to imprecision, unclear selection and reporting bias, and the data were obtained from only one unpublished trial.

3.4.7.3.8 Receiving breast milk at discharge home

Only one trial, Romano-Keeler 2016, included the outcome measure of receiving breast milk at discharge home in 99 enrolled preterm infants (OPC: 48; control: 51infants). This outcome was described for two subgroups, received any fortified breast milk at discharge and any unfortified breast milk, and reported as two separate outcomes. There were no statistically

significant differences between OPC and control groups for both types of feeding; receiving any fortified breast milk at discharge and receiving any unfortified breast milk at discharge (Figure 3.15). Combining these outcomes meta-analysis showed an effect of OPC on receiving any breast milk at discharge compared with controls (Figure 3.14. The quality of evidence was very low because data were obtained from only one not blinded study with a small sample size. Although meta-analysis is not appropriate for a single study, Figure 3.14 presents information drawn from two reports of the same study.

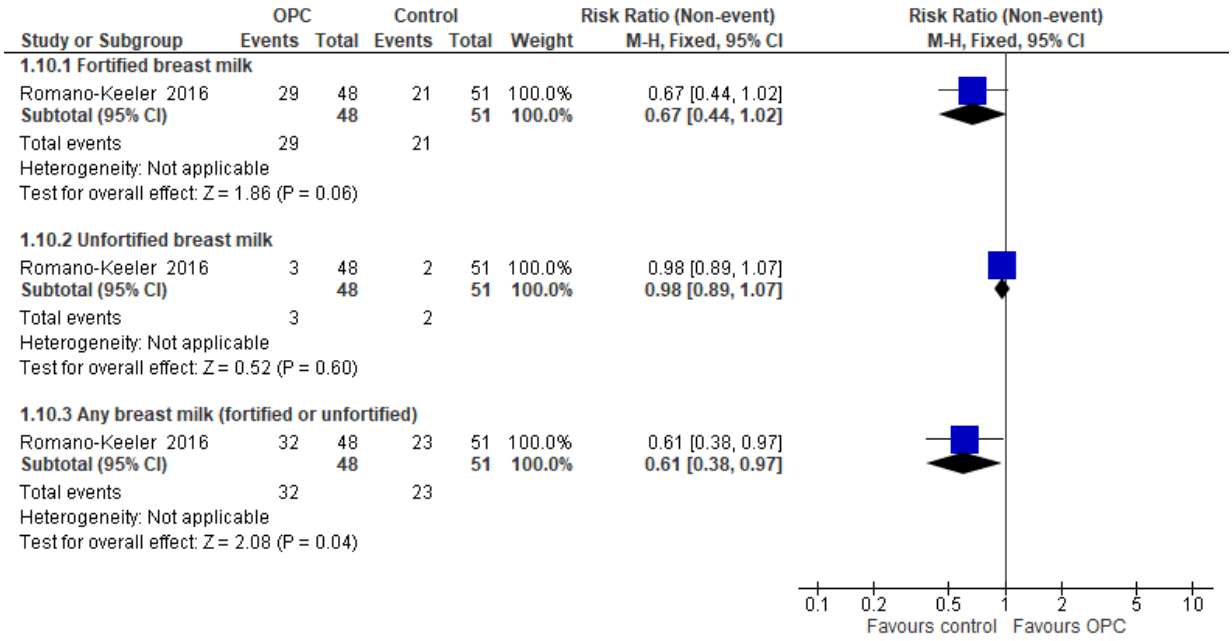


Figure 3.15 Forest plot comparing receiving breast milk at discharge home for preterm infants receiving OPC or control

A single trial reported the outcome receiving breast milk at discharge home. Width of the line: 95% CI; Blue box: mean effect of estimate. Black diamond: overall effect of estimate (upper and lower angles: effect size; width: 95% CI); the black diamond at the bottom of the graph: overall effect of estimate for combing the two outcomes (fortified and unfortified breast milk); M-H: Mantel-Haenszel; Fixed: analysis model; CI: confidence interval; Chi^2: Chi-square; df: degrees of freedom; P: p value; I^2: I-square: statistical heterogeneity; Z: test used for statistical significance; OPC: oropharyngeal colostrum.

3.4.7.3.9 Retinopathy of prematurity (ROP)

Two trials; Rodriguez 2011 and Mota Ferreira 2016, reported the rate of ROP in 165 enrolled preterm infants; (OPC: 90; control: 75 infants). There was no statistically significant difference in the incidence of ROP between the two groups (Figure 3.16). There was no

heterogeneity across the trials, $I^2 = 0\%$. The quality of evidence was very low due to imprecision and performance and reporting bias.

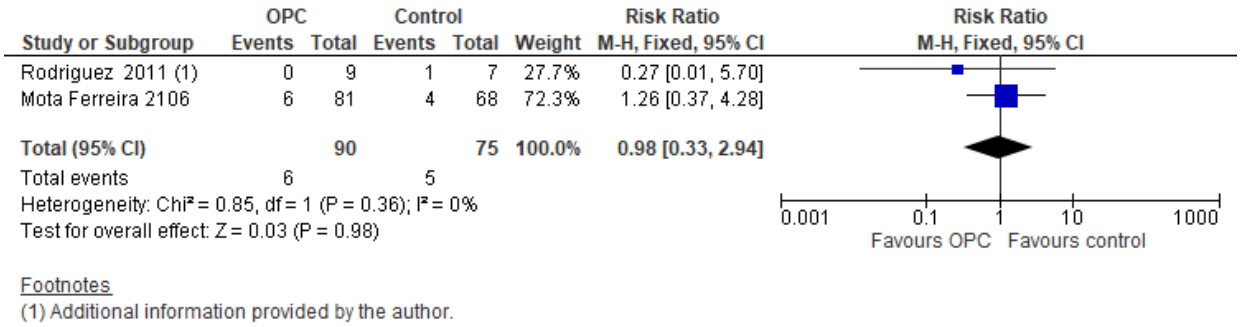


Figure 3.16 Forest plot comparing Retinopathy of prematurity for preterm infants receiving OPC or control

Each trial is represented by a horizontal line; width of the line: 95% CI. Blue box: mean effect estimate and the weight assigned to the trial. Middle vertical line (1): no effect. Black diamond: overall effect of estimate (upper and lower angles: effect size; width: 95% CI); M-H: Mantel-Haenszel; Fixed: analysis model; CI: confidence interval; Chi^2: Chi-square; df: degrees of freedom; P: p value; I^2: I-square: statistical heterogeneity; Z: test used for statistical significance; OPC: oropharyngeal colostrum.

3.4.7.3.10 Reported adverse effects

Five trials; Rodriguez 2011, McFadden 2012, Romano-Keeler 2016, Mota Ferreira 2016 and Glass 2017, reported adverse effects narratively indicating that all infants tolerated the intervention and no adverse events were observed during the study period. No specific definitions for adverse effects were described by the studies. However, two reports only briefly described the adverse effects; Rodriguez 2011 reported "no recorded episodes of apnoea, bradycardia, desaturation or other adverse effects" but did not define the adverse effects. Glass 2017 stated, “Apnoea and bradycardia events during the procedure as well as the occurrence of aspiration pneumonia were charted according to unit policy”. As no numerical data were provided by these trials, a meta-analysis was not performed, and the adverse effects were narratively presented in the SoF table (Table 3.5). The quality of evidence was very low owing to imprecision and high to unclear risk of bias.

3.4.8 Quality of evidence

All outcomes with pooled data from the included trials were assessed for the quality of evidence applying the GRADE approach. Based on the recommendation of the CNG seven outcomes were only included in the SoF table. Assessment of the quality of evidence for each outcome is presented in the SoF table. (Table 3.5).

The overall quality of evidence ranged from low to very low across all the outcomes of interest for this review. It was downgraded due to concerns about allocation concealment and blinding in the highest weighted studies, concerns about incomplete outcome data, small sample sizes with few events, wide confidence intervals crossing the line of no effect in almost all the outcomes and high heterogeneity in some outcomes.

Table 3.5 Summary of Finding (SoF) table:
Oropharyngeal colostrum (OPC) compared to control (water, saline, or no intervention) in preterm infants

Outcomes	Absolute effects* (95% CI)		Relative effect (95% CI)	Participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with control	Risk with OPC				
Incidence of necrotising enterocolitis (NEC)	3 per 100	4 per 100 (2 to 12)	RR 1.42 (0.50 to 4.02)	335 (6 RCTs)	⊕◯◯◯ VERY LOW	The quality of evidence was downgraded to very low due to very serious risk of bias (four out of six studies were not blinded, and two studies had unclear randomisation). Very serious impression (small sample size and wide CI)
Incidence of late-onset sepsis	20 per 100	17 per 100 (11 to 26)	RR 0.86 (0.56 to 1.33)	335 (6 RCTs)	⊕◯◯◯ VERY LOW	The quality of evidence was downgraded to very low due to very serious risk of bias (four out of six studies were not blinded, and two studies had unclear randomisation). Very serious impression (small sample size and CI crossed the line of no effect).
Death before discharge to home	6 per 100	5 per 100 (2 to 11)	RR 0.76 (0.34 to 1.71)	355 (6 RCTs)	⊕◯◯◯ VERY LOW	The quality of evidence was downgraded to very low due to very serious risk of bias (75% of the included studies were not blinded, and two studies had unclear randomisation). Very serious impression (small sample size and wide CI)

Outcomes	Absolute effects* (95% CI)		Relative effect (95% CI)	Participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with control	Risk with OPC				
Days to full enteral feed	Mean =10-25 days	MD -2.58 lower (-4.01 lower to -1.14 lower)	-	355 (6 RCTs)	⊕◯◯◯ VERY LOW	The quality of evidence was downgraded to very low due to very serious risk of bias (75% of the included studies were not blinded, and two had concerns about allocation concealment and unclear randomisation). Serious impression (small sample size and CI crossed the line of no effect in three trials).
Length of hospital stay	Mean = 47-86 days	MD 0.81 days higher (-5.87 lower to 7.5 higher)	-	293 (4 RCTs)	⊕◯◯◯ VERY LOW	The quality of evidence was downgraded to very low due to very serious risk of bias (50% of the included studies were not blinded and had concerns about allocation concealment. One study had unclear randomisation). Serious impression (small sample size and CI crossed the line of no effect).
Pneumonia	6 per 100 (3 to 52)	7 per 100 (1 to 45)	RR 2.08 (0.54 to 8.06)	57 (3 RCTs)	⊕⊕◯◯ LOW	The quality of evidence was downgraded to low due to very serious risk of bias (75% of the included studies were not blinded, and had concerns about randomisation). Serious impression (very small sample size, variable effect size and very wide CI).

Outcomes	Absolute effects* (95% CI)		Relative effect (95% CI)	Participants (studies)	Quality of the evidence (GRADE)	Comments
	Risk with control	Risk with OPC				
Reported adverse effects	No pre-defined adverse effects have been described by all the studies. Adverse effects were narratively reported as no adverse effects with the intervention. No numerical data were provided. One study reported ‘‘no recorded episodes of apnoea, bradycardia, desaturation or other adverse effects’’ but without defining the adverse effects. A second study stated that ‘‘no adverse events were noted’’, and another mentioned in the method section that ‘‘apnoea, bradycardia events and aspiration pneumonia were charted according to unit policy.’’			335 (6 RCTs)	⊕◯◯◯ VERY LOW	The quality of evidence was downgraded to very low due to very serious risk of bias (75% of the included studies were not blinded, and two contained concerns about randomisation and allocation concealment). Neither definitions of adverse effects nor the methods used for monitoring were reported. A narrative report was conducted without a clear statement of adverse effect, and estimates were not precise.

* The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI).

“GRADE Working Group grades of evidence
High quality: We are very confident that the true effect lies close to that of the estimate of the effect
Moderate quality: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
Low quality: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
Very low quality: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.”
CI: Confidence interval; RR: Risk ratio; MD: Mean difference; RCT: randomised controlled trials; GRADE: Grading of Recommendations Assessment, Development and Evaluation

3.5 Discussion

3.5.1 Key findings

Based on predefined eligibility criteria, this review included six RCTs that considered the effects of using OPC in preventing mortality and other morbidities in preterm infants (357, 358, 417, 502, 505, 506). These trials were recent; conducted between 2010 and 2016 and therefore, the results are relevant to the current practice of the care for preterm infants. All of these studies were small and from single centres, involving 335 infants with sample sizes of between 12 and 149 patients. Participants had gestational ages ranging from 25 to 32 weeks' gestation and birth weights between 410 to 2500g (Table 3.2).

As no other comparisons have been studied by RCTs, this review focussed on only one comparison; namely the early use of OPC versus control in preterm infants. All the included studies administered OPC within 48 hours of birth, and own mother's colostrum was used except for one trial. Mota-Ferriera 2016 (505) used human donor milk for infants assigned to the colostrum group if mother's colostrum was not available, this could introduce bias into the results as preterm infants fed with own mother's milk have a better outcome than those who fed donor milk (507, 508); which could partly be explained by the effect of pasteurisation on the bioactive factors and nutrients of the milk (509).

Generally, the included studies were of low methodological quality (high to unclear risk of bias). Most of the included studies were not blinded and contained concerns about allocation concealment, and one study, Mota Ferreira 2016 (505), was unpublished data. The overall quality of evidence ranges from low to very low across almost all the outcomes of interest for this review.

3.5.2 Primary outcomes

All the included trials reported the primary outcomes of the review; 'incidence of NEC', 'incidence of LOI and 'death before discharge home'. Meta-analyses of the available trial data for these outcomes showed no significant differences between OPC and controls in the

‘incidence of NEC’ (p = 0.51), ‘incidence of LOI’ (p = 0.43) and ‘death before discharge home’ (p = 0.51) in preterm infants <37 weeks’ gestation. These are expected findings as all the included studies were small trials with insufficient power to detect statistically significant differences but clinically important effects in the incidence of NEC, LOI, nor death. These results are however consistent with the biological plausibility of OPC administration improving outcomes as suggested by previous studies (353, 355, 377). There is an ongoing trial (352) that aims to recruit 489 extremely preterm infants with birth weight <1250 grams, during the first 96 hours of life. Once available, including the results of this trial in the meta-analysis might modify the overall estimates of effects and the conclusions.

3.5.3 Secondary outcomes

Several secondary outcomes were considered in this review including, days to full enteral feeds, length of hospital stay, pneumonia, CLD, days of antibiotic therapy, days of parenteral nutrition, weight gain at discharge home, ROP, receiving any breast milk at discharge home. There were no differences between OPC and the controls for the secondary outcomes except for the outcome; ‘days to full enteral feeds’. Six trials reported on days to full enteral feeds and found that infants who received oropharyngeal colostrum established full enteral feeding faster compared with those who received the control (Figure 3.9). However, at the study level, only two trials (Rodriguez 2011 (417) and Mota-Ferreira 2016 (505)) demonstrated a reduction in time to full enteral feeds in the OPC group compared to the control. There was some variability across the trials as indicated by the statistical test of heterogeneity (I^2=53%); albeit heterogeneity, it could be explained by variability in defining time to full enteral feeds between the included studies (100-150 ml/kg/day). Although there was a moderate heterogeneity, a fixed-effect model was used for analysing this outcome because it is more powerful and estimates the best effect, which is needed for best practice, for an intervention. Furthermore, two studies reported the data as median and interquartile range; hence, the means and SD were estimated; therefore, the observed variation in the standard deviations between the included studies can reflect the difference in the reliability of

outcome measurements; therefore, using fixed-effect model was appropriate (453). Also, using a random-effects model is primarily intended for unexplained heterogeneity. Moreover, there is more uncertainty in the I^2 when there are few studies, such as in this review (453).

This result supports the findings of previous studies in very low birth weight infants (VLBW) which reported earlier attainment of full enteral feeding with OPC use (353, 377, 419). However, these studies were small, observational studies. Another study found no difference in time to reach full enteral feeds between OPC and control (366), but this was also an observational study (before and after the adoption of OPC in a neonatal unit) that included only a total of 218 preterm infants. This reduction in time to attain full enteral feeds indicates the potential benefits of the use of OPC in preterm infants as achieving full enteral feeding earlier may have positive impacts on the incidence of NEC and infection, the leading causes of death in preterm infants (133).

Furthermore, attainment of full enteral feeding promotes the growth of the infant and may consequently improve long-term outcomes for preterm infants such as neurodevelopmental outcomes. Earlier attainment of full enteral feeding is also associated with cost-saving by minimising the use of parenteral nutrition, reducing stay in the intensive care unit, and shorten hospital stay (47, 67). Therefore, even a small effect would reduce the burden for the health services and society. As the included trials did not provide information concerning the feeding status of the participating infants during OPC administration, it is possible that a confounder effect could be created by the strategy of infant's feeding. Of note, the included trials did not show consistent evidence of an effect on length of hospital stay. The findings should be cautiously interpreted with consideration of the low quality of the evidence because the included trials had a high risk of bias, and serious impression due to small sample sizes with few events and a wide confidence interval.

Although the included studies in this review stated that, no reported adverse effects related to the procedures of OPC administration to preterm infants in the first few days after birth, selective reporting bias (an outcome non-reporting bias (490)) is a potential concern. As all

the studies had no pre-defined adverse effects and described adverse events narratively such as no adverse events with the intervention and no numerical data were provided, therefore, the estimates are not precise, and meta-analysis was not conducted. Additionally, the methods used in monitoring adverse effects, and the duration and frequency of monitoring were not detailed by the authors. However, the lack of data does not necessarily indicate that the intervention is safe. Furthermore, the studies were not blinded, have small sample sizes, and OPC was provided by different procedures. Some methods could be associated with a higher risk of adverse effects, such as using a syringe to provide fluid to an infant may increase the risk of aspiration compared to using a swab (510). Previous studies have suggested the safety of OPC but so far studies were not powered or designed to assess adverse effects (353-355, 422).

All the included trials were able to administer OPC within the first 48 hours of life; this is indicating that collection of sufficient volume of colostrum within the first 48 hours of life is possible which support the feasibility of OPC. Likewise, previous studies also have reported the feasibility of OPC (354, 366, 422).

3.5.4 Agreements and disagreements with other reviews

Based on a comprehensive search strategy, this is the first Cochrane review addressing the use of OPC in preterm infants. This review focuses on OPC administration within the first 48 hours of life. There is a previous systematic review (413) published in 2014 that assessed the effect of oral therapy with colostrum compared with no colostrum in ill newborn infants. The authors concluded that there was no strong evidence for supporting the efficacy of colostrum oral care in reducing morbidity and mortality for sick neonates. Although the previous review included different study designs (observational, retrospective, RCTs, non-randomised studies) without meta-analysis unlike this review which only included RCTs and undertook meta-analysis, the findings were generally consistent with this Cochrane review.

Of note, a recent systematic review was published in July 2018 while this Cochrane review was in the process of publication. The recent review (511), was a narrative review conducted

to address the impact of OPC on the health of preterm infants; similarly, the authors' s conclusion was generally consistent with this Cochrane review; however, it included studies with variable methodology; RCTs, non-RCTs and observational studies. This narrative review had some drawbacks such as failure to retrieve many studies (355, 358, 366, 419, 422) which were potentially relevant to its criteria.

3.5.5 Overall completeness and applicability of evidence

Participants in the included trials were very or extremely preterm infants with very low birth weight. One study reported that five of 30 participants were small for gestational age (Glass 2017) while all the included trials did not indicate small for gestational age or compromised in utero such as abnormal maternal Doppler as exclusion criteria. The majority of the trials included ventilated infants, and only Rodriguez 2011 included the need for "vasopressor medications at a dosage of > 10microg/kg/min" as an exclusion criterion. Therefore, the review findings should apply to most preterm and very low birth weight babies. Additionally, all the included trials were conducted in high-income countries. Therefore, studies from low-income countries would improve the generalisability of future studies.

3.5.6 Potential bias and limitations

Potential publication bias was the main concern with this review. An extensive search with no language and regional restrictions was conducted including reference lists of the included trials and relevant studies. Proceedings and abstracts of major perinatal conferences were also searched for any unpublished study, an attempt to minimise any publication bias. However, it is possible that some relevant studies may still have been missed. There were insufficient studies to perform a funnel plot analysis to explore possible publication or reporting bias (495). However, for this review publication bias could not be assessed as all of the included studies are small, and most of the trials have been registered in a clinical trials registry. Moreover, that these studies reported insignificant results makes it less of likely as publication bias arises when there is a tendency for the publication of large trials and

acceptance and publication of manuscripts based on positive results rather than negative or null results (512, 513).

Reporting bias was another concern; incomplete reporting of the results by some of the included trials. To minimise this bias trials authors were contacted for additional information when needed, many of them provided missing data which were reported in the review. The majority of data included in the analyses were obtained from study reports (published or unpublished), and additional information was provided by study authors. Mota Ferreira 2016 reported continuous outcomes as a median (IQR), and mean (standard deviation (SD)) was not available on request. Therefore, a normal distribution was assumed and mean ± SD was estimated (494). Moreover, intention-to-treat analysis (ITT) was not conducted by some studies. ITT analysis, which is highly endorsed to estimate the unbiased effect of an intervention in RCTs (514, 515), was applied when missing or unclear data were provided by the study authors.

Furthermore, to ensure that any bias in the review is avoided, all the stages (study search, screening of search result, data extraction and analysis and assessment of the risk of bias and quality of studies) of the review were conducted independently by the review authors.

The review results were limited by lack of participants with just of few eligible small trials enrolling a low number of participants and events and some outcomes being reported only by one trial.

3.6 Conclusion

Despite the risk of bias and the low quality of the studies, this systematic review presents updated information regarding the available evidence on the use of OPC in preterm infants and provides data for designing future clinical trials, which are still needed.

3.6.1 Implications for practice

Based on the currently available studies, there was insufficient quality evidence to ascertain if oropharyngeal administration of colostrum to preterm infants during the first 48 hours of life

can reduce the risk of NEC, late-onset infection, or death until discharge in preterm infants. There was a trend towards earlier attainment of full enteral feeding with OPC but no impact on the length of hospital stay. Results from an ongoing study may alter these conclusions.

3.6.2 Implications for research

Does OPC prevent mortality and morbidities in preterm infants? Could OPC be implemented as a part of the standard care for preterm infants? There appears that no high-quality evidence to answer those questions and additional, larger RCTs are required to answer these questions conclusively and assess the efficacy and safety of OPC in the care of preterm infants. Therefore, large well-designed randomised controlled trials are needed to assess the efficacy and safety of OPC in the care of preterm infants.

Future trials should be powered to assess the effects of OPC on clinically critical and important outcomes such as NEC and LOI. A preceding consensus on dose and procedure of OPC administration, inclusion of the most immature and smallest (including growth-restricted) infants with other intensive care needs (such as mechanical ventilation and inotropic support) will enable broader application of the evidence to groups at highest risk. Trials should also aim to evaluate the impact of OPC on prematurity-related long-term morbidities such as neurodevelopmental outcomes and chronic lung disease. Large, observational, prospectively designed studies can be undertaken to address adverse effects and long-term outcomes. As OPC can be provided by parents and caregivers, the involvement of parents and infants carers in designing trials may highlight important outcomes especially those related to the satisfaction of the parents.

Chapter 4. The impact of oropharyngeal administration of mother's colostrum on the clinical outcomes of preterm infants: a case-control study

4.1 Chapter overview

Chapter 2, showed that the UK neonatal units have adopted the use of OPC despite lack of evidence and recommended by the neonatal professionals. The Cochrane systematic review (Chapter 3) also showed that no published randomised controlled trial (RCT) investigated the effects of OPC in preterm infants in the UK. The study described in this chapter was then designed to investigate whether there is an association between OPC and clinical outcomes of preterm infants in the UK. It was prospectively designed and utilised data collected from patient medical records (medical notes and electronic patient records) using the UK National Neonatal Database (BadgerNet Neonatal) and the UK National Health Services (NHS) Trust's Digital Health Records (DHR)). This study had a favourable opinion from the Faculty of Medicine & Health Sciences Research Ethics Committee, University of Nottingham (UoN): A09102016 Audit 16-086C (Appendix 6).

4.2 Background

Preterm infants (born before completed 37 weeks of gestation) are a population at high risk of morbidity and mortality. Although advances in neonatal care have led to increases in survival rates, premature infants carry a high burden of short and long-term morbidities. Further discussion of prematurity-associated complications is described in Section 1.1.8.

OPC is a new practice introduced to deliver colostrum to preterm infants. It was postulated that early OPC (i.e. during the first week of life) could stimulate the immune system and protect the infant from infections and other conditions such as necrotising enterocolitis (NEC) (353, 356, 358, 516). More details about the compositions and benefits of colostrum and OPC were given in sections 1.1 1 and 1.16.

4.2.1 Case-control study

A case-control design is a category of observational studies. Observational studies are classified under analytical designs and subdivided into cohort, case-control and cross-sectional studies. In observational studies, there is no intervention conducted by the investigators; the researchers observe and assess the association between exposure and outcomes or disease (517).

The case-control study was first recognisably used in 1926 in a study, evaluating risk factors for breast cancer (518) and it became more famous in the biomedical research after the publication of a study that reported the association of smoking and lung cancer in 1950 (519).

In a case-control study, cases are identified by a predefined exposure, treatment or outcome which could be an intervention such as drug treatment or a surgical procedure, faced an adverse effect or suffered from a disease. Data from these cases are then compared with those of selected controls from the same population as the cases but without the exposure, treatment or outcome. Data are usually collected retrospectively. A case-control study is a useful design for evaluating associations between diseases and risk factors, and investigating rare diseases and outcomes (520); however, unrecognised confounders may bias the findings. RCTs are the gold-standard design for evidence-based medicine and classified as Level-I evidence (521). RCTs could bridge the gap regarding efficacy, safety and feasibility for interventions, however, the generalisation and implementations of the intervention in the standard care remain under the health services and in certain situations, RCTs might be questioning to conduct (522). As health services focus on more broad populations, they still depend on observational studies especially in evaluating practices, regional variations and national health outcomes (523).

Although observational studies (classified as Level-II and III evidence (521)) such as case-control studies can be especially useful when RCTs would be unethical, but they may also be used to generate hypotheses and data for future studies (442, 520). Moreover, they have the

advantages of being less expensive, quicker to conduct and usually need fewer cases in comparison to cohort and cross-sectional studies. However, case-control studies have been criticised because of the potential risk of selection and recall bias (517) and the inability to determine causal relationships.

4.2.2 Use of secondary data in research

Secondary data was defined as data that are collected for purposes other than the research for which they have been used and originally were collected by other individuals (such as universities, governments, institutions and hospitals) who were not involved in the research (524). Whilst collecting data by the researcher is more useful to answer a specific research question; however, it is not possible nor feasible to obtain all the required data, especially in longitudinal observational studies. Therefore, using secondary data is a practical tool for data collection and usually represents larger population or all population of a country and provide more comprehensive data sets (524); these may broaden the external validity of the research.

Using secondary data is cost-effective and saves time as most of the secondary data have pre-set statistical software that could provide the researcher with ready coded data in downloadable files for analysis (525). The breadth and the depth of secondary data strengthen the quality of the data especially governmental and national data sets (526); this advantage may power the quality of the research which, used those secondary data especially retrospective studies.

Secondary dataset usually designed and weighted by individuals who are specialised and experienced in the topic of the data set (524). For instance, data representing a subgroup of a population, such as the National Neonatal and Statistics Database, and specific disease registries, may provide the researchers with data that are more detailed and a larger sample regarding their target population especially if the target sample of low prevalence. Moreover, using secondary data might assist the researchers with long-term follow up as some of them are longitudinal data that have been collected over a long period (524). Secondary data also

offers the researchers with opportunities to compare their results with others who used the primary and secondary data. Therefore, secondary data analysis has been used in this study.

However, using secondary data has some disadvantages; lack of data related to the research questions and how the data was collected or the data may have different definitions for research specific variables (525). Therefore, it is important to know what the primary objective of the secondary data was. In contrast to primary data, requesting additional data, or follow-up data could not be conducted when using secondary data (526) and it may not provide information regarding a very recently adopted guidelines or policies.

4.2.3 Electronic Health Records (EHRs)

Information technology (IT) has been identified as an important empower to improve health care services. Electronic health record was defined as a digital recording of patients and population health information on a longitudinal pattern. It was emerged after an initiative from the Institute of Medicine's Quality of Health Care in America to improve and innovate the health care delivery system in 2001 (527). EHRs is patient-centred records that allow secure and instant availability of patient's information for authorised peoples. EHRs comprise very comprehensive information about patients' medical histories, laboratory tests, diagnostic images, medications and progress reports from all clinical specialities that involved in the care of a patient (528, 529). A patient EHR is a real-time record that collates the patient's current and past health information in one record, available anytime and anywhere; hence; the researchers could study health issues and interventions as occur in actual practice that may facilitate clinical implication of the findings (530).

Furthermore, EHRs could be managed and assessed by multiple organisations and health providers; helping coordination between health services and institutions. Therefore, EHRs are useful tools for improving the availability and security of information and promising methods for national and international comparisons that would lead to improved health care (531). EHRs have many advantages such as (529):

- easy and quick access to a patient's records
- reduction of medical errors and empowering a safer and reliable prescription
- cost-effective by reducing paperwork, repetitions of laboratory tests and diagnostics images
- improved decision making by incorporating the patient's data from various sources
- provide updated patient's information
- allow better communications between different health care services
- save time by easier centralised patient management and provide specific-topic queries
- enhance organisations and accuracy of health information
- facilitate clinical audits, quality improvement and support research

The use of EHRs also has some limitations such as (524, 532):

- different primary purposes from the research questions
- variability in the recorded patient's data
- design of the EHRs might have an impact on data extraction especially if the data entered in a text-free format
- missing or insufficient data or both
- accessibility to specific databases as authorisation is usually required.

4.2.4 Neonatal databases in the UK

Neonatal networks approach improves the quality of neonatal care (533), in 2003, the UK Department of Health highly recommended that neonatal units collaborate in the form of formally structured clinical, networks to provide safe and efficient maternal and neonatal care (403). Since 2004, neonatal data have been shared across neonatal units through EHRs (534). Initially started regionally and subsequently extended nationally in BadgerNet neonatal database. In 2007, the National Neonatal Research Database (NNRD) was established with the aims of improving clinical data records and neonatal services and assisting research, and currently, approximately 190 UK (England, Wales and Scotland) neonatal units contribute

data to NNRD (531, 535). The NNRD includes very comprehensive and precise data on every neonatal admission across the UK and covers all episodes of hospital stay for every newborn infant (Neonatal dataset, is an NHS approved Information Standard (535)). The BadgerNet neonatal is the primary source of data within the NNRD. The NNRD is approved by the National Research Ethics Committee; hence, for this study, the BadgerNet neonatal database was used as a source for data collection.

4.2.4.1 BadgerNet neonatal database

BadgerNet Neonatal Electronic Patient Record is a national database that provides an electronic recording of patient data in neonatal units throughout the UK (536). It was designed to assist the paperless recording of patient's information within a neonatal unit and connect to BadgerNet data from other neonatal units; also permits daily recording of events occurring during the same period. BadgerNet Platform is produced and managed by a commercial medical software company; Clevermed Limited (537), which has an agreement with the UK Trust to provide perinatal data management services for the BadgerNet platform (536). Clevermed provides secure data, live reporting of data and connects health networks in the UK. Moreover, it regularly updates the software according to the latest IT technology.

BadgerNet neonatal offers comprehensive, detailed records for neonatal units; including detailed clinical and nursing notes, charting and handover, fluids management, procedures, medications, trend monitoring and clinical reviews. It also provides clinical summary reports such as admission, pregnancy and labour details and detailed discharge letters from recorded data during the stay at the neonatal unit (531).

BadgerNet is a useful tool for data collection in clinical research, and most of the recorded items are similar to those required for research purposes and expected to be more valid than administrative data (538, 539). Furthermore, BadgerNet neonatal has been designed to offer easily searchable and extractable data for the researchers. However, missing data, lack of standardisation for some clinical variables could be significant limitations. In BadgerNet

neonatal, this issue has been minimised by using a structured list of items (540) and providing a descriptive dictionary for the data.

Of note, data within the Badger Neonatal database are regularly assessed by the National Neonatal Audit Programme (NNAP), a project carried out by the Royal College of Paediatrics and Child Health (RCPCH) to assess and monitor the quality of care provided by the neonatal units in England, Scotland and Wales (414). The quality of the data is also monitored by the performing and publications of studies that evaluate the accuracy, validity and quality of the recorded data (541). Therefore, BadgerNet neonatal has been recognised to be an appropriate, accessible and cost-effective source of data.

4.2.5 Rationale of the study

According to a survey of neonatal professionals presented in Chapter 2, OPC use is progressively increasing among UK neonatal units with variable practice between the units. However, to the best of my knowledge, no previous studies have explored the clinical impact or feasibility of using OPC in preterm infants in the UK. In this chapter, I evaluate the effects of OPC administration on the health outcomes of preterm infants and assess the feasibility of performing this practice in the UK neonatal units.

4.2.6 Hypothesis and aim

This study hypothesised that administrating mother's colostrum by the oropharyngeal route to preterm infants during the early neonatal period would lead to improved feeding tolerance of preterm infants, shorten hospital stay and improve nutritional outcomes while decreasing the incidence of prematurity-related morbidities.

This study aimed to evaluate the impact of OPC during the early neonatal period on the short-term clinical outcomes of preterm infants and to generate pilot data to guide potential future research.

4.2.7 Objectives

4.2.7.1 Primary objectives

The primary objectives of the study were:

- to assess the hypothesis that oropharyngeal administration of mother's own colostrum is associated with improved feeding tolerance in preterm infants and shorter time to full enteral feeds
- to evaluate the feasibility of assessing the efficacy in preterm infants.

4.2.7.2 Secondary objective

The secondary objective of this study was:

- to determine if there are relationships between OPC administration and short-term clinical outcomes of preterm infants such as NEC, sepsis and nutritional outcomes.

4.3 Methods

In February 2017, in the UK, the Nottingham Neonatal Service (i.e. the Neonatal Units of the Queen Medical Centre (QMC) & City Hospital (CH) Campuses of Nottingham University Hospitals NHS Trust (NUH)), adopted OPC for the care of preterm infants as a quality improvement project supported by a guideline (396); Appendix 7). The two Nottingham neonatal units are neonatal intensive care units (NICUs), specialised in the management and care of preterm and sick newborn infants. They are the neonatal lead units for the Trent Perinatal Network; one of the clinically managed Operational Delivery Networks for the UK neonatal services (401). Together, the two neonatal units provide neonatal intensive care, high dependency care (24 beds) and special care (14 beds). The average annual admission of babies to the units is approximately 1500 babies with preterm infants (less than 37 weeks gestation) constituting about 45% of the total yearly admissions.

4.3.1 Study design

The study design was an observational, historical case-control study that compared preterm infants before, and after, the implementation of oropharyngeal colostrum guideline in the care

of preterm infants. It was a single centre study conducted at the two Nottingham neonatal units (QMC and CH) in the UK.

A matched case-control design was chosen to minimise potential confounding by reducing baseline differences between the two groups. Controls were matched for the well-known risk factors that influence the outcomes of preterm infants (151, 542). The controls were matched to OPC cases by sex, gestational age and the closet birth weight, in a ratio of 2:1 to increase the power of the study and ensure comparability between the study groups (543). Pre-defined inclusion and exclusion criteria were used to avoid possible selection bias that can limit the validity of research using this type of study design (517).

The study compared two groups: an intervention group (OPC) and a control group (Pre-OPC). The OPC cohort included preterm infants admitted to the neonatal units after the adoption of OPC (February, 2017) and received OPC by the unit guideline. The control group (Pre-OPC) was identified from preterm infants admitted to Nottingham neonatal units before the adoption of OPC use.

4.3.2 Participants

Participants were preterm infants < 32 weeks' gestation or/ and ≤1500g birth weight. For the intervention (OPC group), eligible infants were identified from the neonatal units at the QMC and City hospitals. For the control (Pre-OPC) group, participants were identified from the list of preterm infants admitted to the two Nottingham neonatal units before the adoption of OPC using the BadgerNet Neonatal for the Nottingham neonatal service.

4.3.2.1 Inclusion criteria

- Gestational age less 32 weeks.
- Birth weight of 1500g or less.
- Admission to neonatal units within 96 hours of birth.

4.3.2.2 Exclusion criteria

- Major congenital anomalies.

- Maternal HIV infection.
- Maternal drug use as a known contraindication to breastfeeding.

Other exclusion criteria for enteral feeding in the neonatal units, such as inotrope use did not constitute exclusion criteria for this study.

4.3.3 Data collection

4.3.3.1 Source of data

Data were collected from the infants' medical records. For the OPC group, the data were collected prospectively from the infant's medical notes, nursing charts and records. Badger Neonatal database and the NHS Trust's DHR were also used as needed such as when the infant discharged to a postnatal or paediatric ward. For the Pre-OPC group, all data were primarily extracted from the Badger Neonatal database. The NHS Trust's DHR has also been used if data were missed or unavailable in the Badger database.

4.3.3.2 Types of data

The following demographic and clinical data of the participants were collected:

- Date of birth, date & time of admission to the neonatal unit, gestational age, sex, birth weight, date of hospital discharge.
- Clinical characteristics of each infant such as:
    - mode of delivery, multiple gestations, delivery room resuscitation, 1 & 5-min Apgar score, non-invasive ventilation, mechanical ventilation, central line placement, nasogastric tube, use of parenteral nutrition and medications
    - detailed feeding history, including; type of milk, mode of feeding, date of commencement, volume received during the intervention, date of attainment of full enteral feeding and discharge feeding
    - morbidities during the hospital stay, including; NEC defined as Bell's criteria stage II or more, clinical or culture-proven late-onset sepsis, pneumonia and any serious complications
    - death
    - weight and clinical status on discharge home.

- Administration of oropharyngeal colostrum included; time of starting, the frequency of OPC, the number of doses and total volume received by an infant, days of receiving OPC and adverse effects related to the OPC procedure.
- Maternal history, depending on data that were available from the infant's record and included; age, multiple pregnancies, pre-eclampsia, premature rupture of membrane, prolonged rupture of membranes (>12hours), antibiotic therapy, antenatal steroid administration, antenatal infection, other medical illness.

4.3.4 Outcome measures

4.3.4.1 Primary outcomes

- Days to attain full enteral feeding defined as (150 ml/kg/day) sustained for 72 hours.
- Feasibility of OPC administration defined as an infant received OPC within 96 hours of life and received 50% or more of the planned doses.

4.3.4.2 Secondary outcomes

- Length of hospital stays to discharge home (days).
- Days to start enteral feeds (gavage, oral feeding or both).
- Days of parenteral nutrition received by the infant.
- Days of mechanical ventilation the infant had.
- Incidence of NEC (defined as modified Bell's criteria ≥ II (167).
- Late-onset sepsis (LOS): clinically suspected and culture confirmed after 72 hours of life.
- Adverse events within 60 minutes of OPC administration:
    - bradycardia - decrease in heart rate to <100/min
    - tachycardia - increase in heart rate to >200/min
    - tachypnea - increase in respiratory rate to >80/min
    - apnoea - cessation of breathing for >20 seconds
    - decrease in oxygen saturation (SpO_2 %) to <80%

- milk aspiration (an episode of choking/milk in the mouth with consistent chest X-ray changes).

Data related to OPC administration was not available in the electronic databases (Badger and Trust DHRs), data for adverse events were exclusively and prospectively collected from the infants' record charts at the neonatal units. Nursing reports and medical notes were also used for collecting these data. A study-specific form was created for collecting data for OPC administration, including the adverse events (Appendix 8).

- Incidence of death before discharge to home.
- Rates of breastfeeding at hospital discharge to home (exclusive/mixed/none).
- Weight at hospital discharge. Weight-for-age Z (WAZ) score was used to assess weight against weight for age percentiles. WAZ score was calculated using clinical actual age percentile Z-score calculator (544).

Weight-for-age, height-for-age and weight-for-height, Z-scores are widely recognised as the best approach to analyse and present anthropometric data (545). The Z-score is the number of standard deviations (SD) above or below the reference mean or median of a dataset (545). It can compare results across age groups. Z-score allows combining sex and age groups and can be computed as summary statistics such as means and SD that can be used to describe nutritional status for population (545). The cut-off point of <-2 SD to >+2 SD was used by the World Health Organization (WHO) to classify nutritional status child growth and malnutrition (546).

4.3.5 Blinded Endpoint Reviews (BERs)

BERs were performed for the outcome incidence of NEC during the hospital stay to determine definite NEC cases and ensure they have met the pre-specified criteria. Endpoint refers to a targeted outcome of a clinical study that can be measured objectively to investigate if the studied intervention has effects, such as survival, the incidence of disease, and quality of life (547). BERs are used to reduce the risk of variation in important clinical outcomes.

NEC is a critical clinical outcome with significant short and long-term sequelae in preterm infants. Lack of consistent case-definition may potentially lead to difficulty deciding if an infant had NEC (548), and may also lead to variation in reporting NEC cases in the Badger neonatal database where data are documented as part of routine clinical care.

Two clinicians (subspecialist neonatal registrars) independently reviewed the information of all cases of a possible diagnosis of NEC among the study cohort. The reviewers were also masked/blind to the study groups. The two reviewers were independent of the study to avoid any possible perception bias. If a consensus could not be reached, cases would be discussed with a third reviewer (consultant neonatologist). Based on the final decisions of the reviewers, NEC has been recorded as confirmed or not occurring.

A BERs form (Appendix 9), containing data on all cases potentially diagnosed with NEC, was prepared and submitted to the reviewers as either as a paper or electronic copy based on reviewer preference. Every reviewer assessed each case and completed the form and returned it to the study investigator. The two independent reviews were compared for each case, and whenever the reviews did not agree, this was clarified by discussion between the two reviewers.

4.3.6 Statistics

4.3.6.1 Sample size

This study was originally powered to demonstrate a likely difference in the primary outcome (time to full enteral feeds). The sample size was based on the results of previous studies [estimated mean ± SD, Pre-colostrum: 29.3 ± 15.6; Colostrum: 25.3 ± 12.8 days); p = 0.02 (353)] and [mean ± SD, Placebo: 24.17 ± 8.66; colostrum: 14.29 ± 5.74); p = 0.03 (417)], assuming a mean difference in the time for attainment of full (150ml/kg/day) enteral feeds of four days between the two groups. With a significance level of 0.05 and power of 80%, 111 infants would be needed to detect a clinical difference of four days in the primary outcome (days to full enteral feeds). It was also based on the cases being matched by a 1:2 ratio with

controls (2 controls per 1 OPC case; 37 for the OPC group and 74 for the pre-OPC group) to increase the strength of the study.

After the study commenced, it became apparent that hazard ratio analysis was a more appropriate and powerful way to analyse these data. A post hoc power calculation was therefore; performed before assessing the data using this method. Based on two to one allocation ratio, an alpha of 5% (nQuery software power calculation (46)), found that the study sample size of 111 infants provided approximately 80% power to detect a hazard ratio of 0.5 or 90% power to detect an HR of 0.45, assuming minimal drop-outs.

4.3.6.2 Statistical analysis

Statistical analysis was conducted using the Statistical Package of the Social Science version 23 (IBM 2013, (549)) for Windows and statistical significance described with a p-value of <0.05.

Continuous variables were tested for normality using histograms. Data were described according to the data distribution as a mean ± standard deviation (SD) for normal distribution and median (interquartile range (IQR)) for skewed data. Comparisons between the study groups, for continuous variables, and differences in outcomes were summarised using independent t-tests for parametric data and Mann Whitney U tests for non-parametric data.

Categorical data were presented using descriptive analysis (frequencies and percentages), and the Pearson Chi-Square test or Fishers' exact tests were used for comparison between the two groups.

Survival analysis was used to analyse time to event variables such as time to reach full enteral feeds, length of hospital stay, days of parenteral nutrition and days of mechanical ventilation. Multivariate analysis was conducted to address potential confounding when there were statistically significant differences between the study groups.

Reliability analysis, using Krippendorff alpha (Kalpha), was conducted to evaluate agreement between the reviewers of the BERs and the study investigator. Kalpha (550) is a reliability

coefficient that used in content analysis to determine agreement among independent raters, observers or measuring instruments; however, it is applicable where two or more approaches are performed to the same unit of analysis. Kalpha differs from other reliability coefficients, such as Cohen's Kappa, in that it can be applied to a various number of observers, categories and sample sizes. It also applies to any scale or measurement levels such as nominal, ordinal, interval or ratio and allows for reliability with missing data (550). Kalpha' s range is $1 \geq \alpha \geq 0$, Kalpha > 0.8 indicates strong inter-rater reliability (551).

4.3.6.3 Dealing with missing data

As this study was a single centre, matched case-control study, participants were similar for most of the baseline characteristics. Therefore, data were analysed by the complete case basis; for each variable; only those infants with complete data are included in the final analysis.

Sensitivity analyses were also performed for missing data. A variable was considered incomplete if ≥ 5% was missing. Sensitivity analysis is another approach to assess the effect of missing data on the final analysis. In this analysis, possible values are imputed for the missing data using different scenarios such as worst-case and best-case scenarios and hot deck imputation (552, 553). Worst and best case scenarios involve replacing missed values with favourable outcomes in one group and poor outcomes in the comparison group. Hot-deck imputation is a method (552) that involves replacing the missed data with a value from a similar available case within the same study. In this study, I used the data of the corresponding matched case for every missing value. If the results are consistent with each scenario, the results should be more robust.

4.3.7 Ethical considerations

4.3.7.1 Ethical approval

The study was conducted as a clinical audit of practice against the current, Nottingham Neonatal Service Guideline (396), for the administration of OPC to infants in the NICUs

within the Nottingham University Hospitals NHS Trust (Audit number: ID 16-086C). According to the Health Research Authority (HRA) decision-tool (554), this study did not require the full Ethics Committee review. A favourable opinion was also received from the Faculty of Medicine & Health Sciences Research Ethics Committee, UoN (reference No: A09102016 Audit 16-086C (Appendix 6)). The research was conducted according to the laws and regulations of the UK and the HRA (373).

4.3.7.2 Data management and confidentiality

The policy regarding the data of the study was as follows:

- ensure the confidentiality of participants
- ensure the secure storage of data.

Each participant was allocated a study identity number (ID), for use on data collection sheets, and in the study electronic records, which also included a second anonymous identifier (Badger ID) as is considered best practice. A separate confidential record of the participant's name, date of birth, Badger ID, local hospital number or NHS number, and the study ID was made to permit identification of all participants enrolled in the study in case additional follow-up was required. The master file linking the study ID with the infants' identifiable information was kept securely and separately at the Division of Child Health, Obstetrics & Gynaecology (COG) of the UoN.

The data collection sheets were handled securely according to ethical regulations and best practice. The sheet was filled by hand using a black pen. Data were imported anonymously to electronic records, and all paper forms were stored in a locked cabinet in an authorised access restricted area in the Division of COG, UoN, according to the University of Nottingham Code of Research Conduct and Research Ethics (372). Only members of the study team have access to this cabinet.

The electronic data collection file was password-protected and stored in a secure dedicated web server (Z-drive, UoN) and a password-protected computer provided by the UoN. All

study documents were updated on 21. 05. 2018 according to the UK new General Data Protection Regulation (GDPR) (55) and Data Protection Act 2018 (56).

4.3.7.3 Safety considerations

The intervention was part of the care of preterm infants in Nottingham neonatal units. The study did not include samples or investigations beyond those considered as standard care in the neonatal units. All study data were routinely recorded clinical items that can be collected from infant health records. There was no direct contact with the participants' families nor carers.

4.4 Results

From March 1st 2017 to February 6th 2018, 1481 babies were admitted to Nottingham neonatal units at the Nottingham University Hospitals NHS Trust; QMC and City Hospital, UK of whom 593 infants were born before a gestational age of 37 weeks. One hundred sixty-three infants were born < 32 weeks of gestation, and these infants were expected to receive OPC according to the unit guidelines. Infants were included in the study as soon as the infant received OPC.

Based on the infant's records, fifty-two infants who received OPC were identified, of these, 15 infants were excluded (Figure 4.1). Thirty-seven infants who received OPC were therefore included in the study as the OPC group. The control (Pre-OPC) group consisted of 74 matched preterm infants who were admitted before the implementation of OPC in the neonatal units; from December 2012 to December 2016 (Figure 4.1).

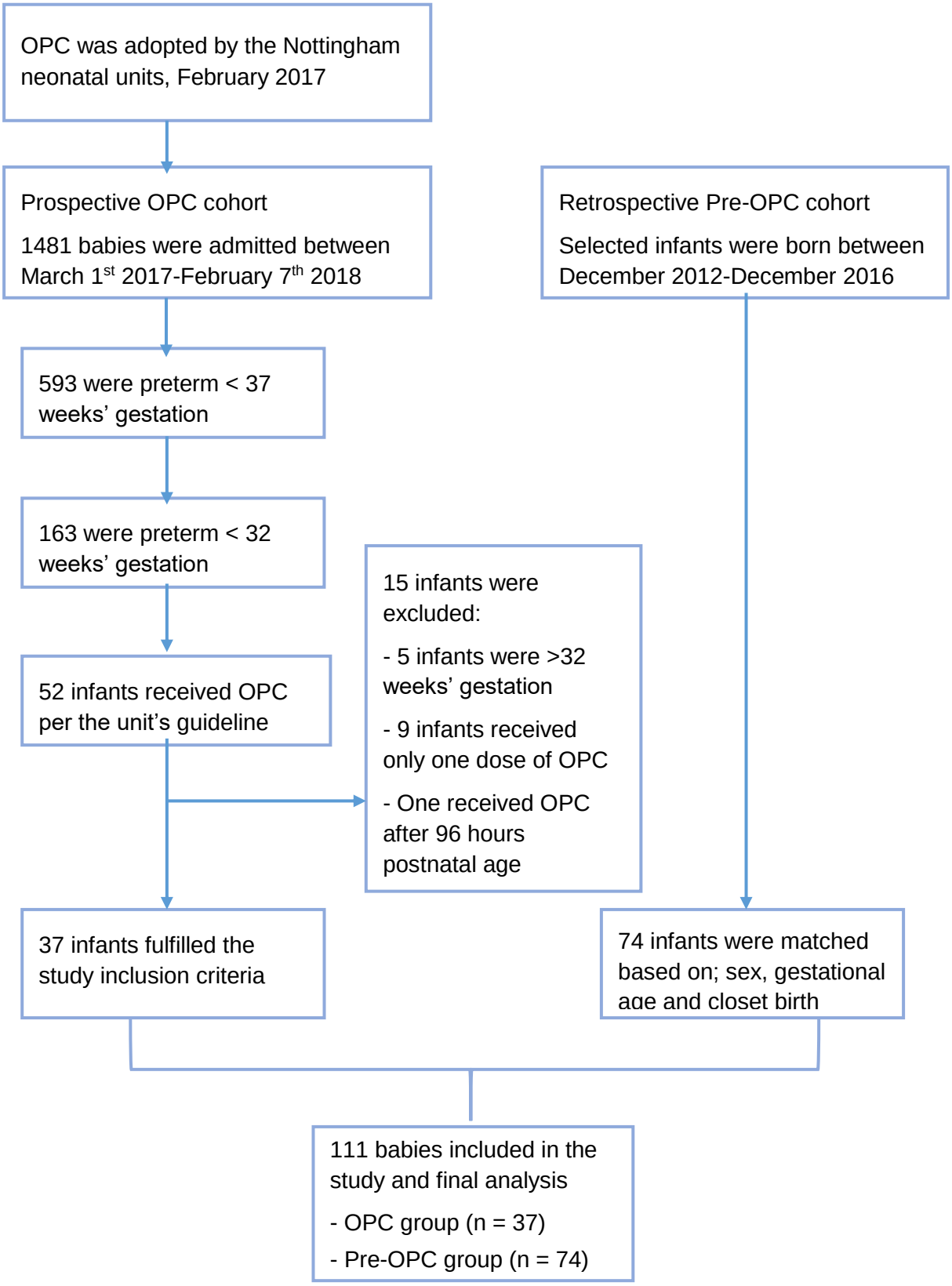


Figure 4.1 Study flow chart

Selection of participant infants. n: number of infants; OPC (oropharyngeal colostrum) group; the intervention; Pre-OPC group: the control

4.4.1 Baseline characteristics

4.4.1.1 Infants' characteristics

The study sample was predominately male (64.9%). The mean ± SD gestation age was 27.6 ± 2.37 weeks, and the mean ± SD birth weight was 1042.5 ± 343.51 g. As matched groups, gestational age, gender and birth weight were similar between Pre-OPC and OPC groups (Table 4.1). There were no statistically significant differences in the baseline characteristics of the infants between the Pre-OPC group and the OPC group. Table 4.1 compares the infants' baseline characteristics by study group.

Table 4.1 Infants baseline Characteristics

Characteristic	Pre-OPC (n = 74)	Post-OPC (n = 37)	p value
Male*, n (%)	50 (67.6%)	25 (67.6%)	1.0[f]
Gestational age*, week Mean± SD	27.6±2.37	27.5±2.41	0.98[t]
Birth weight* g, Mean ± SD	1041.2 ± 337.98	1045.6 ± 359.04	0.95[t]
Weight/GA, n (%) AGA SGA	 70 (94.6) 3 (4.1%)	 34 (91.9%) 2 (5.4%)	1.0[f]
Weight Z score at birth, Mean± SD	0.039 ± 0.70	0.035 ± 0.76	0.97[t]
Agar at 1 min, Median (IQR)	6 (4, 8)	6 (4, 8)	1.0[U]
Apgar at 5 min, Median (IQR)	8 (7, 9)	8 (6.75, 9)	0.71[U]
First Admission temperature Mean ± SD	36.59 ± 0.56	36.52 ± 0.30	0.69[t]
Inotropes, n (%)	30 (40.5%)	14 (37.85%)	0.83[f]
IUGR, n (%)	9 (12.2%)	4 (10.8%)	0.87[f]
Major congenital anomalies, n (%)	5 (6.8%)	3 (8.1%)	1.0[f]
Postnatal steroid therapy, n (%)	14 (18.9%)	6 (17.6%)	1.0[f]
CLD	39 (52.7%)	12 (35.3%)	0.12[f]
Received EBM during stay in neonatal unit	72(97%)	37 (100%)	0.55[f]

OPC: oropharyngeal colostrum; n: number; SD: standard deviation; AGA: appropriate for gestational age; SGA: small for gestational age; LGA: large for gestational age; IQR; interquartile range; IUGR: intrauterine growth restriction; CLD: chronic lung disease; EBM: expressed breast milk; t: independent t-test; U: Mann-Witney test; f: Fischer's Exact test.
*: Matching criteria

4.4.1.2 Maternal baseline characteristics

Most maternal characteristics recorded were also similar between the two groups, Table 4.2.

Table 4.2 Maternal baseline characteristics

Characteristic	Pre-OPC, (n = 74)	Post-OPC (n = 37)	P value [f]	Missing data
Multiple pregnancies, n (%) Singleton Twins, Triplets	 53 (71.6%) 20 (27%) 1 (1.4%)	 28 (75.7%) 9 (24.3%) 0 (0%)	0.88	0%
Fetal presentation Cephalic, n (%) Breech, n (%)	 38 (52.1%) 29 (39.7%)	 23 (62.2%) 13 (35.1%)	0.55	6.4%
Mode of delivery SVD, n (%) CS, n (%)	 34 (45.9%) 38 (51.4%)	 20 (54.1%) 18 (48.6%)	0.48	1.8%
Antenatal steroid, n (%)	64 (86.5%)	35 (94.6%)	0.47	0.9%
Prolonged rupture of membrane, n (%)	29 (39.2%)	8 (21.6%)	0.16	6.3%
Chorioamnionitis, n (%)	6 (8.1%)	2 (5.4%)	0.02	9.9%
Preeclampsia, n (%)	9 (12.2%)	5 (13.5%)	0.08	4.5%
Smoking during pregnancy, n (%)	14 (18.9%)	4 (10.8%)	0.29	9.9%
Abnormal antenatal Doppler AREDF, n (%)	5 (6.8%) 42%	3 (8.1%) 47%	0.26	49%
Intrapartum pyrexia, n (%)	13 (17.6%)	1 (2.7%)	0.01	8.1%

OPC: oropharyngeal colostrum; n: number; SVD: spontaneous vaginal delivery; CS: Caesarean section; AREDF: Absent or reverse end diastolic flow; [f]: Fischer's Exact test. Values: frequencies (%)

There was a statistically significant difference between the two groups for the incidence of chorioamnionitis (p = 0.02). However, there were missing data for 9.9% (where data were not

recorded in the Badger database nor in the infants' medical notes) unbalanced between the groups for these variables. Therefore, a sensitivity analysis was conducted to evaluate the effect of the missing data. Assuming the data were missing at random, using complete-case analysis yielded different results for the variable chorioamnionitis (from p = 0.02 to 1.0). I imputed the missing values using different scenarios; worst case (had chorioamnionitis and coded as yes) and best case (did not have chorioamnionitis and coded as no) as shown in Table 4.3.

Table 4.3 Sensitivity analysis for chorioamnionitis

Assumption	Pre-OPC with missing data (n=3)	OPC with missing data (n=8)	Chorioamnionitis		P value [f]
			Pre-OPC	OPC	
Worst Case Analysis	Yes	Yes	9 (12%)	10 (27%)	0.06
Best Case Analysis	No	No	6 (8.1%)	2 (5.4%)	1.0
Best-Worst Imputation	No	Yes	6 (8.1%)	10 (27%)	0.01
Worst-Best Imputation	Yes	No	9 (12%)	2 (5.4%)	0.33[f]
Hot Deck Imputation [m]	NO/Yes	NO/Yes	6 (8.1%)	5 (13.5%)	0.72

OPC: oropharyngeal colostrum; n: number; Yes: Worst (had chorioamnionitis); No: Best (did not have chorioamnionitis); [f]: Fischer's Exact test; [m]: missed values are imputed based on values of the matched cases.

Similarly, there was a statistically significant difference between the two groups for the incidence of intrapartum pyrexia (p = 0.01). However, there were missing data for 8.1%. The complete-case analysis yielded different results; the p value changed from 0.01 to 0.06. Missing data were also imputed; worst case (had intrapartum pyrexia and coded as yes) and best case (did not have intrapartum pyrexia and coded as no) as shown in Table 4.4. This indicates that the missing data may have an impact on the significant differences for these two variables.

Table 4.4 Sensitivity analysis for Intrapartum pyrexia

Assumption	Pre-OPC with missing data (n=3)	OPC with missing data (n=6)	Intrapartum pyrexia		P value [f]
			Pre-OPC	OPC	
Worst Case Analysis	Yes	Yes	16 (21.6%)	8 (21.6%)	1.0
Best Case Analysis	No	No	13 (17.6%)	2 (5.4%)	0.13
Best-Worst Imputation	No	Yes	13 (17.6%)	8 (21.6%)	0.61
Worst-Best Imputation	Yes	No	16 (21.6%)	2 (5.4%)	0.03
Hot Deck Imputation [m]	NO/Yes	NO/Yes	13 (17.6%)	2 (13.5%)	0.13

OPC; oropharyngeal colostrum; n: number; Yes: Worst (had Intrapartum pyrexia); No: Best (did not have Intrapartum pyrexia); f: Fisher's Exact Test; [m]: missed values are imputed based on values of the matched cases.

There were also missing data (per the study protocol, > 5%) for other maternal characteristics namely, fetal presentation, prolonged rupture of membrane, smoking during pregnancy and abnormal antenatal Doppler. There were no significant differences between the study groups for these variables (Table 4.2). However, a sensitivity analysis was conducted using complete-case analysis, which did not change the significant differences between the two groups for these variables. Therefore, further sensitivity analyses were not conducted.

4.4.2 Administration of OPC

The OPC group included 37 infants. OPC administration was started within the first 48 hours of life in 62 % of the participant infants (Figure 4.2).

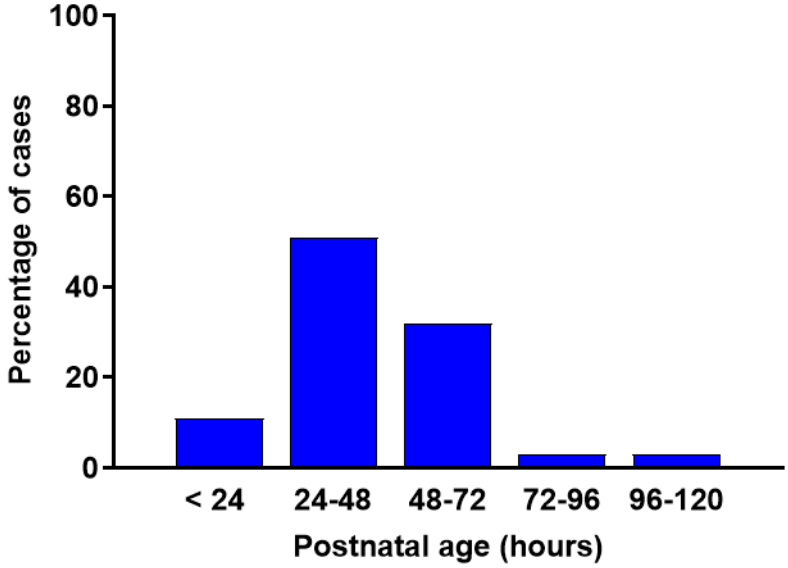


Figure 4.2 Postnatal age of receiving OPC
Postnatal age (hours) when preterm infants (<32 weeks) started OPC. Bar: percentage of infants; <24: (n=4); 24-48: (n=19); 48-72: (n=12); 72-96: (n=1); 96-120: 9n=1).
OPC: oropharyngeal colostrum

Based on the Nottingham Neonatal Service's guideline for OPC, each infant would be expected to receive a total of 3.6 ml of colostrum divided into18 doses over 3 days. The median (IQR) volume of colostrum received by an infant was 2 (1.3, 2.8) ml. The Median (IQR) of OPC doses received was 10 (6, 12) doses (10/37 (27%) infants received <50% of the planned doses; 14/37 (38%) received 50-70% and 13/37 (35%) >70% doses).

The mean ± SD duration of receiving OPC by an infant was 2.6 ± 0.7 days (Figure 4.3). During the period when OPC has been provided, only 7/37 (19%) infants received trophic feeding by nasogastric tube along with OPC administration.

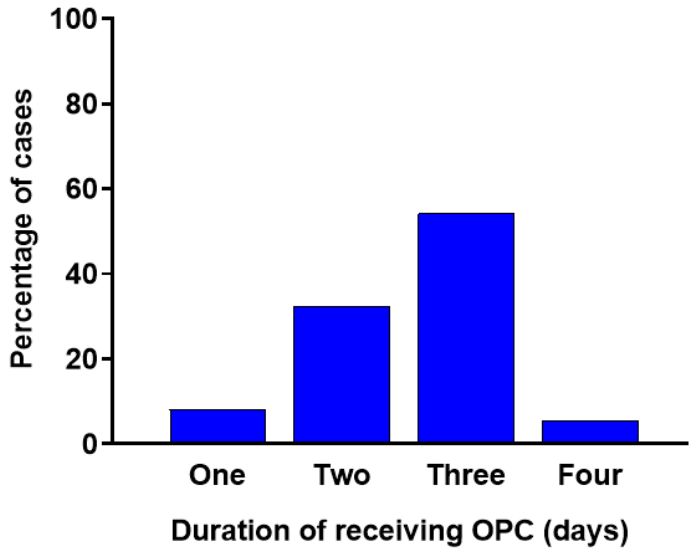


Figure 4.3 Duration of receiving OPC
Period (days) during which preterm infants (<32 weeks) received OPC; Bars: percentage of infants; one day: (n=3); 2 days; (n=12); 3 days: (n=20); 4 days: (n=2).
OPC: oropharyngeal colostrum

4.4.2.1 Adverse effects with the OPC procedure

Adverse events defined per the study protocol (section 4.3.4.2) and recorded within 60 minutes of OPC administration were reported.

Based on the infants' clinical records and using the adverse effects study-specific form, there were no adverse effects reported during the administration of OPC. Specifically, there were no bradycardia, tachycardia, tachypnea, hypotension, oxygen saturation decreases, and choking or aspiration events during or soon after, the OPC administration procedure.

4.4.3 Primary outcomes

4.4.3.1 Days to full enteral feeds

Infants who received OPC achieved full enteral feeds faster compared to those who did not receive OPC. There was a statistically significant difference between the study groups in days to full enteral feeds, the median (95% confidence interval (CI)); (Pre-OPC: 18 (95% CI; 13.49 to 22.50); OPC: 14 (95% CI; 9.61 to 18.38); p=0.004) Figure 4.4.

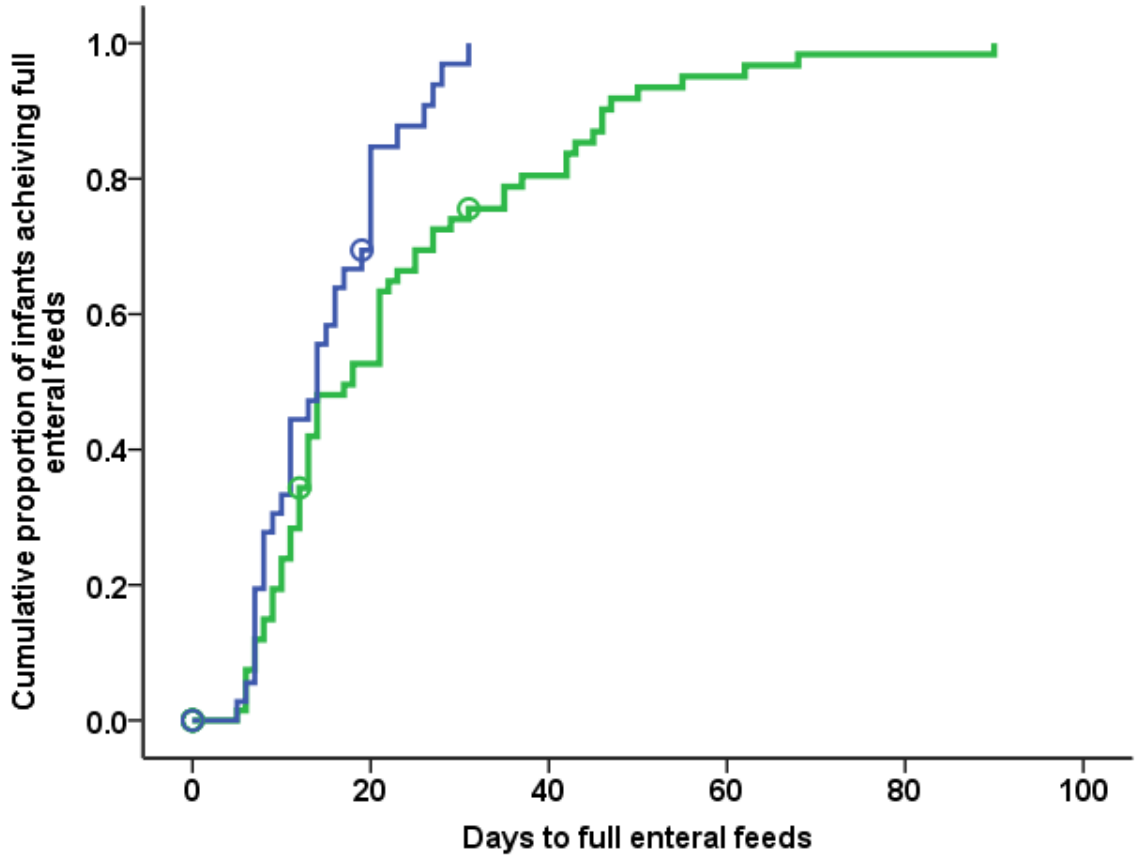


Figure 4.4 Days to full enteral feeding

Kaplan-Meier plot demonstrating the probability of days to attain full enteral feeds among preterm infants (<32 weeks) who received OPC and those who did not receive OPC. The median for each group corresponds to the probability of 0.5. Green line: Pre-OPC group (n=74); Blue line: OPC group (n=37); Circle marker on each line: censored (died); p= 0.004 (Log-rank test); OPC: oropharyngeal colostrum.

Most infant and maternal characteristics were similar between the two groups except for maternal chorioamnionitis and intrapartum pyrexia. Therefore, Cox regression analysis was conducted to investigate the effect of maternal chorioamnionitis and intrapartum pyrexia on days to full enteral feeds. Cox regression showed that the effect of OPC did not change by adjusting for maternal chorioamnionitis (adjusted p = 0.004) and intrapartum pyrexia (adjusted p = 0.005), Table 4.5. However, the variables maternal chorioamnionitis and intrapartum pyrexia had significant numbers of missing data (9.9% and 8.8% respectively), which were unbalanced between the two groups (Table 4.3, Table 4.4). Regression analysis for maternal chorioamnionitis, using complete case analysis, did not change the estimate of the OPC effect (unadjusted Hazard ratio (HR): 1.83 (p = 0.01); adjusted HR: 1.81 (p = 0.01)).

NEC is a critical clinical outcome for preterm infants, and the study cohort had a high NEC rate that was reported more often in the Pre-OPC group. In univariate analysis, NEC was a significant predictor of days to full enteral feeds. There was a statistically significant difference for the days to full feeds between infants with NEC (median (95% CI): 37 (95% CI; 15.8 to 58.2) and infants without NEC: 13 (95% CI; 11.6 to 14.4) days; p = 0.001), Figure 4.5.

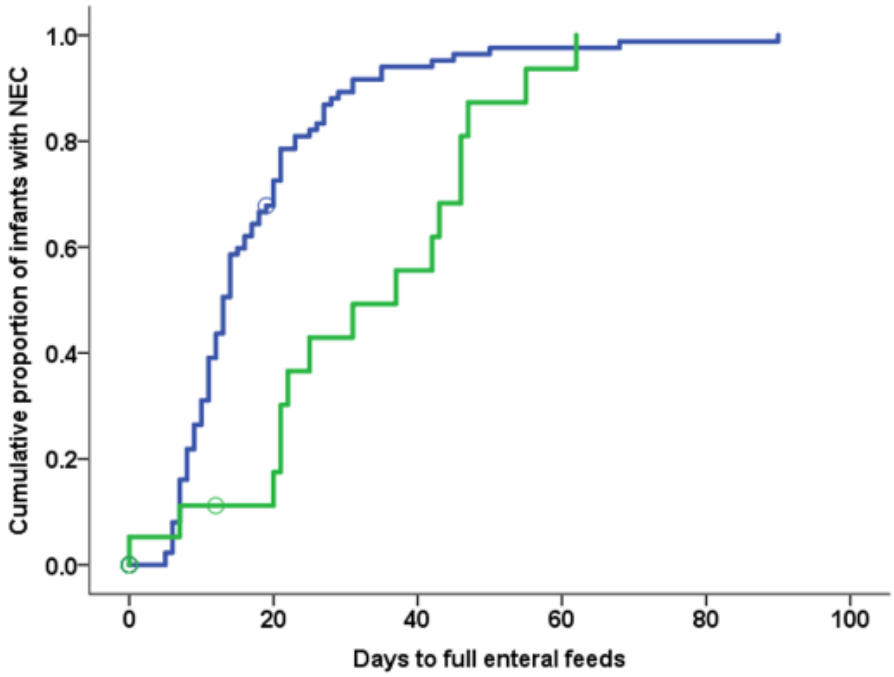


Figure 4.5 Days to full enteral feeding and NEC
Kaplan-Meier plot demonstrating the probability of days to attain full enteral feeds among preterm infants (<32 weeks) who had NEC and those who did not have NEC. The median for each group corresponds to the probability of 0.5. Green line: NEC (n=18); Blue line: no NEC (n=93); p= 0.001(Log-rank test); Circle marker on each line: censored (died); NEC: necrotising enterocolitis

Although there was no statistically significant difference in the incidence of NEC between the study groups, another model was undertaken to investigate whether NEC might confound the effect of OPC on days to full enteral feeds. In a multivariate Cox regression, adjusting for NEC slightly decreased the effect of OPC (adjusted p = 0.02). OPC remained a significant dependent predictor for days to full feeds; however, NEC was a weak confounder effect on this association. In a multiple regression model to further control for the covariates; confirmed NEC, maternal chorioamnionitis and intrapartum pyrexia made a slight difference to the

effect estimate (unadjusted HR: 1.90; adjusted HR: 1.71; adjusted p=0.02), OPC remained a significant factor (Table 4.5).

Table 4.5 Cox regression for the effect of OPC on days to full enteral feeds in preterm infants (<32 weeks gestation)

	HR (95% CI)	P value
Unadjusted analysis	1.90 (1.22, 2.96)	0.004
Adjusted for NEC	1.74 (1.12, 3.15)	0.022
Adjusted for Maternal chorioamnionitis	1.99 (1.26, 3.06)	0.004
Adjusted for maternal intrapartum-pyrexia	1.98 (1.25, 3.14)	0.005
Fully* adjusted analysis	1.71 (1.06, 2.74)	0.026

HR: hazard ratio; CI: confidence interval; OPC: dependent variable
*: multiple regression model adjusted for confounders NEC, maternal chorioamnionitis and intra-partum pyrexia; NEC: necrotising enterocolitis

4.4.4 Secondary outcomes

4.4.4.1 Length of hospital stay

The mean ± SD of the length of hospital stay for the study population was 67.6 ± 34.54 days. Survival analysis showed no significant difference between the two groups in the length of hospital stay, median (95% CI); (Pre-OPC: 73 (95% CI; 61.5 to 84; OPC: 62 (95% CI; 58.4 to 65.5); p = 0.84). The Kaplan-Meier graph also showed close approximation and crossing of the two curves (Figure 4.6). Therefore, Cox regression analysis to explore potential confounders could not be performed.

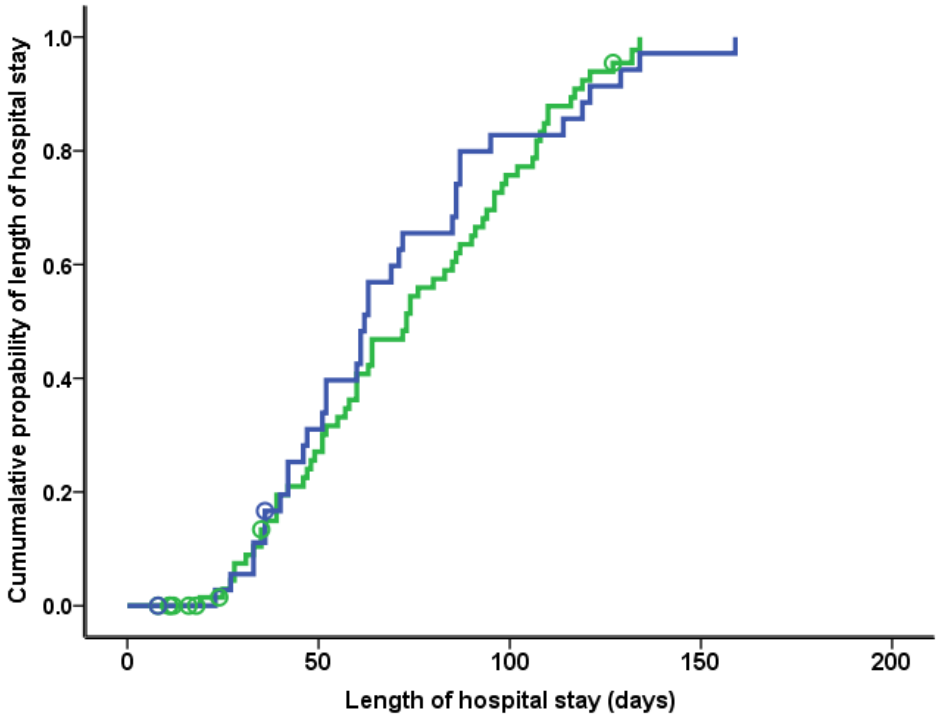


Figure 4.6 Length of hospital stay

Kaplan-Meier plot demonstrating probability of the length of hospital among preterm infants (<32 weeks) who received OPC and those who did not receive OPC. The median for each group corresponds to the probability of 0.5. Green line: Pre-OPC group (n=74); Blue line: OPC group (n=37); p = 0.84 (Log-rank test); Circle marker on each line: censored (died); OPC: oropharyngeal colostrum.

4.4.4.2 Postnatal day of starting enteral feeding

The number of infants available for this analysis was 109/111 (98%) infants; (Pre-OPC: 73/74; OPC: 36/37 infants) as two infants died before receiving any enteral feeds. There was a statistically significant difference between the two groups for postnatal days of starting enteral feed, median (IQR) (Pre-OPC: 5 (3,10) days; OPC: 4 (2, 5) days; p = 0.006), Figure 4.7.

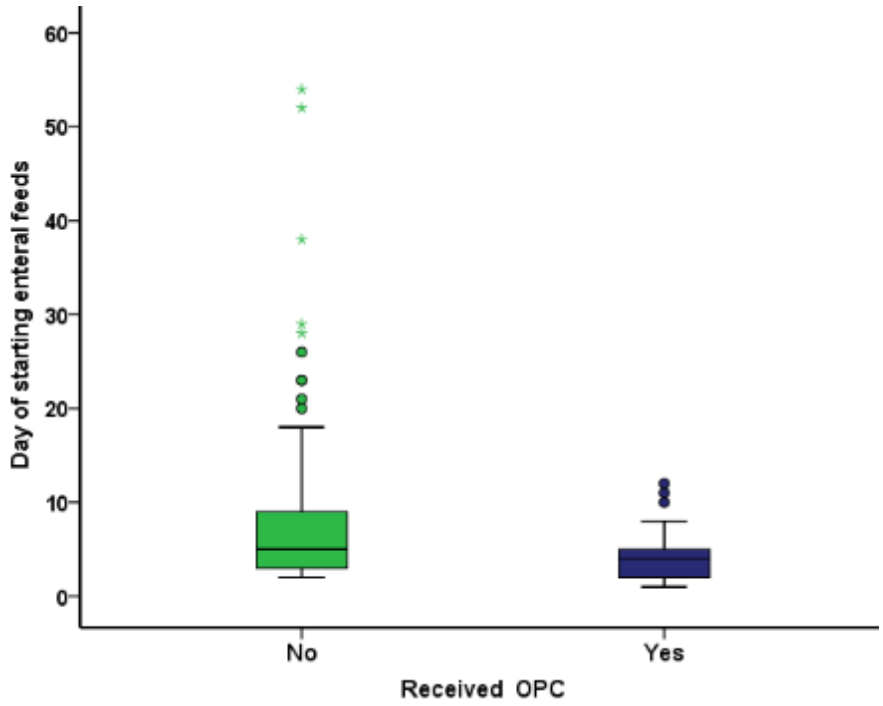


Figure 4.7 Days of starting enteral feeding

Box & whisker plot displaying, the median days of starting enteral feeding for the Pre-OPC and OPC groups. The top and bottom sides of the box represent IQR. The horizontal line inside the box is the median. Green box: Pre-OPC (n = 73); Blue box: OPC group (n =36); P = 0.006 (Mann-Whitney U test); Asterisks and circles: extreme values (outliers). OPC: oropharyngeal colostrum; IQR: interquartile range

4.4.4.3 Days of parenteral nutrition

There was no difference between the study groups in days of parenteral nutrition, median (95% CI) (Pre-OPC: 15 (95% CI; 10.77 to 19.22) days; OPC: 15 (95% CI; 10.34 to 19.65) days; p = 0.30). The Kaplan-Meier graph showed partial overlapping and crossing of the two curves, which may point to an inconstant hazard ratio over time (Figure 4.8). Therefore, Cox regression analysis could not be conducted.

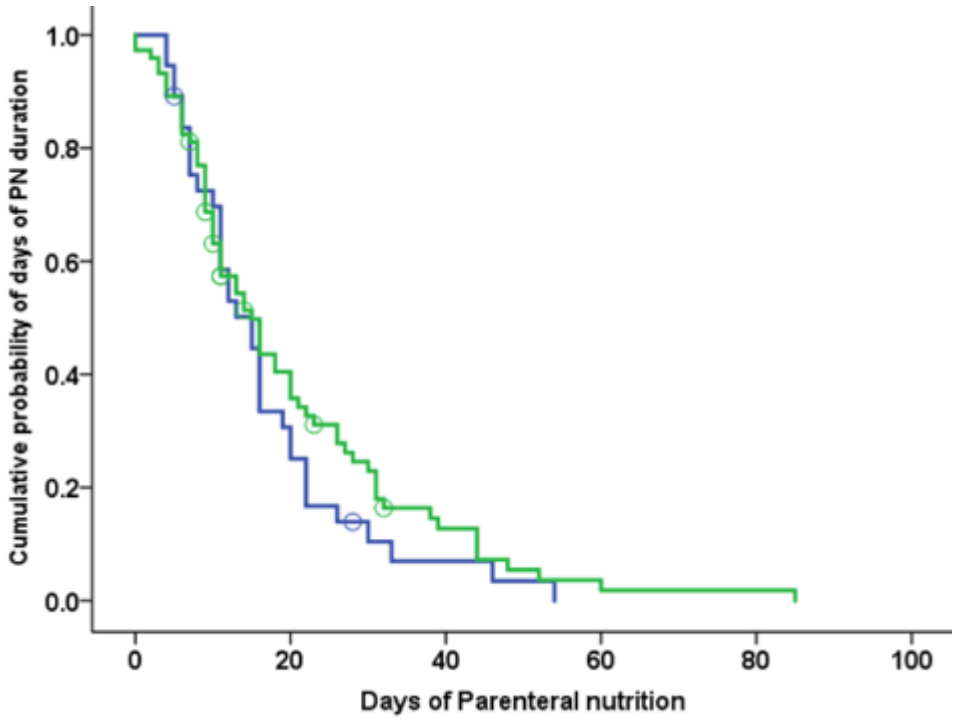


Figure 4.8 Days of parenteral nutrition therapy

Kaplan-Meier plot demonstrating probability of days of parenteral nutrition (PN) among preterm infants (<32 weeks) who received OPC and those who did not receive OPC. The median for each group corresponds to the probability of 0.5. Green line: Pre-OPC group (n = 74); Blue line: OPC group (n = 37); p = 0.30 (Log Rank test); Circle marker on each line: censored (died); OPC: oropharyngeal colostrum.

4.4.4.4 Days of mechanical ventilation

There was no statistically significant difference between the two groups in days of mechanical ventilation, the median (95% CI); (Pre-OPC group: 8 (95% CI, 3.91 to 12.08 days; OPC; 5 (95% CI, 0.43 to 9.56 days); p = 0.22). Although there was a separation of the two curves between 5 to 40 days that indicating fewer days of mechanical ventilation in the OPC group, there was a close approximation in the extremes, which may point to an inconstant hazard ratio over time (Figure 4.9).

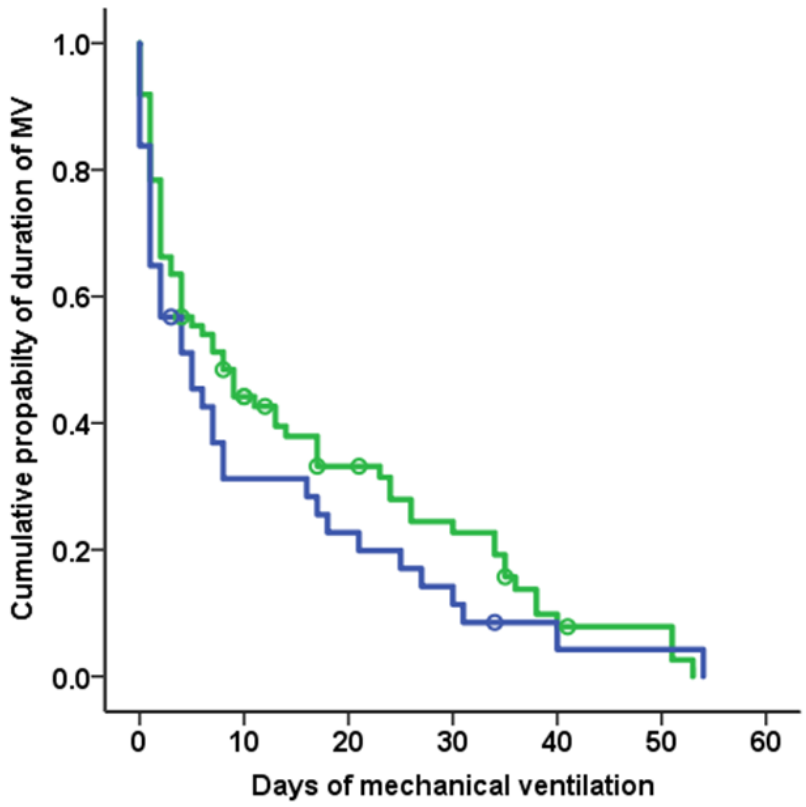


Figure 4.9 Duration of mechanical ventilation

Kaplan-Meier plot; demonstrating probability of days of mechanical ventilation (MV) among preterm infants (<32 weeks) who received OPC and those who did not receive OPC. The median for each group corresponds to the probability of 0.5. Green line: Pre-OPC group (n=74); Blue line: OPC group (n=37); p =0.22 (Log Rank test); Circle marker on each line: censored (died); OPC, oropharyngeal colostrum.

Further comparison showed no significant difference in days of mechanical ventilation between 5-40 days of age, median (IQR); (Pre-OPC: 17.5 (9, 30); OPC: 17 (7.5, 27.7) days; p = 0.58). Gestational age, birth weight and sex were similar between the two groups for those infants (Table 4. 6).

Table 4.6 Characteristics of infants received mechanical ventilation for 5-40 days

Characteristic	Pre-OPC (n = 35)	Post-OPC (n = 14)	p value
Male, n (%)	25 (71%)	9 (64%)	0.73[f]
Gestational age, weeks median (IQR)	25.5 (25. 27.5)	25.2 (24.6, 27.2)	0.40[U]
Birth weight, g median (IQR)	800 (720, 995)	795 (724, 1000)	0.91[U]

OPC: oropharyngeal colostrum; n: number; IQR: interquartile range; f: Fischer's Exact test; [U]: Mann-Whitney U test; statistical significant: p<0.05

4.4.4.5 Incidences of NEC and LOS

The incidences of suspected NEC and clinically suspected LOS in the study population were 53.2% and 96.4% respectively. There were no statistically significant differences between the study groups in the incidences of clinically suspected, NEC and LOS (p =0.84; p =0.6 respectively).

The incidences of confirmed NEC and culture proved LOS in the study cohort were 16.2% and 33.3% respectively. There were no statistically significant differences in the incidence of confirmed NEC and confirmed LOS between the study groups as demonstrated in Table 4.7.

Table 4.7 Incidences of NEC and LOS

Outcome	Pre-OPC group n= 74	OPC group n=37	P value (f)
Clinically Suspected NEC	40 (54%)	18 (51%)	0.84
Clinically Suspected Sepsis	72 (97%)	35 (95%)	0.60
Confirmed NEC	15 (20.3%)	3 (8%)	0.08
Culture proved LOS	27 (36%)	10 (27%)	0.39

OPC: oropharyngeal colostrum; n: number of infants; NEC; necrotising enterocolitis; LOS: late-onset sepsis; values: frequencies (percentage); f: Fisher's Exact test; statistical significant: p<0.05.

Of note, in the study cohort NEC was commonly seen (18/111 (16.2%); surgical NEC: 11/18 (61.1%); medical NEC: 7/18 (38.9%)). The Pre-OPC group showed a higher NEC rate than the OPC group although this did not reach statistical significance (p = 0.08; Table 4.7). Further analysis showed that in the two study groups, infants with NEC were inborn and admitted within the first day of life. Table 4.8 demonstrates the criteria of NEC cases by the study groups.

Table 4.8 Criteria of infants with NEC

Criteria	Pre-OPC (n=15)	OPC (n=3)	P value [f]
Gestational age*	25.9 (25, 28.3)	25 (24.6, 26.7)	0.44[U]
Birth weight*	790 (710, 955)	740 (687.5, 770)	0.44[U]
IUGR, n (%)	2/15 (13.3%)	1/33 (33.3%)	0.55
Twins, n (%)	4/15 (26.7%)	0 (0%)	0.55
Medical NEC, n (%)	7/15 (47.7%)	1/3 (33.3%)	1.0
Surgical NEC, n (%)	8/15 (53.3%)	2/3 (66.7%)	1.0
Death, n (%)	3/15 (20%)	1 (33%)	0.55

*: median (interquartile range); n = number; f: Fisher's Exact test; [U:] Mann-Whitney test; statistical significant: p<0.05; NEC: necrotising enterocolitis; OPC: oropharyngeal colostrum

To identify definite NEC cases, blinded endpoint reviews were conducted to assist in data validation. Blinded data for twenty cases with a possible diagnosis of NEC were reviewed by two clinicians. The two reviews were matched; two cases were excluded, and further information was requested for one case. A consensus was reached and the occurrence of NEC was confirmed for 18 infants who were included in the analysis of the outcome incidence of NEC during a hospital stay. There was also a strong agreement between the study investigator and the two reviewers (Krippendorff α: 0.86 (95% CI: 0.72 to 1.0).

4.4.4.6 Death before discharge home

There was no statistically significant difference in mortality before discharge to home between the two groups (Pre-OPC: 8/74 (10.8%); OPC: 2/37 (5.4%); p = 0.71).

4.4.4.7 Weight at discharge to home

To report on the weight in a standardised mode, the weight-age-Z score was used to compare the weight gain between groups. At birth, the two groups were similar (p = 0.97), Table 4.1.

At discharge to home, 101 infants who survived were included in the analysis (Pre-OPC: 66/74 (89%): OPC: 35/37 (95%) infants). There was no statistically significant difference between the two groups for WAZ score at discharge to home; median (IQR) WAZ scores (Pre-OPC: -1.40 (-2.22, -0.670); OPC: -1.50 (-2.30, -0.90); p = 0.65) Figure 4.10.

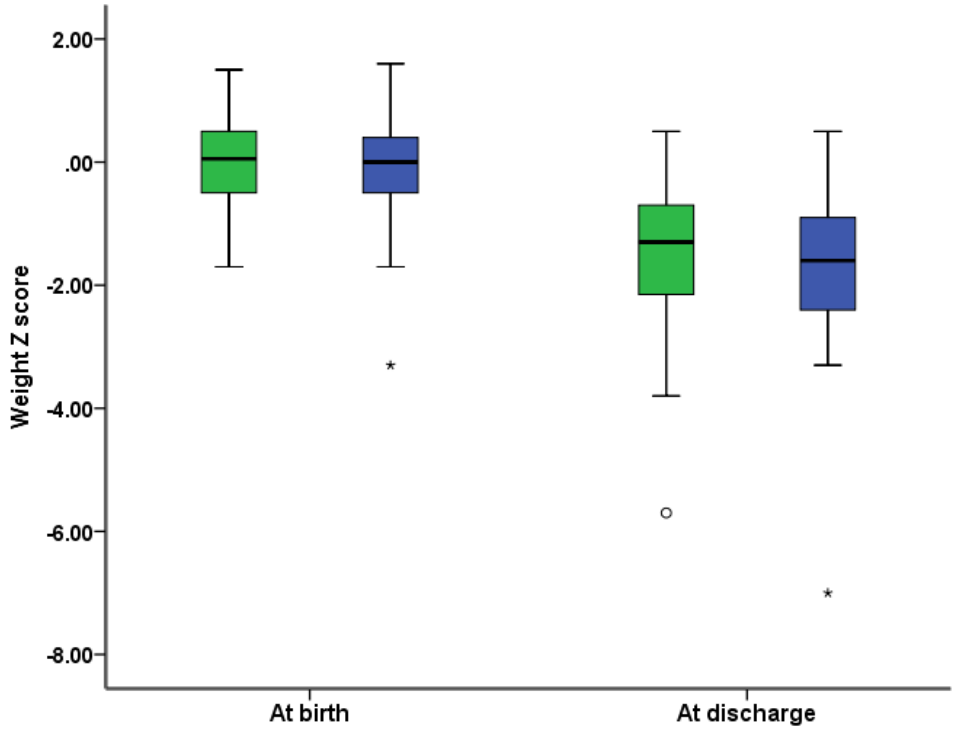


Figure 4.10 Median weight Z score at discharge to home
Box & whisker plot displaying, the median weight Z scores at hospital admission and discharge home for the Pre-OPC and OPC groups. The top and bottom sides of the box represent interquartile range. The horizontal line inside the box is the median. Green box: Pre-OPC; Blue box: OPC group. At Birth: Pre-OPC: n = 74; OPC: n=37 (p = 0.97); At discharge: Pre-OPC: n = 66; OPC: n = 35 (p = 0.65) (Mann-Whitney test U); OPC: oropharyngeal colostrum.

4.4.4.8 Breastfeeding at hospital discharge

Ten infants died before hospital discharge, therefore; analysis of this outcome included 101/111 infants (8 infants from the Pre-OPC group and 2 form the OPC group).

4.4.4.9 Receiving any breast milk at discharge to home

There was a statistically significant association between OPC use and receiving any breast milk at discharge to home (Pre-OPC: 35/66 (53.0%); OPC: 28/35 (80.0%); p = 0.01

(Fischer's Exact test)), Figure 4.11. There was no significant difference between the study groups in receiving expressed breast milk (EBM) during stay in the neonatal unit (p = 0.5; Table 4.1). However, the volumes of EBM received by the infants were not analysed as data were not available for the Pre-OPC group.

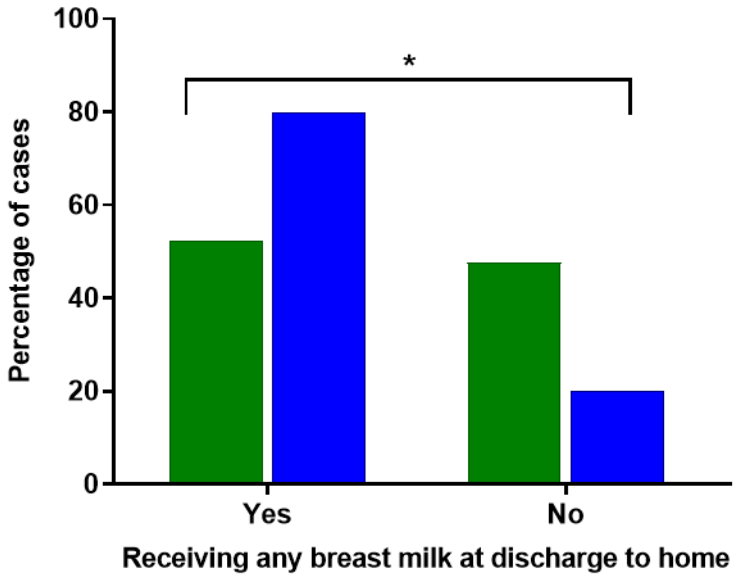


Figure 4.11 Receiving any breast milk at discharge home
Bar chart comparing receiving any breast milk at discharge home among preterm infants (<32 weeks' gestation) who received OPC and those who did not receive OPC; Bar: percentage of cases; Green column: Pre-OPC group (n = 66); Blue column: OPC group (n = 35); Yes: infants received any breast milk; No: infant did not receive any breast milk;
* p = 0.01 (Fischer's Exact test); OPC: oropharyngeal colostrum

4.4.4.9.1 Type of milk and method of feeding at discharge home

Overall, there was a statistically significant difference between the study groups in the type of milk at discharge home (p = 0.04; Figure 4.12). Post hoc pairwise comparisons demonstrated that the difference in the proportion of infants in the groups who were discharged to home on formula milk and mixed types of milk remained statistically significant (corrected p = 0.02).

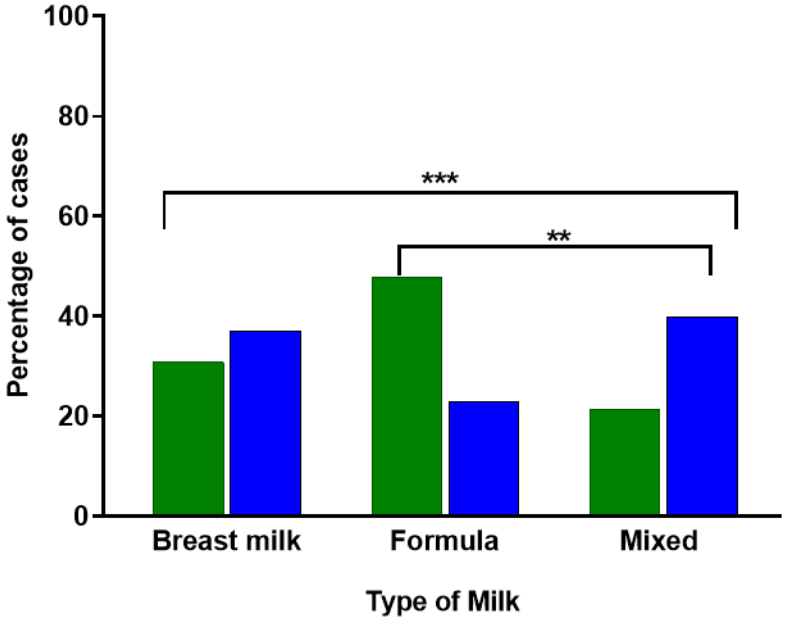


Figure 4.12 Type of milk at discharge

Type of milk at discharge home among preterm infants (<32 week's gestation) who received OPC and those who did not receive OPC. Bars: percentage of infants; Green column: Pre-OPC group (n = 66); Blue column: OPC group (n = 35). Mixed: breast milk and formula; *** p = 0.04; **: p = 0.02 (Fisher's Exact test); OPC: oropharyngeal colostrum

Whilst, there was a significant difference between the two groups for the type of milk being used at discharge to home, there was no statistically significant difference (p = 0.17) in the method of feeding at discharge home as demonstrated in Table 4.9.

Table 4.9 Methods of feeding at discharge home

Method	Pre-OPC group (n = 66)	OPC group (n = 35)	P value (f)
Suckling at breast	9 (13.6%)	4 (11.4%)	
Bottle	38 (57.6%)	21 (60%)	
Multiple	16 (24.2%)	10 (28.6%)	0.17*

OPC: oropharyngeal colostrum; n: number of infants; Multiple: > one method (breast and bottle or breast and cup or nasogastric tube (NGT); f: Fisher's Exact test; *: overall p value. Values: frequencies (percentage).

4.5 Discussion

4.5.1 Key findings

OPC administration was associated with earlier commencement of enteral feedings and faster attainment of full enteral feeds in preterm infants <32 weeks' gestation. Rates of breastfeeding and breast milk use were also higher at discharge to home in those infants who received OPC in the first 96 hours after birth. There were no significant differences in confirmed NEC, the length of hospital stay, and weight Z score (at discharge home) between the study groups. The occurrence of microbiologically proven sepsis and death was similar between the OPC and Pre-OPC groups. Infants and maternal characteristics that could be anticipated to contribute to the study outcomes revealed no significant differences between the study groups except for maternal chorioamnionitis and intrapartum pyrexia (Table 4.2).

4.5.2 Primary outcomes

The OPC group achieved full enteral feeds faster than the Pre-OPC group at 14 versus 17 days respectively and started enteral feeds earlier than the Pre-OPC group. A higher rate of maternal chorioamnionitis was found in the Pre-OPC group compared to the OPC group. Maternal chorioamnionitis has been recognised as a potential risk factor for NEC (555-557), which might be initiated by exposure to infection in the uterus (558), thus, may indirectly impact infant feeding as withholding feeds is one of the medical treatment of NEC (146). However, in the Cox regression analysis, adjusting for maternal chorioamnionitis, OPC remained a significant predictor for time to achieve full enteral feeds. Though, this earlier achievement of full enteral feeds associated with the administration of OPC could be confounded by other factors such as NEC. Although there was no statistically significant difference between the study groups in the incidence of NEC, a higher percentage of NEC was noted in the Pre-OPC group. A univariate analysis showed that NEC was a significant factor associated with days to full enteral feeds. NEC also remained a significant independent predictor after multivariate regression (Table 4.5).

Earlier initiation of enteral feeds and quicker achievement of full enteral feeds (defined here as 150 ml/kg/day in this study) have also been reported in previous studies conducted to assess outcomes of VLBW infants after implementation of a standardised feeding protocol including OPC use (353, 377, 417). Nonetheless, other clinical trials that were on-going in the period of this study could have affected the results. For instance, the Speed of Increasing milk Feeds Trial (SIFT), a multicentre RCT which evaluated the impact of two speeds of advancing the rate of milk feeds in preterm infants (<32 weeks gestation) (559). The SIFT trial had been conducted and completed enrollment (From June 2013 to June 2015) during the Pre-OPC period. The SIFT reported that infants who were fed faster (30ml/kg/day) reached full enteral feeds quicker than those infants who were fed 18ml/kg/day (7 versus 10 days) (conference abstract had been granted by the author) (559). However, the enteral feeding guidelines in the NICUs did not change along with the adoption of OPC practice in Nottingham neonatal units. Similarly, a meta-analysis from a Cochrane review (560) (Chapter 3) also showed an association between OPC and faster attainment of full enteral feeds in preterm infants (<37 weeks' gestation); however, the studies included in the review were low-quality evidence. In contrast, other recent studies reported no difference in the time to start enteral feeds or to achieve full enteral feeds between preterm infants who received OPC and those who did not (356, 358, 366). However, these previous studies were small unblinded RCTs and one was an observational study before and after the introduction of OPC in the care of VLBW infants.

The earlier commencement of enteral feeds and faster achievement of full enteral feeds might be related to the exposure of the oropharyngeal mucosa to immune and growth factors found in colostrum (236, 238). These bioactive factors are present in higher concentrations in colostrum from mothers who deliver preterm infants than mothers who deliver full-term infants (192, 274, 275, 561) suggesting the importance of providing mother's colostrum in the early neonatal period. Although the current study demonstrated a significant difference between the Pre-OPC and OPC groups in days to full enteral feeds, the confidence interval was wide, indicating that the sample size was small. Studies with larger sample sizes with

complete data on variables for risk adjustment could support the association between OPC and shortened days to full enteral feeds.

Despite, faster achievement of enteral feeds was associated with OPC use, there was no statistically significant difference in weight Z score at discharge to home between the two groups. Likewise, there was no statistically significant difference (p= 0.21) between the study groups in the length of hospital stay. Previous studies (353, 417, 506) also reported that OPC use was not associated with a reduction in the length of hospital stay. In contrast, a recent randomised controlled trial (358) assessed the effects of OPC on the oral immuno-microbial environment in preterm infants < 32 weeks of gestation reported a statistically significant reduction in the length of hospital stay in infants who received OPC compared to controls (40 versus 56 days). However, the study did not explain the causality of the reduced hospital stay associated with OPC use, but other variables would be expected to be dealt with by the randomisation.

4.5.3 Secondary outcomes

There was no statistically significant difference in the incidence of confirmed NEC between the study groups. Overall, the whole study population had a reasonably high NEC rate of 16.2 %, which might be in part due to the low gestational age and birth weight (mean 1040g) in the study cohort as the incidence of NEC is inversely related to the gestational age (78). Approximately 70% of NEC cases in this study were <27 weeks' gestation with a mean birth weight of 750g (Table 4.7). Since infants born <28 weeks or VLBW infants are at a higher risk of complications including NEC (164, 170), which was estimated to affect 10-15% of those infants (78), this finding is plausible and suggests the likelihood of incorrect attribution is low.

Although there was no statistically significant difference between the study groups, confirmed NEC was reported more often in the Pre-OPC group; this could be related to a higher rate of maternal chorioamnionitis among this group compared to the OPC group (p=0.02). Chorioamnionitis has been indicated as a potential risk factor for developing NEC (556, 557),

though a systematic review was inconclusive about the effect of chorioamnionitis on the risk of NEC (555). Some previous studies showed high NEC rates in VLBW and ELBW infants. In Canada, a prospective cohort study reported that the incidence of NEC was 14% for infants 22-25 weeks gestation and 10% for 26-28 weeks infants; a 5.9% reduction was associated with the use of colostrum and EBM as a part of Quality Improvement Program (562). However, the program involved multiple interventions; therefore, reduced NEC rate might be attributed to the whole program, as the authors did not adjust for individual intervention. Likewise, a retrospective study of quality improvement found a significant reduction in the incidence of NEC after the implementation of a standardised feeding protocol, which included OPC; NEC was reduced from 18% to 3% in VLBW and from 35% to 8% in ELBW infants (377). However, other factors, such as an occurrence of other interventions at the same time, may confound this association.

NEC rate that was reported in this study contrasts to a recent study that including data from the UK, reported a very low incidence of NEC (3.15%) in infants < 32 weeks gestation, however, the authors reported only on severe cases of NEC that confirmed by surgery or autopsy, or death (80). Conversely, EPICure, a population-based study conducted to determine survival and morbidities for extremely preterm infants in England, reported 8% of laparotomy confirmed NEC among infants between 22-26 weeks' gestation (59). However, EPICure did not report on medically treated NEC; this may underestimate the incidence of NEC in those infants. The NEC (defined as Bell stage 2 or 3) rate was similar at 10% in the Probiotics in Preterm Infants Study (PiPS), an RCT, which investigated the effect of probiotic on the rate of NEC, sepsis and death in infants <31 weeks of gestation (142). However, the incidence of NEC is variable between hospitals, nationally and internationally, a recent systematic review reported NEC rates ranging from 5% to 22% in infants with a birth weight <1000g among developed countries (365).

The current study presented NEC episodes documented in electronic patient records (BadgerNet Neonatal), which included medical and surgical NEC. Lack of consistent case-definition may potentially lead to difficulty deciding if an infant had NEC, this may lead to an

overestimation/underestimation of NEC rate. For example, diagnosing feeding intolerance as a stage of NEC or medically treated spontaneous intestinal perforation might be erroneously diagnosed as NEC (548). Similarly, some of the Bell's staging criteria, such as pneumatosis intestinalis and portal vein gas, are less manifested in extremely preterm infants (563). However, to minimise variation in reporting NEC case in Badger neonatal database, additional sources such as radiology, histopathology reports and death certificates, were used to determine definite NEC cases.

Furthermore, Blinded Endpoint Reviews (547) were performed to determine definite NEC cases and ensure they have met the pre-specified criteria. BERs (also referred to as Endpoint Adjudication) (547) is an important part of clinical research for validating data, especially when the endpoints are subjective and require expertise to assess and apply a complex definition. The reviews are conducted by clinicians who have expertise in the relevant area for the study (564). These reviewers should also be trained in keeping with the principles of Good Clinical Practice (GCP) and compliant with the Data Protection Act. Such endpoint assessment can facilitate a study achieving higher scientific quality (564). Noteworthy, the sample size of this study was too small to infer the incidence of NEC as conclusive.

OPC use was also associated with a higher rate of feeding any breast milk at discharge to home (80% versus 53%) although similar rates of EBM were used during the hospital stay in the two groups (Table 4.1). Additional detail on EBM volumes received by infants may have provided further information on the effect of OPC administration on the type of milk at discharge home. As these data were not available for the Pre-OPC group, such an analysis was not possible. This might be important as the benefits of human milk have been shown to have a dose-response effect (281, 284).

Breastfeeding and breast milk use at discharge to home were higher in the OPC group while significantly fewer infants in this group were receiving formula alone at discharge. In a recent RCT, Romano-Keeler et al. also observed a statistically significant effect of OPC on receiving

any breast milk at discharge home in infants <32 weeks GA compared to controls (358). However, this was a small unblinded RCT. Similarly, a prospective observational study (565) found an association between OPC use and receiving breast milk as the main enteral feed at six weeks postnatal age, and at hospital discharge in VLBW infants. Conversely, a recent Cochrane review (Chapter 3) did not demonstrate an effect of OPC on receiving any breast milk at discharge to home in preterm infants (<37 weeks) (560).

The rate of breast milk feeding in the Pre-OPC group is comparable to reported UK rates (58-60%) of feeding any breast milk at discharge home for infants less than 33 weeks gestation from 2012 to 2016 (414). It is also similar to the rates of receiving any breast milk at discharge home in the Nottingham population (54%) according to 2016 data from Trent Perinatal & Central Newborn Neonatal Networks (414). The observed association of OPC with a higher rate of receiving any breast milk might be explained by the encouragement of the mothers to express breast milk as early as possible to provide OPC to their infants. Interestingly, the Nottingham Neonatal Service's guidelines to support breastfeeding and educate mothers on the benefits of breast milk did not change over the study period, and the feeding guideline has not been updated since 2014. This supports the change in the breastfeeding at discharge being due to OPC introduction.

However, breastfeeding and the use of breast milk has risen since the 1990s; the WHO reported that globally >80% of newborn infants receive breast milk during the neonatal period and exclusive breastfeeding rates were increased by 10% from 1993 to 2013 (566). The UK EPICure2 studies also found that in 2006, the use of any breast milk was increased by 10% from 1995 in infants < 26 weeks gestation; approximately 96% of those infants received some EBM during a stay in the neonatal units, and 43% were receiving breast milk at discharge to home (59). Additionally, the UK implemented the Baby Friendly Initiative, a global program of the United Nations International Children's Emergency Fund (UNICEF) and the World Health organization (WHO) for improving practice to support breastfeeding (567). Hence, in this study, the pattern of breast milk feeding that was observed for the OPC group might reflect a continuation of an existing trend.

There were no statistically significant differences between the two groups in the incidence of proven sepsis, death before discharge home, and days of PN or mechanical ventilation. While some studies (353, 377) have found that OPC might reduce the risk of these common prematurity-related morbidities, other studies have not shown effects on some of these morbidities (356, 357, 417, 506). However, these previous studies were not powered to detect significant differences in prematurity-related complications such as NEC or sepsis. There is an ongoing multicentre RCT to evaluate the impacts of OPC on the incidence of NEC, sepsis and death in extremely preterm infants (target sample, n = 498 infants) (352).

4.5.4 Feasibility of OPC use in neonatal units

OPC appears to be a safe intervention in the care of preterm infants as no adverse events were reported for the infants during the OPC procedure.

Although data regarding adverse events were collected from routinely reported data on the clinical records, every effort was made to minimise this potential reporting bias; by using all available clinical records including nursing charts and reports and the medical notes. Moreover, a study-specific adverse events form was created to collect these data prospectively (Appendix 8). Nevertheless, this result is in line with growing evidence suggesting the safety and feasibility of OPC administration in very low birth weight (VLBW) and extremely preterm infants (354, 358, 422, 506). Despite inconsistencies in methods and sample sizes, these studies were consistent in demonstrating that the OPC procedure was not associated with adverse effects such as bradycardia, a decrease in O2 saturation or aspiration. However, the current study and the previous studies were not powered to evaluate the safety of OPC use in preterm infants.

The practice of OPC administration was feasible, and mothers were able to provide colostrum at the planned time, as approximately 60% of the infants who received OPC did so within the first 48 hours of life. In previous studies, it was possible to start OPC within the 48 hours after birth (353, 354, 417, 506, 561) while other researchers were able to administer

OPC after the second postnatal day and reported that it was impractical to apply OPC within the first 24 hours after birth (356, 419).

OPC feasibility was also determined by the percentage of the planned doses and the frequency of administration (352, 356, 422). Completed OPC administration was defined as receiving more than 70% of the expected doses. In this study, approximately 73% of the infants received 50%-70% of the doses; some of the missing doses could be explained by unavailability of the mother's colostrum at the scheduled dose. Nine infants had been excluded from the study because they received only one dose of OPC as it was assumed that receiving one dose will not provide enough information to conclude. Exclusion of those infants may have affected the feasibility result. However, OPC administration was stopped to use colostrum for trophic feeding rather than for providing OPC once trophic or enteral feeding started. This possible non-feasibility could be attributed to understanding and adherence to the clinical guideline for OPC administration and a decision of the treating team as OPC administration is still under the discretion of the clinical team of the infant. Although the exclusion of those infants may have influenced the feasibility findings, availability of mother’s colostrum and an attempt to provide OPC within 96 hours indicate potential feasibility in the excluded infants.

Of note, in some infants, OPC was discontinued before the completion of the planned period for its administration when trophic/enteral tube feeds were started despite guidance to the contrary. This could be related to the very recent adoption of OPC practice in Nottingham neonatal units, and that the educational and training program supporting staff to implement this new guideline, may have been insufficient to ensure consistent adoption of the practice across the two Nottingham units.

Clinical guideline implementation can be hindered by a variety of barriers that may lead to failure of adherence to the guidelines (568, 569). However, to enhance the implementation of the OPC guideline, reminder letters along with the OPC guideline were sent to the attending Nottingham neonatal unit consultant at the time of their service (Appendix 10). A Nurse

Information sheet was also created and distributed (Appendix 11). It is likely that OPC use could be further improved by educational or quality improvement strategies that influence the attitudes, awareness and understanding of the guideline by relevant professionals. This is especially the case for neonatal nurses, as OPC is mainly administrated by nurses.

4.5.5 Strengths and limitations of the study

To the best of my knowledge, this is the first study in the UK evaluating the impact of early OPC administration on the clinical outcomes of preterm infants from birth to discharge to home. Whilst other studies have assessed the effects of OPC in different settings, almost all of these studies evaluated the use of OPC in very low and extremely birth weight infants and extremely preterm infants, this study included infants up to 32 weeks of gestation. Moreover, the current study included comprehensive data analysis from a single centre limiting variations in treatment with just one guideline. Another strength was that most of the data were extracted from a neonatal database which uses a national standard coding system and predefined data items to enhance the accuracy and comparability of the data (531).

In order to minimise differences, eliminate bias and ensure comparability between the cases, the study was designed as a matched case-control study. Each case was individually paired with a control infant relating to sex, gestational age and birth weight; as these criteria are well-known factors that influence the outcomes of preterm infants (143, 151, 542); also, the matched cases were selected from the closest year to the period of the study. Moreover, two controls per case were used to increase the power of the study so that, even though the sample size of this study was small, the results may generate hypotheses and preliminary data for future studies; particularly for sample size calculations.

Finally, to enhance transparency, ensure complete reporting and minimise potential reporting bias, Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) (570) recommendation was used in writing up the study findings. STROBE constitutes a checklist of 22 items to help improve the appropriate reporting of cohort, case-control and cross-sectional studies to strengthen completeness and transparency in analysing and reporting of

observational studies (571). STROBE statement has been endorsed by many biomedical journals and by the International Committee of Medical Journal Editors (572).

This study had several limitations. It compared two different groups of preterm infants before and after the adoption of OPC use. The non-parallel comparison may bias the findings due to possible differences in the care of preterm infants between the two study periods (517). Therefore, the findings might not be entirely attributable to the OPC use. However, in the current study, the feeding protocol, management of NEC and sepsis did not differ between the two eras.

Other research studies that were conducted during the same period of this study might have altered the outcome of preterm infants independent of the effect of OPC practice (534). The finding of the SIFT trial could have influenced the comparison between the Pre-OPC and OPC groups in the current study (559). Enteral lactoferrin supplementation for very preterm infants (ELFIN), a recently published RCT (208) that evaluated the effects of enteral lactoferrin supplementation on late-onset sepsis in preterm infants (< 32 weeks gestation), was also on-going during period of the present study. ELFIN trial found that enteral lactoferrin had no effects on late-onset sepsis and its related complications. It appears that ELFIN results were unlikely to affect the results of my study.

As an observational study, a causal relationship could not be assessed and, as data from the Pre-OPC group was collected retrospectively, it was susceptible to information bias (442, 520).

OPC use also faced some challenges; such as administration of OPC often being discontinued when enteral feeds were started despite guidance to the contrary. The OPC protocol used in this study had some differences from the protocol used in previous research particularly in the frequency and duration of the OPC administration. These differences might have contributed to the disagreement with some of the previous study findings. The OPC protocol was chosen to fit in with the nursing practices in the units as there was insufficient evidence to determine the optimal regime. Additionally, Nottingham neonatal units adopted

OPC shortly before this research study, and although a guideline supported it, the application of OPC remained the responsibility of the treating clinical team, providing the opportunity for individual decision making and practice. With more education and regular reviewing of the OPC guideline along with wider adoption, it is likely that results could be further improved.

4.6 Conclusion

OPC appears to be a feasible practice in the care of preterm infants. It was possible to collect the mother's colostrum within the first 48 hours after birth, and approximately 60% of the infants (included in the study) received OPC during the first 48 hours of life.

OPC was associated with significantly reduced days to initiate enteral feeds and days to reach full enteral feeds. A higher rate of breastfeeding at hospital discharge was also observed. Although this study did not find statistically significant differences in the length of hospital stay, days of mechanical ventilation, the incidence of NEC and sepsis, and deaths before hospital discharge, with larger sample size, these may have been significant.

The study provided insight into the implementation of a new guideline and if it was well integrated into the standard care. The study found that OPC delivered to the preterm infants was often not in keeping with the current OPC guideline. Improvements could likely be achieved by more and repeated education of the healthcare professionals to establish a better OPC practice in the neonatal service. Furthermore, parents could be encouraged to administer OPC to their baby, this practice may offer the parent an active role in providing care for their baby and ensuring it is given more often.

4.6.1 Implications for clinical practice

It is apparent from the results of this study, and previous studies that using OPC in preterm infants appears to have positive impacts on the time to start enteral feeds and achieve full enteral feeds. Therefore, despite, high-quality evidence being limited, OPC seems to have a promising role in the standard care of preterm infants. Moreover, the well-known benefits of mother's colostrum for preterm infants and the potential safety and low cost of OPC

procedure may outweigh the high risks of mortality and morbidities, such as devastating NEC and infection, in these infants.

4.6.2 Implications for future studies

There is still uncertainty whether OPC administration could improve health outcomes for preterm infants. Meta-analysis of available trial data in a Cochrane review including high-quality studies is a worthwhile venture and is presented in Chapter 3 of this thesis. Depending on the findings of the meta-analysis, further research using large, adequately powered, randomised controlled trials are required to assess the efficacy of this intervention. Such studies also offer the potential to identify and elucidate the mechanisms of the effect of OPC.

Additionally, studies comparing different protocols concerning procedural method, doses, frequencies and duration of the intervention are needed to optimise the practice of OPC and to set a standard protocol for administration of mother's colostrum by the oropharyngeal route. Of note, as OPC does not require advanced technology, it can be implemented even in low- income countries where the rate of preterm birth is high (23, 177). Therefore, it is worth obtaining data from middle and low-income countries in future studies and synthesising these in meta-analyses to ensure generalisability of the results.

Chapter 5. Gut hormone response to oropharyngeal administration of mother's colostrum to infants in neonatal intensive care

5.1 Chapter overview

In Chapter 3 (Cochrane review) and Chapter 4 (case-control study), it was concluded that using oropharyngeal administration of mother's colostrum (OPC) in preterm infants appears to have positive impacts on time to start enteral feeds and to achieve full enteral feeds. With the introduction of trophic feeds, there are surges in the circulating gut hormones (306). Gut hormones (GutH) have essential role in the postnatal adaptations of the gastrointestinal tract (GIT) to prepare the infant for enteral feeding (573).

In this Chapter, I present a study that was conducted to investigate the effects of OPC administration on plasma gut hormone concentrations in preterm and sick infants requiring Neonatal Intensive Care (NIC).

5.2 Background

Infants admitted to neonatal intensive care units require invasive therapies and the consequences of their underlying clinical condition be compounded by additional risks arising from the medical and nursing care they require. This research will focus on one aspect of preventing the complications which can accompany clinical care, i.e. feeding intolerance and the subsequent withholding of oral feeding during the early neonatal period.

5.2.1 Feeding of newborn infants receiving intensive care

Feeding is a significant challenge for preterm infants and those with congenital GIT conditions and an important factor affecting nutrition, growth, and later outcomes (290, 293). Unfortunately, the complex clinical conditions of some infants requiring NIC does not permit provision of enteral feeds in the critical days after birth. Withholding enteral feeding, and delays in achieving full enteral feeds promote intestinal atrophy and abnormal bacterial

colonisation of the bowel, leading to disturbance in gut hormones. This may contribute to the pathogenesis of necrotising enterocolitis (NEC) (574).

Preterm infants especially extremely preterm (EXP) and extremely low birth weight (ELBW) infants are prone to poor postnatal growth. These infants are often born in a negative-energy balance; therefore, after birth, they have to catch-up a favourable growth, but at discharge from NICU, their weights are often below the 10th percentile for their completed weeks of gestation (332). In preterm infants, inadequate growth during the postnatal period was associated, with long-term consequences such as growth retardation, metabolic bone disease, and poor neurodevelopmental outcomes (103, 137). Similarly, rapid postnatal growth has been linked to obesity and insulin resistance that increase the risk for chronic adverse outcomes such as diabetes and cardiovascular diseases (107, 108). Therefore, interventions which have the potential to influence enteral feeding and energy balance that may enhance achievement to full enteral feeds, could also promote growth and improve outcomes for infants requiring NIC.

5.2.2 Gut hormones during the neonatal period

The GIT represents the largest endocrine gland in the body (575) containing many specialised cells secreting multiple regulatory peptides in response to nutrients (576). These multiple regulatory peptides, known as gut hormones, have important effects on the growth and functions of the GIT. The neuroendocrine cells of the gut produce hormones such as gastrin, gastric inhibitory polypeptides (GIP), peptide tyrosine tyrosine (PYY), glucagon-like peptide (GLP), insulin and pancreatic polypeptide (PP), which regulate gastrointestinal functions such as digestion, mucosal growth, blood flow, motility, repair and maintenance of mucosal integrity (577).

After birth, the GIT is still growing and maturing; enteral nutrition is vital for intestinal growth and normal GIT function. This effect could be reflected by varying changes in the circulating concentrations of GutHs that may play a crucial role in postnatal adaptations of the gut. During the neonatal period, with the initiation of enteral feeds, there are significant elevations

in some of the plasma concentrations of GutHs (578, 579). This postnatal surge of different gut hormones is related to the infant's feeding status rather than the infant's gestational or postnatal age and occurs even with trophic feeding where a minute volume of milk <1ml/kg/hr is given via gastric tube (580). This normal postnatal increase in gut hormones is absent in those infants who do not receive enteral feeds (306, 574). High levels of certain GutHs have been linked with earlier attainment of full enteral feeds in preterm infants < 33 weeks of gestation (581). Some gut hormones such as GIP, PYY, GLP and ghrelin have been proposed to be potential predictors for feeding intolerance, NEC, and postnatal growth of preterm infants (333, 582).

The physiological and structural changes of the GIT, which occur after birth are complex processes. Gut hormone secretion in response to enteral feeds is one of the factors that influence these processes and circulating gut hormones might reflect enterocytes' functions and the GutH axes (581). Therefore, understanding these regulatory hormones may contribute to improving the feeding strategies for preterm infants.

5.2.2.1 Gut hormones studied

5.2.2.1.1 Peptide tyrosine tyrosine (PYY)

PYY is a peptide hormone secreted by the enteroendocrine cells of the terminal ileum and colon in response to ingestion of nutrients. PYY is a potent inhibitor for gastric acid and pancreatic secretions and gut motility (583, 584). PYY also has a central action binding to receptors in the brain to inhibit appetite (583). In newborn infants with the beginning of enteral feeding, there was a profound increase in the circulating PYY that reached higher concentrations compared to the adults' levels, and it is further higher in preterm infants (584). The pattern of feeding during the neonatal period might explain these higher levels in PYY. Even though PYY has inhibitory effects on gastrointestinal motility, rising plasma PYY concentrations during the first week of life were associated with less days to attain full enteral feeds in preterm infants (581, 585). The rise in plasma PYY is believed to balance the effects of gastrin, which stimulates gastric acid secretion at birth and within the first 48 hours of life

(584). The inhibitory action of PYY on gastrointestinal motility could also be an adaptive response to allow a longer time for digestion and absorption (584), to prepare the newborn infants for enteral feeding. Therefore, PYY may be a potential indicator for the GIT transit regulation (581).

5.2.2.1.2 Glucose-dependent insulinotropic polypeptide (GIP)

GIP is also known as 'Gastric inhibitory polypeptide'. GIP is one of the essential incretins, a peptide secreted by the small intestine (duodenum and jejunum) in response to ingestion of nutrients. It stimulates insulin secretion in response to the intake of food, particularly carbohydrates. It also enhances the generation of beta-cells of the pancreas (586). GIP is present in the brain, bone and adipose tissue, where it has trophic effects on the cells.

Lucas et al. in a study of 100 preterm infants demonstrated that postnatal insulin response to enteral feeding might be related to the onset of GIP release. GIP was indicated as the primary effector of the enteroinsular axis (587) and its plasma concentrations during the neonatal period had a positive relationship with days to achieve full enteral feeds in preterm infants (581). Therefore, plasma GIP could be a potential marker for the integrity of the GutH axes and gut maturation to accept enteral feeds (581).

5.2.2.1.3 Glucagon-like peptide 1 (GLP-1)

GLP-1 is also an incretin hormone and is mainly secreted by the mucosa of the small intestine (terminal ileum) and the colon in response to nutrient intake. It is also secreted by the pancreas and the brain. GLP-1 stimulates glucose-dependent insulin secretion from the pancreas (588) and promotes proliferation of the pancreatic β-cells (589). GLP-1 has potent anabolic effects through its stimulation of insulin secretion; hence higher plasma concentrations may be beneficial for the metabolism and energy storage (590). Other functions of GLP-1 include inhibitions of glucagon secretion, gastrointestinal secretions and motility (588) and food intake (583), and it has neurotrophic effects on the brain (591).

Preterm infants have higher fasting concentrations of GLP-1 than full-term infants (592). This higher GLP-1 concentrations in preterm infants could be attributed to the immaturity of

dipeptidyl peptidase (DPP)-IV; an enzyme degrades the active GLP-1 in the blood (593). GLP-1 is inversely related to the gestational age, and its concentrations increase in response to enteral feeding during the first few weeks of life reaching a peak higher than the adults (584, 590). Therefore, it was suggested that GLP-1 is an important peptide in the postnatal development and adaptation of the GIT (593).

5.2.2.1.4 Ghrelin

Ghrelin is a peptide hormone which is mainly secreted by the gastric mucosa with a small fraction produced by other organs such as the small intestine, pancreas, brain, heart, kidney and placenta (594). Ghrelin is a potent stimulant for growth hormone secretion. The widespread presence of ghrelin in many organs indicates that it has broad effects. Ghrelin is also an orexigenic hormone; it acts centrally at the hypothalamus to stimulate appetite, and high circulating ghrelin was found during fasting statuses (583). Ghrelin contributes to the regulation of diverse processes including control of energy balance and body weight, metabolisms of glucose and fat, and modulation of GIT, and some cardiovascular, pulmonary and immune functions (595).

After birth, the circulating ghrelin was low or even undetectable (581, 590); however, higher ghrelin concentrations were detected in the cord blood of small for gestational age (SGA) infants compared to appropriate for gestational age (AGA) infants (596). During the postnatal period, ghrelin release starts to increase at 2 to 3 weeks, and it is not correlated to enteral feeding (597, 598) but is inversely related to the anthropometric parameters of the infants (599, 600). The late increase of ghrelin in comparison to other hormones might be related to the requirement of ghrelin, as a potent growth hormone stimulant (335), during this stage of life when growth hormone starts to exert its actions. This pattern of ghrelin secretion could indicate that Ghrelin may play a role in intrauterine and postnatal growth (599), and its plasma concentrations might reflect the energy balance and postnatal catch-up growth of the infants (596, 601).

5.2.2.1.5 Insulin

Insulin is a peptide secreted by the beta-cells of the pancreas in response to the blood glucose levels. It is the principal anabolic hormone and plays a fundamental role in metabolic regulation of the body. Insulin enhances cellular transport of glucose and stimulates glycogen synthesis and storage in the tissues (602). It also stimulates lipogenesis and protein synthesis. Insulin has inhibitory effects on the breakdown of glycogen in the liver and muscles and decreases fatty acid oxidation (603).

Newborn infants have inefficient insulin secretion in response to changes in the concentration of blood glucose compared to older children and adults; this insufficient response is more manifested in preterm infants (604). In preterm infants, blood glucose concentrations are influenced by the administration of glucose rather to plasma insulin nor glucagon. Therefore, preterm infants are at higher risk for hypoglycaemia and hyperglycaemia at the neonatal period (605). The incretins, such as GLP-1 and GIP, stimulate insulin secretion through direct action on the β-cells of the pancreas; consequently, factors which influence GLP-1 and GIP secretion, promote insulin release (605). As, the patterns of insulin secretion during the neonatal period may programme the consequent later metabolic regulations (579, 606), the extent of insulin sensitivity of preterm infants during the neonatal period has been proposed as a potential indicator for long-term insulin-resistance (607).

5.2.3 Immunoassay

Immunoassay is a technique that involves the use of specific antibodies for identification and quantification of particular molecules in a sample. Immunoassays enable specific and sensitive detection of biomolecules in biological samples for research and clinical diagnostics. Traditional enzyme-linked immunosorbent assays (ELISA) was the most popular immunoassay procedure since the 1970s and is widely used in diagnostic medicine, quality control and research (608). ELISA is easy to perform, a very sensitive and specific technique for detecting and quantifying molecules and could be run at high throughput (609). Whilst

ELISA has been the standard method, it identifies and quantifies only a single marker per assay and to identify several molecules, multiple analyses result in longer time and requires a larger sample volume (610).

In certain clinical situations, quick analyses of multiple biomarkers are highly demanded, which may assist clinicians in earlier diagnosis and decision-making regarding treatment of a patient's conditions. For example, advances in oncology led to the discovery of varieties of biomarkers for different cancers. Detection of those markers facilitated early pre-clinical diagnosis and had a remarkable impact on clinical management and prediction of outcomes (611). Moreover, in epidemic incidents such as cholera, identification of the bacteria and toxins early is exceptionally vital to protect the population (612). The need for fast simultaneous analysis of multiple markers, with high sensitivity, requires further methods being investigated to conduct immunoassays. Therefore, the multiplexed assay was developed as another method for immunoassays.

5.2.3.1 Multiplexing technology

Multiplex technology allows simultaneous analysis of multiple different molecules in a single sample. There are two approaches available to perform multiplex analysis namely, microarray-based technology, and bead-based, which utilising micro-carriers, such as microbeads. (613), For the study presented in this chapter, I focus on the microbeads' approach.

Microbeads-based multiplex assays enable simultaneous identification and quantification of different molecules in one sample and process several samples at the same time (610). Microbeads immunoassays have been used to analyse cytokines, hormones and growth factors in various samples (plasma, serum and tissue culture) acting as a direct approach for detecting biomarkers and currently being the most advanced multiplex immunoassay (614, 615).

As this study was included neonates (preterm and term infants) and investigated five GutHs, a method that can assay multiple hormones in a single small sample is worthy of use;

therefore, the magnetic-beads multiplex technique has been used for analysing the targeted GutHs. This method has been used in previous research that investigated gut hormones and biochemical metabolites in preterm infants (581, 590, 616, 617). Moreover, some of the different gut hormones have direct relationships with each other (585, 598); it would be advantageous to measure such hormones simultaneously from the same sample. The magnetic-beads multiplex assay will be further described in Section 5.3.8.3 and Section 5.3.8.4.

5.2.4 Rationale for the study

As described in Section 1.9.2, colostrum contains many trophic factors such as epidermal growth factor, insulin-like growth factor and transforming factor, which have trophic effects on the growth and maturation of the GIT (234-236), potentially leading to the earlier establishment of enteral feeding (618). Colostrum growth factors may also exert indirect trophic effects by increasing the concentrations of some of the circulating gut hormones (580); these trophic factors are present in colostrum expressed by mothers of full-term infants as well as and preterm infants, and are further higher in colostrum of those who have delivered preterm infants (195, 201, 274).

Using the oropharyngeal route to coat the oropharynx with a small volume of colostrum could continue the effects of the amniotic fluid in utero, as described in Section 1.11 (158, 277). Furthermore, OPC administration can be used to provide the benefits of mother's colostrum to all infants whose care requires that they do not receive milk enterally such as infants with GIT immaturity or anomalies (oesophageal atresia, gastroschisis) and those requiring mechanical ventilation. Although, research investigating the effects of using OPC on outcomes of preterm infants are progressing (359, 503, 619, 620), to the best of my knowledge, no ongoing or published study has investigated gut hormone secretion in response to OPC administration. Moreover, most of the previous research has studied OPC in EXP and ELBW and VLBW infants; this study included all preterm infants' categories and other sick infants requiring NIC.

Some studies have investigated gut hormones in preterm infants and their relationship to trophic and enteral feedings during the neonatal period (580, 592, 621), these studies did not assess the effect of providing mother's colostrum by the oropharyngeal route on gut hormones.

The Cochrane review (Chapter 2) and the case-control study (Chapter 4), as well as previous studies (353, 417), suggested that OPC administration can shorten the time to reach full enteral feeds. However, the mechanism through which colostrum administered by the oropharyngeal route enables the GIT to adapt to its postnatal function is currently unclear.

The present study, therefore, evaluated some GutHs in the early postnatal period to assess the influence of OPC administration on gut hormone concentrations in preterm and sick infants. Changes in plasma concentrations of five different gut hormones were evaluated over the first two weeks of life using multiplex technology. Table 5.1 summaries the rationale for the gut hormones which were measured in this study.

Table 5.1 Rationale for the gut hormones studied

Gut hormone	Rationale
Peptide tyrosine tyrosine (PYY)	Potential biomarker for predicting feeding intolerance (FI) in preterm infants. May reflect energy/weight balance (585) and it may predict impaired neuroendocrine responses and intestinal growth in infants at risk for FI (582).
Gastric inhibitory polypeptide (GIP)	Potential biomarker in predicting FI. May predict impaired neuroendocrine responses, and intestinal growth in infants at risk of FI (582, 593). Biomarker for entroinsular axis (587).
Glucagon-like peptide (GLP-1)	A regulatory signal between enteral feeding and GIT adaptation and potential biomarker in predicting FI (582).
Ghrelin	Reflects energy balance and postnatal catch-up growth (601).
Insulin	Reflects the metabolic state and an important growth regulator (622). A potential predictor of long-term insulin resistance (607).

5.2.5 Hypothesis and aims

I hypothesised that oropharyngeal administration of the mother's own colostrum to infants requiring neonatal intensive care is associated with changes in plasma concentrations of gut hormones which are known to promote the development of GIT and tolerance to enteral feeds. I also hypothesised that postnatal changes in different gut hormones are associated with growth rates in the infants.

5.2.5.1 Aim of the study

The purpose of this study was to evaluate the impact of OPC administration on gut hormone concentrations during the neonatal period. It was a pilot study to provide data that might inform appropriate sample size calculations for future studies with the power to assess the secondary clinical outcomes.

5.2.5.1.1 Primary objective

To evaluate whether early administration of OPC to preterm and unwell infants requiring NIC results in beneficial (increase/decrease or both) changes in plasma gut hormone concentrations during the first few postnatal weeks.

5.2.5.1.2 Secondary objectives

To investigate if there are relationships between changes in the plasma gut hormone concentrations and:

- the growth trajectory of the infants
- the clinical outcomes of these infants during the stay in neonatal units.

5.3 Methods

This study received a favourable opinion from the East Midlands - Leicester South Research Ethics Committee (REC) (Appendix 12) and Human Research Authority (HRA) approval (reference number: 17/EM/0323) (Appendix 13). The study protocol was published on the HRA website (623). As a participating National Health Services (NHS) organisation, the

Nottingham University Hospitals (NUH-NHS) trust confirmed the Capacity and Capability for conducting the study on the Nottingham neonatal units.

5.3.1 Study design

It was an observational, non-randomised study; compared infants who received own mother colostrum by the oropharyngeal route (OPC group) during the early neonatal period with those infants who did not receive OPC (No-OPC group). The neonatal units of the Queen's Medical Centre (QMC) and the City Hospital (CH) at the NUH-NHS trust were the study site.

5.3.2 Participants

5.3.2.1 Eligibility criteria

Infants were eligible for inclusion if they required NIC and were not able to receive enteral feeding.

While all mothers were encouraged to express colostrum for their infant, this was not always available for administration in the 96 hours of life. Based on the standard care of the neonatal unit, infants who received OPC were assigned to the 'OPC' group, and those who did not receive OPC were assigned to the 'No-OPC' group. As the clinical conditions of, and NEC risks for, preterm and near-term/term infants differ, infants who had been recruited into the study were stratified for analysis into gestational age groups (<34 weeks (preterm) and >34 weeks of gestation (near-term/term).

5.3.2.2 Inclusion criteria

Infants were considered for inclusion in this study if they have been:

- admitted to one of the Nottingham's two neonatal units, and
- their developmental maturity or clinical condition or both requires that they do not receive enteral feeding, and
- they are eligible to receive OPC according to the guideline of the neonatal units (Appendix 7), and

- their parents gave informed written consent.

5.3.2.3 Exclusion criteria

Infants were not eligible for inclusion in the study if:

- they had major congenital anomalies, except those affecting the GIT, or
- death was considered likely within the first 72 hours, or
- there were contraindications for the infant to receive their mother's milk as per the unit guideline (such as HIV infection), or
- they were already receiving oral feeds, or
- there was no informed parental consent to participate in the study.

5.3.2.4 Involvement of the participants

The participants were enrolled in the study after written informed consent was obtained from the parents and continued until the infant was discharged from the neonatal unit.

5.3.3 Recruitment

The researcher identified participants who were eligible for inclusion in the study from the ward list and the clinical database (Badger Neonatal) of the participating units. The participant's clinical team who were nominated by the Chief Investigator (CI), appropriately trained in keeping with the principles of Good Clinical Practice (GCP) and entered onto the delegation log, approached the parents of eligible infants in the neonatal units and the postnatal ward at the QMC and CH Campuses of the Nottingham University Hospital NHS Trust. Detailed instructions and a study flow chart were provided to the clinical team and were available in the Investigator Site Folder (ISF), which included copies of all the study documents, at each participating unit. When it was reasonable after delivery, the clinical team approached the mothers, explained the study in detail and answered any question provided by the parents.

Parents were offered Parent Information Sheets (PIS) (Appendix 14) approved by the ethics committee. The PIS described the objectives of the study, including the study process, how

the infant would be involved and the benefits and risks of taking part in the study. The PIS also described the infants' rights, including the confidentiality of their information, voluntary participation and withdrawal from the study. Contact details of the research team were also provided. The PIS was only available in the English language in paper format in the neonatal units. When it was needed, an interpreter assisted with a discussion of the study. All parents were given sufficient time to read the PIS and to decide whether to participate in the study. The clinical team then introduced those who were interested in the study to the researcher.

5.3.4 Informed consent

To keep with the principles of GCP (624) and protect the participants' rights, informed written consent was obtained from parents of all the participants before the enrolment of their infants in the study. The process for obtaining parental informed consent was according to the REC guidance, and GCP and the regulations of the University of Nottingham (UoN) (372).

As mothers automatically have parental responsibility for their babies, written informed consent was gained from the infant's mother. However, an agreement to participate is ideally sought from both parents of an eligible infant. Babies admitted to the neonatal unit within the first week of age, have very rarely received Birth Registration under UK law (https://www.gov.uk/parental-rights). Although the infant's father to whom the infant's mother is married/civil partnered at the time of the infant's birth is legally permitted to give consent to studies involving his baby, as this study included information on the mother and her pregnancy, consent was considered valid when the mother gave consent.

The consent form included data collection from the medical records, collection and storage of blood samples and the parent's and infant participant's rights (Appendix 15).

Consent was received by an appropriate person in keeping with the principles of GCP and listed on the delegation log of the research. The consent form was completed and signed by the infant's mother then signed and dated by the person who received the consent. According to the regulations of the UoN, the consent form was prepared in three copies; one

copy was given to the infant's parent to keep, one was kept securely by the investigator, and a third was retained in the patient's hospital records. The decision regarding participation in the study was entirely voluntary. It was emphasised to the parents that consent regarding study participation could be withdrawn at any time without consequences on the quality or quantity of their babies' medical care.

5.3.5 Intervention

Administration of OPC is part of the clinical care of infants on the neonatal units, according to the Nottingham Neonatal Service Guideline on the use of OPC (Appendix 7). Small volumes (0.2ml) of own mother's colostrum were given by the oropharyngeal route every four hours for three days. OPC was started as early as possible within the first 96 hours of birth with the timing was dependent on the availability of the mother's colostrum. All aspects of care and OPC use were at the discretion of and were the responsibility of, the clinical team treating the infant.

5.3.6 Study regimen

Once the signed written informed consent form was obtained, each participant was allocated a study identity code number (SID) to be used on the data collection forms and electronic data. A separate confidential record of the participant's name, date of birth, local hospital number Badger unique number, and the SID was made to permit identification of all participants enrolled in the study, in case additional follow-up was required.

The master file linking the study ID with the infants' identifiable information was kept securely and separately at the Division of Child Health, Obstetrics & Gynaecology (COG) of the UoN. The study ID was used to label blood sample containers and all participant data records.

5.3.6.1 Data collection

With informed written consent, the researcher (AN) collected the study data from routinely recorded clinical items obtained from the clinical records. The data were recorded in the data

collection forms (DCFs) by hand using a black ballpoint pen. Next, the data were entered into encrypted anonymised electronic records.

The completed DCFs were treated as confidential documents and to protect the rights of the study's participants to privacy, the researchers adhered to the Data Protection Act, 1998. The study protocol, PIS and the consent form were updated to ensure compliance with the UK new General Data Protection Regulation (GDPR) (406) and Data Protection Act 2018 (407). A supplementary information sheet was provided to the parents who participated in the study before the new law commenced. Only the necessary required information for the study was collected on the DCF, and all the DCFs were held securely, in a locked room and locked cupboard in the Division of COG at the UoN. Access to the information was limited to the researchers and any relevant regulatory authorities. All data were stored on a computer Z drive; a dedicated web server for the UON, which was securely password protected.

5.3.6.1.1 Source of data

The data were collected from the infants' medical records including, the medical notes and the Badger neonatal database and Trust's Digital Health Record (DHR) when it was necessary. Where an infant admitted to the Nottingham NICU was transferred to a local neonatal unit at another hospital Trust for ongoing care, data on outcomes to discharge from hospital were collected using the Badger database of that unit and the DHR system with the agreement of the local neonatal unit.

5.3.6.1.2 Type of data

The collected data included the following:

- infants' demographic information; date of birth, date & time of admission to the neonatal unit, gestational age, sex, birth weight and date of discharge from the neonatal unit
- clinical characteristics of the infants such as mode of delivery, multiple gestations, delivery room resuscitation, 1 and 5-min Apgar score, non-

invasive ventilation, endotracheal intubation & mechanical ventilation, central line placement, nasogastric tube, use of parenteral nutrition and medications

- infant's feeding history including, type of milk, mode of feeding, date of start, volume received during the intervention, date of attainment of full enteral feeding and type of milk received during admission and at discharge to home.
- any morbidities during a hospital stay, such as NEC, sepsis, pneumonia, any other serious complications and death
- results of the infant's routine blood glucose testing (if undertaken)
- infant's growth parameters, weight, head circumference, at admission, throughout the infant's care in the neonatal unit and before discharge as is standard care. It was also planned to include the length of the infants, however, measuring the length was not routinely recorded clinical items in the Nottingham neonatal units
- OPC administration including, date of starting, frequency, number of doses received, total volume of colostrum received by the infant, duration of OPC, parent's participation in administration, concomitant feeding regimen and any reported adverse effects
- maternal and pregnancy medical history such as, medical and pregnancy-related illness including diabetes, pre-eclampsia, infection, premature and/or prolonged rupture of membranes, antibiotic therapy, antenatal steroid use. Maternal data were also collected from the infant's medical records. As part of standard care for infants on the NICU, these maternal data are routinely transferred into the infant records.

5.3.7 Outcome measures

5.3.7.1 Primary outcome

- Postnatal changes in plasma concentrations of gut hormones during the first two postnatal weeks. These will be evaluated by measuring plasma concentrations of PYY, GIP, GLP-1, ghrelin and insulin at baseline (before or within 24 hours of first receiving OPC) and at around 7 and 14 days from baseline.

5.3.7.2 Secondary outcomes

The following outcomes were assessed from birth till discharge from the neonatal unit:

- Infant's growth during a stay in the neonatal unit. Weight Z scores were calculated using clinical actual age percentile and Z-score calculator (544). Head circumference (HC) Z scores were not calculated as HC was not measured at birth. Length Z score was also not calculated as no data were available for the infant's length in the medical records because the length is not routinely measured during stay in the neonatal unit. The difference between birth weight Z-score and weight Z-score at discharge from the neonatal units were used to assess the growth trajectory of the infants
- days to reach full enteral (milk) feeds (defined as enteral milk at 150ml/kg/day or more sustained for 72 consecutive hours)
- days of parenteral nutrition (PN)
- days to first enteral feed
- days of mechanical ventilation
- length of stay in NICU, high dependency and low dependency units
- death before discharge home
- days of antibiotic use
- the rate of breastfeeding and the type of milk at discharge

- feeding intolerance (defined as delayed commencement or/and delayed advancement of enteral feeding)
- incidence of NEC (defined as Bell's stage ≥ II)
- incidence of clinically suspected or microbiologically-confirmed late-onset sepsis.

5.3.8 Measurement of Gut hormones

5.3.8.1 Blood sampling

Blood samples for analysis of gut hormones were collected after written informed consent. Samples were withdrawn by the clinical team caring for the infant in line with routine blood sampling of the infant according to the clinical indications to prevent any additional discomfort or disturbance to the infant. Three samples were collected from each infant participant: at baseline (GH1) before administration of OPC (or a maximum of 24 hours after first OPC administration), around 7 and 14 days (GH7 & GH14). Each sample was a small volume of blood (around 250-500µl), in addition to that required for routine laboratory testing in the clinical care of the infant. The blood samples were collected in standard, manufactured tubes containing ethylene diamine tetra-acetate (EDTA) as an anticoagulant as used in clinical practice. A sample collection instruction sheet (Appendix 16) was prepared, and copies were kept in the ISF at the two study centres. The researcher (AN) collected and transported the blood sampling tubes on Ice from the neonatal units to the laboratory at the Division of COG at the UoN. Whilst waiting for collection the blood sample tube was immediately stored in a universal container within Ice bag in the refrigerator at the neonatal unit. A member of the laboratory team processed the blood samples immediately according to the manufacturer's instructions. Samples were labelled using a combination of study ID, and two unique study identifiers (e.g. GH0-245) to permit accurate linkage to study data and the consent form. All samples were registered and stored according to the Human Tissue Authority's Code of Practice (HTA) for Research, at the Division of COG (QMC and CH), UoN (625).

5.3.8.2 Sample preparation

The blood samples were collected in tubes containing EDTA as an anticoagulant. To counterbalance the normal degradation of GIP and GLP-1 by dipeptidyl peptidase IV (DPP-IV) enzyme (626), 10 µl protease inhibitors (Catalogue number: DPP4-010, Millipore) and 10 µl of serine protease (Catalogue number: 565000-1VL, Millipore), which stabilises ghrelin (627), were immediately added to every 100 µl of blood in the EDTA tube. DPP-IV is a protease enzyme present mainly in the endothelial cells, and it also exists in the circulation. DPP-IV rapidly inactivates bioactive peptides such as the incretin hormones (628). Therefore, inhibition of the DPP-IV prolongs the half-life of the GIP and GLP-1 in the plasma; this effect allows better detection of these hormones. The blood samples were then centrifuged for 10 minutes at 2,000 g at 4°C. The plasma fractions of the samples were divided into aliquots and stored at -80°C until assayed. The cellular fractions were disposed of according to the HTA guidelines (625). Processing of the blood samples was carried out by members of the COG laboratory team (Dr Lesia Kurlak, Dr Hiten Mistry, Dr Ian Bloor, Mr Mark Pope) or a laboratory research student (Mrs Layla Albustanji).

5.3.8.3 Quantification of plasma gut hormones

Plasma concentrations of the study target hormones were measured using microsphere-based immunoassay such as MILLIPEX MAP assay.

5.3.8.3.1 Magnetic beads-based immunoassay

Magnetic beads (MBs) are small sized microspheres having a diameter ranges from 1 to 5 micron (629). They consist of an iron core surrounded by solid spherical particles and a mixture of two coloured dyes that adjusted to provide multiple distinct colours to enable simultaneous measurement of multiple biomolecules in a single microplate well (630). MBs immobilise molecules such as proteins, enzymes, peptide and nucleic acids to separate them from a sample and act as a sold surface where the assay reaction takes place (631).

Microbeads classified into two basic categories, nonmagnetic and magnetic. Magnetic beads have fluorescent and magnetic features and are compatible with all currently available Luminex-based equipment, which is commonly used as a platform in the commercial assay (632)

5.3.8.3.1.1 Principle of MBs immunoassay

The principle of the assay is analogous to a sandwich ELISA. Sandwich immunoassay is a technique using a pair of antibodies specific for the target molecule (e.g. peptide, protein, and antigen); one is attached to a solid surface to capture the target, it is referred as a capture antibody. The second antibody (detection antibody) binds to the target at a different site; thus, the target molecule is grasped between the two antibodies (633).

In microbeads multiplex assay, sets of microbeads uniquely coded with a two fluorescent dye. The captivating antibodies directed against the target biomolecule are covalently combined with the beads. Coupled beads react with the sample containing the molecule of interest, and a sandwich compound is formed after adding biotinylated (conjugated with biotin) detection antibodies specific for the target molecule. Finally, the biotinylated detection antibodies bind with Phycoerythrin-Streptavidin conjugate, a reporter dye, which acts as the fluorescent indicator (Figure 5.1). The intensity of emitted light quantifies the relative amount of the molecule bound to the bead, and the quantity of the molecule found is directly proportional to the fluorescent signal (634).

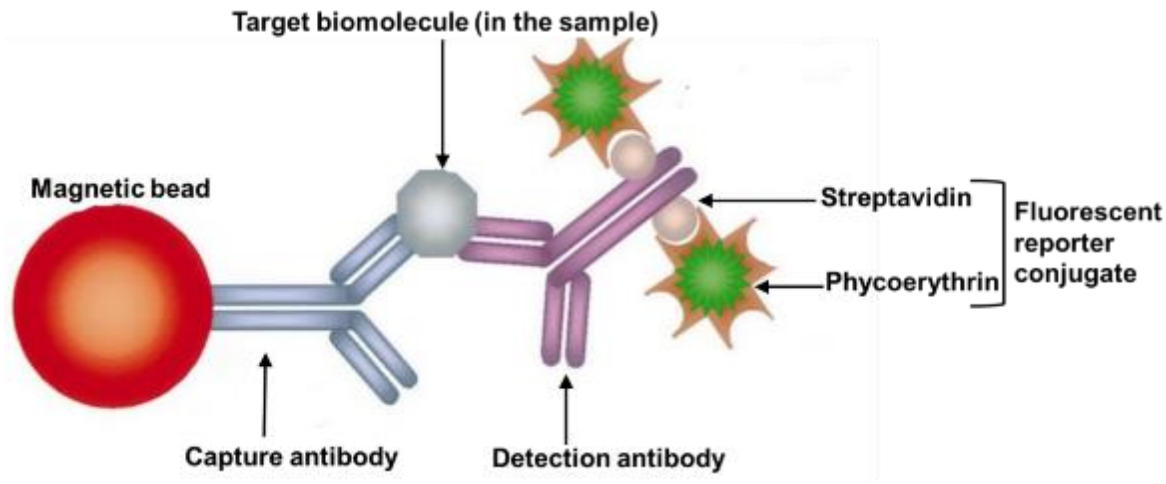


Figure 5.1 Magnetic-bead immunoassay general principle
Schematic diagram presents the sandwich magnetic-bead immunoassay. Magnetic bead: internally coloured with fluorescent dyes (red and infrared). Capture and Detection antibody: specific for a target molecule. Fluorescence reporter: Streptavidin-Phycoerythrin conjugate for detection of molecule via light emission that is directly proportional to the concentration of the bound molecule. Diagram adapted from Vignali 2000 (635).

5.3.8.3.1.2 Advantages of magnetic-bead based assay (610)

MBs immunoassay have many advantages include:

- MBs have an optimum binding capacity even in small volume due to their small size and large surface area that allow the entire particle to react rapidly to stimuli with a high reaction rate. Therefore, may generate higher signals than if the same reaction is conducted in solutions (636)
- Microbeads shorten the analysis time due to their low viscosity and high mobility that makes them more effective in the transport and delivery of the molecules to the reactive surface
- MBs enhance the sensitivity of the assay because of their central iron core which, prevents trapping of the antibodies
- MBs minimise sample loss, as centrifugation is not needed, which may lead to loss of immune complexes or breaking weak antibody-antigen bonds. Therefore, accuracy and reproducibility of the results will be ensured

- small sample volume is required for simultaneous analysis of multiple molecules that make the analysis more cost-effective (632).

The multiplex assay has some limitations, such as non-specific binding of antibodies that present in some samples may confound the measurement. However, many methods are available to remove these antibodies and preserve the integrity of the analysis (637). Sensitivity in the very low concentrations of some biomolecules and reference values for newborn infants remain a challenge for the researchers. Additionally, the multiplex assay requires specialised equipment, which creates high costs for initial installation (638).

5.3.8.4 Gut hormone multiplex assay

The target GutHs (Table 5.1) were measured using a Human Metabolic Hormone Magnetic Bead Panel (MILLIPLEXR Map # HMHEMAG-34K, 2013 EMD Millipore Corporation, Billerica, MA, USA.). The kit was obtained from a commercial company (Merck Millipore, U.K.) (https://www.merckgroup.com/uk).

Bio-Plex® 200 (BIO-RAD) system consisting of an analyser, a computer station and a Bio-Plex Manager software, was used as a platform for the analysis. BIO-RAD is a life science commercial company, providing a range of technological products for different areas such as life science research, clinical diagnostic, food science, quality control and spectroscopy (639)). Bio-Plex 200 is a flow cytometry-based detection system capable of conducting a qualitative and quantitative analysis of proteins and nucleic acids in a range of matrices. This flow cytometry platform has high sensitivity and allows simultaneous measurement of up to 100 target molecules in a single well of 96-well plate. It is compatible with magnetic beads, used to analyse various biomolecules such as cytokines, hormones, and nucleic acids (640). However, Bio-Plex 200 is a costly system which could not be equipped into a total analysis system (610).

Bio-Plex Manager version 6.1 software (BIO-RAD) was used for the quantitative analysis and data visualisation (641). The Bio-Plex Manager presents the data as median fluorescence intensity and the concentrations of the targeted hormones (pg/mL), which are proportional to

the fluorescence intensity. This software generates statistical reports for multiplex data that were exported in formats compatible with statistical software such as Microsoft Excel, facilitating and accelerating data analysis (641).

5.3.8.4.1 Procedure of the assay

The procedure of the Multiplex assay was carried out by Dr Ian Bloor and Mr Mark Pope at the Life Sceince Department (Biology Building, University Park Campus), UoN. All steps were performed according to the manufacturer's instructions (Appendix 17). Figure 5.2 demonstrates the basic steps of the bead-based immunoassay reactions.

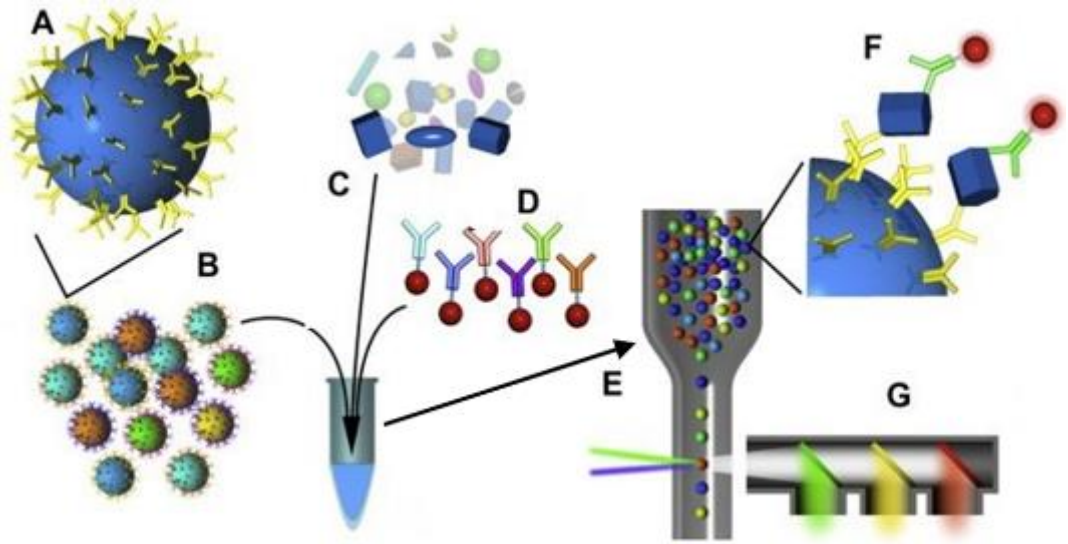


Figure 5.2 Summary of the procedure of beads-based immunoassay
Schematic diagram summarises the basic steps of beads multiplex immunoassay (634)
A: Microbeads are coloured internally with fluorescent dyes and coated with a specific capture antibody.
B: Multiple sets of beads, is made, each bead set is coated with a distinct capture antibody to a target molecule
C: Addition of the sample and a mix of all desired bead sets are combined and incubated.
D: Addition of a mixture of detection antibodies specific for the target molecules conjugated to a reporter dye, which is also specific for the same target molecules.
E: The bead-analyte-reporter mixture is analysed through a flow chamber supporting individual bead separation.
F: Each bead has the potential to have analyte bound to capture antibody, and a specific detection antibody bound to a reporter-dye, which depends on the analyte in the sample.
G: A red laser light stimulates the red and infrared dyes within the microbeads, which identifies and categorises the beads. A green laser stimulates the detection antibody–reporter dye complex that bound to the beads; the intensity of the signal quantifies the relative amount of the target molecule bound to the microbeads.

Each sample was analysed in triplicate with seven standards and two controls, which were included in the kit, to verify the efficiency of the experiment. A blank (Serum Matrix: LHGT-SM, Millipore) that was provided with the kit was included for comparison. Two samples were only analysed in duplicates because there was insufficient plasma volume, which was expected in newborn infants with high haematocrit. For each gut hormone, a standard curve was generated by the Bio-Plex Manager software to determine the hormone concentrations of the samples relating to the mean fluorescence intensity (Appendix 18).

The assay showed good precision (0 to 10.6% intra-assay coefficients of variation (CV)) except for two samples that had %CV of 11.5 and 13.9%. Each participant's set of plasma samples was analysed at the same time to prevent potential errors due to inter-assay variations. The range of the standard recoveries was 83-110 %, except for PYY (S6; 41%). The standards recoveries were determined by back-calculation ((observed concentration/expected concentration) x 100), to assess the accuracy of the assay (acceptable range: 70-130%). The assay sensitivities (minimum detectable concentration) for the measured hormones were as follow: PYY: 28; GIP: 0.6; GLP-1: 1.2; Ghrelin: 13; Insulin: 87 pg/mL. Most of the observed concentration values of the measured hormones fall within the range of the standard curves except for GLP-1 and ghrelin. For GLP only 4/22 (18%) samples were below the range. Fifty per cent of the observed values of ghrelin fall outside of the range; this could be due to that the sample contains no hormone or its concentration below the detectable levels. Out of range values were also could be attributed to small sample volumes or a technical error.

However, these ghrelin values were expected as ghrelin concentrations are very low or undetectable at birth and during the early neonatal period (581, 590). However, the data is still valuable by showing very low concentrations.

5.3.9 Statistics

5.3.9.1 Sample size

The study sample size was calculated using nQuery Advisor + nTrim version 7.0 software (642). Using data from a previous study that investigated gut hormones in preterm infants (590) this study reported an increase in the mean (SD) plasma PYY concentrations from 353.0 ± 457.73 pg/ml to 634.3 ± 580.03 pg/ml after one week of enteral feeding, for an alpha of 0.05, a sample size of 9 per group would give 80% power to detect a difference.

The aim was to recruit 40 infants, 20 infants born at <34 weeks of gestation and 20 infants born at > 34 weeks of gestation (10 per group).

This research was a novel study which has been based on feasibility of completion with the recognition that this study was only powered to demonstrate likely changes in the primary outcome (postnatal changes in plasma gut hormone concentrations).

5.3.9.2 Statistical analysis

The Statistical Package for Social Sciences (SPSS) for Windows (Version 23, Armonk, NY: IBM Cop) (549), and GraphPad (GraphPad Software Inc., La Jolla, CA, USA) (643) were used for performing the statistical tests. A p-value of 0.05 was considered statistically significant.

Participants' demographic and clinical data were summarised using descriptive analysis for categorical variables (frequencies and percentages). Continuous variables were presented according to the data distribution as a mean ± standard error of the mean (SEM) for normal distribution and median and range/interquartile range (IQR) for skewed data. Normality of data was assessed using a histogram plot and Shapiro-Wilks test of normality. A transformation was performed for continuous data that did not demonstrate a normal distribution.

Repeated Measure One-way Analysis of Variance (RM-ANOVA) was used to measure changes in the gut hormone concentrations over time. Correlations of changes in plasma gut

hormones concentrations with feeding volumes and gestational age and birth weight of infants were assessed using Pearson's Correlation Coefficients for parametric data or Spearman's for non-parametric.

Comparisons between the study groups, for continuous data, independent t-test for parametric data, and Mann Whitney U tests for non-parametric data and Fishers' exact test for categorical data are the planned analysis when the study complete.

Survival analysis using the Kaplan-Meier method is also planned to analyse time to reach event outcomes such as full enteral feeding, duration of parenteral nutrition and stay in the neonatal unit. Kaplan-Meier curves will be used to present the difference between the study groups in outcomes of interest. The Kaplan-Meier analysis is a non-parametric estimate; computing the probability of events that occur at a given point of time (644).

Appropriate, multivariate analysis is planned to adjust for potential confounders such as gestational age and birth weights.

5.3.9.3 Dealing with missing data

Several methods have been established to deal with partially missed data such as complete-case analysis and available-case analysis and single imputation. However, such approaches have many limitations; therefore, they are generally not recommended when an unacceptable percentage of data is missed (> 10%) (552). In this study, the data were analysed by the total case basis; for each variable; only those infants with complete data are included in the final analysis.

5.4 Results

From May 1st 2018 to July 19th 2018, 344 babies were admitted to Nottingham neonatal units at the Nottingham University Hospitals NHS Trust; QMC and CH, UK: 70 infants were born before a gestational age of <34 weeks and 274 infants were born >34 weeks of gestation. Parents of 20 eligible infants who were <34 weeks of gestation had been approached to participate in the study. Informed written consents have been obtained from

parents of four infants, however, one of these four infants was excluded because he was transferred to other hospital before collecting the second and third blood samples. Parents of 7 eligible infants who were >34 weeks of gestation were approached, parents of 4 infants consented to enrol their infants in the study (Figure 5.3).

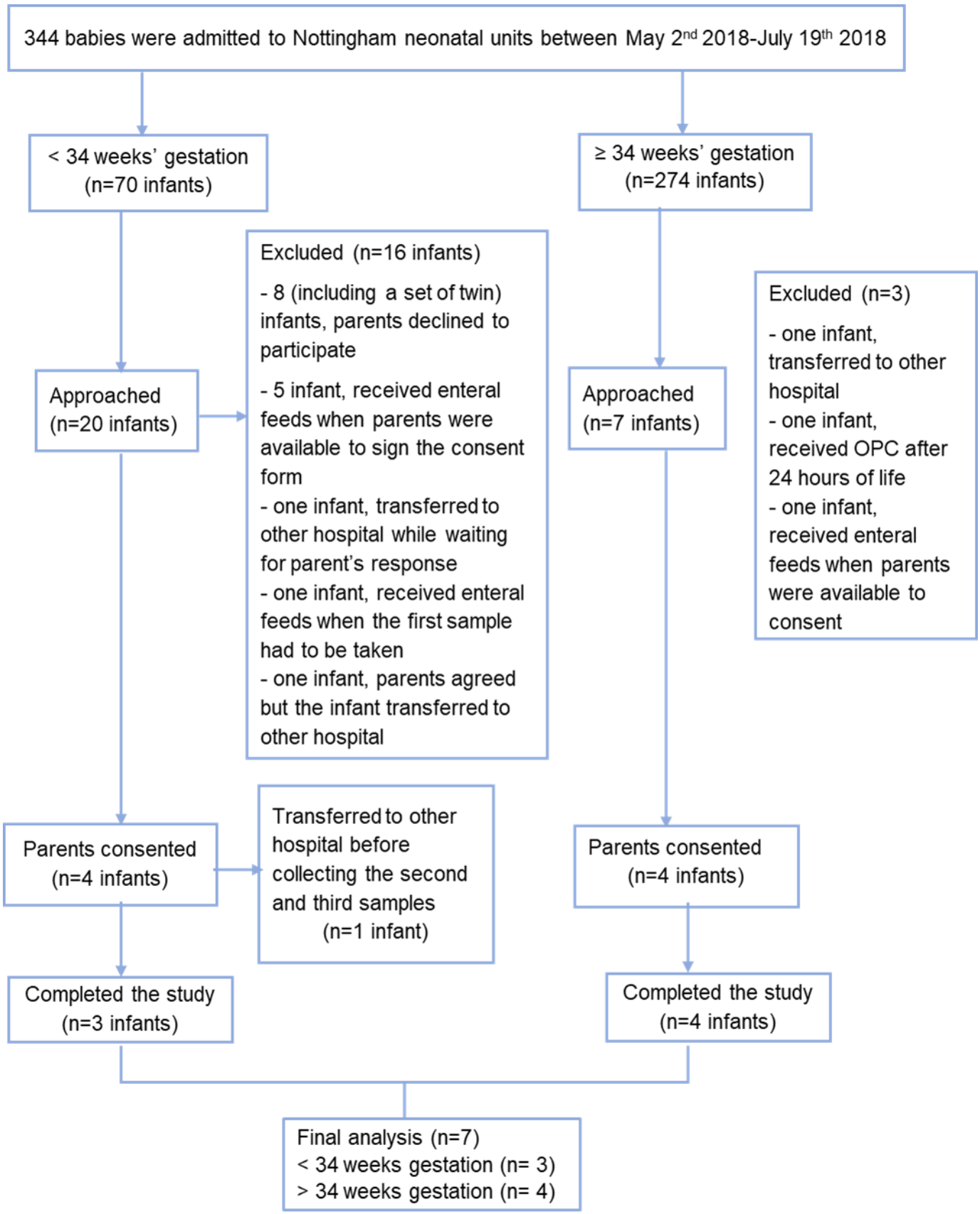


Figure 5.3 Study flow chart

Flow of the enrolled infants in the study and final analysis.
n: number of infants

5.4.1 Characteristics of the included infants

Seven infants were included in the analysis, comprising a heterogeneous group. Most of the infants were male (85%). The mean ± SEM of the gestational age was 32.8 ± 1.5 weeks, and the mean birth weight was 1970 ± 302.20 grams. Six infants received OPC; mean ± SEM, age for starting OPC was 2.2 ± 0.4 (95% CI, 1.14 to 3.19) days; number of doses were received: 11.5 ± 1.4 (95% CI, 8 to 15) doses; colostrum volume was received: 2.3 ± 0.25 (95% CI, 1.7 to 3) ml. OPC was provided for 3 ± 0.25 (95% CI, 2.34 to 3.66) days. Most of the infants did not receive trophic or enteral feeding throughout when OPC has been given except one infant (infant 4) who had started trophic feeding during the second day of OPC administration. Table 5.2 presents the characteristics of the seven infants that participated in the study.

Table 5.2 Characteristics of the participant infants

Criteria	Infant 1	Infant 2	Infant 3	Infant 4	Infant 5	Infant 6	Infant 7
Study group	OPC	OPC	OPC	OPC	No-OPC	OPC	OPC
Gender	F	M	M	M	M	M	M
GA (weeks+ day)	37+1	31+3	35+4	35	36+1	28+2	27+1
Birth weight (g)	2230	1630	2120	1910	3520	1250	1130
Birth weight/GA	SGA	AGA	AGA	SGA	LGA	AGA	AGA
Mode of delivery	NVD	CS	CS	CS	CS	NVD	NVD
Apgar score							
1 minute	3	6	1	10	1	3	7
5 minute	10	6	4	10	7	8	10
Inotropes administration	No	Yes	Yes	No	No	Yes	No
Maternal Antenatal steroid	No	No	Yes	No	No	Yes	Yes
Underlying diagnosis	Duodenal /choanal atresia	Preterm	Gastroschisis	OA/TOF	HIE	Preterm	Preterm
Start feeding (postnatal day)	5	3	6	3	4	4	4
Type of milk	EBM	Formula	EBM	EBM	EBM	EBM	EBM
Feed volume (ml/kg/day)							
GH1	NBM	NBM	NBM	1*	NBM	NBM	NBM
GH7	110	160	15	150	160	100	145
GH14	32	145	16	145	165	125	160

OPC: oropharyngeal colostrum; GA: gestational age; SGA: small for GA; LGA: large for GA; AGA: appropriate for GA; NVD; normal vaginal delivery; CS: caesarean section; TOF: tracheoesophageal fistula; HIE: hypoxic-ischemic encephalopathy; OA: Oesophageal atresia; GH: gut hormone, 1: first sample; 7, 14: days from first sample; NBM: nil by mouth; EBM: expressed breast milk; *: ml/kg/hour.

5.4.2 Gut hormone concentrations over two postnatal weeks

The mean ± SEM postnatal age of the infants was 2.9 ± 2.4 (95% CI, 1.86 to 3.84) days, and almost all of the infants were fasting (6/7 (86%) infants) when the basal blood samples for gut hormone assay were taken.

5.4.2.1 Peptide tyrosine tyrosine (PYY)

Five of seven infants showed a significant increase in plasma PYY concentrations during the first week. By postnatal day 14, plasma PYY did not show a further increase in its concentrations (Figure 5.4). Overall, there were statistically significant changes in plasma PYY concentrations over time (p = 0.02). Bonferroni pairwise comparisons demonstrated that PYY plasma concentrations increased significantly at D7 compared to the basal value (adjusted p = 0.04 (Figure 5.5).

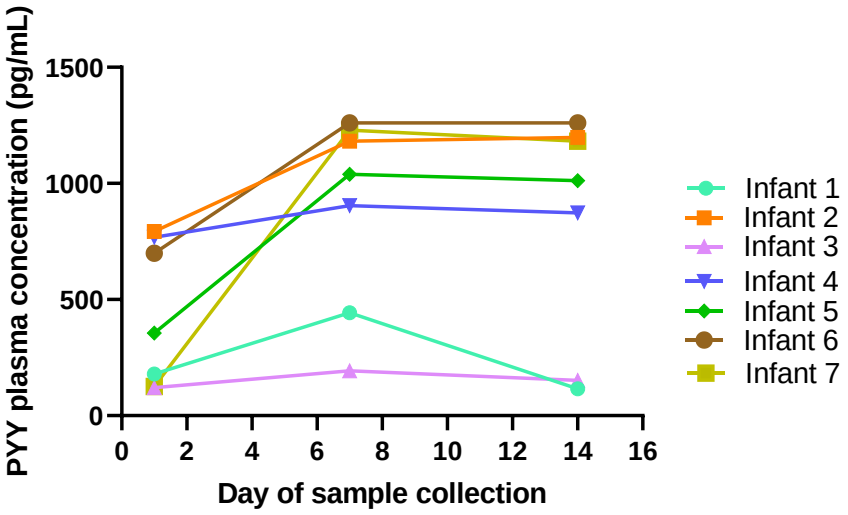


Figure 5.4 Plasma PYY concentrations for individual infant

Postnatal changes in plasma PPY concentrations (n = 7) over a two weeks period. Each line: represents the trend of PYY concentrations for an individual infant. Infants 2, 6 and 7 <34 weeks gestation; infants 1, 3, 4 and 5 > 34 weeks (All infants received OPC except infant 5); Symbols: time point (the day when samples were taken: D1 (baseline), around D7 and D14); PYY: peptide tyrosine tyrosine.

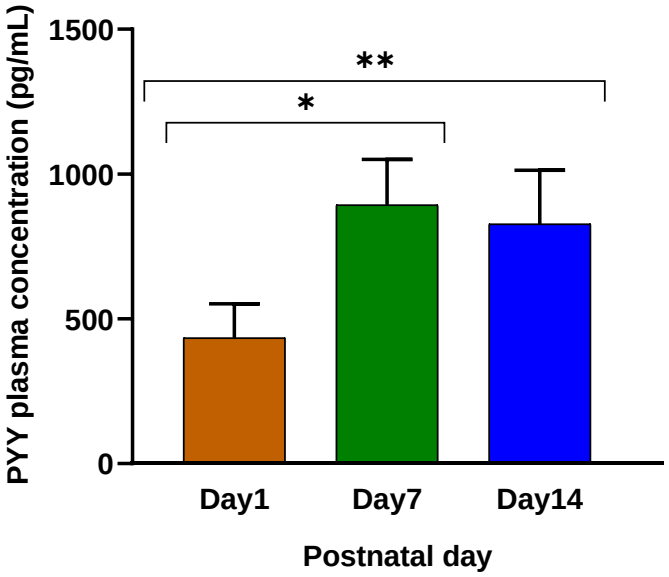


Figure 5.5 Plasma PYY concentration over time

Postnatal changes in plasma PYY over a two weeks period (n = 7). Bar: represents PYY plasma concentrations. Blood samples were taken for analysing PYY, Brown: baseline (around postnatal day 1 of life); Green: around day 7 from baseline; Blue: around day 14; Values are mean ± SEM. Comparisons: repeated measure ANOVA and Bonferroni post hoc test; **: p = 0.02; * p= 0.04; PYY: peptide tyrosine tyrosine.

5.4.2.2 Gastric inhibitory polypeptide (GIP)

The majority of the infants showed a significant increase in plasma GIP concentrations at D7. By D14, plasma GIP concentrations had a decreasing trend (Figure 5.6). Overall, there was a statistically significant increase in the plasma GIP over two postnatal weeks (p = 0.007). Adjustment for multiple comparisons (Bonferroni) demonstrated that the increase of GIP concentrations from the basal value and D7 (adjusted p = 0.02) and D14 (adjusted p = 0.04) remained statistically significant (Figure 5.7).

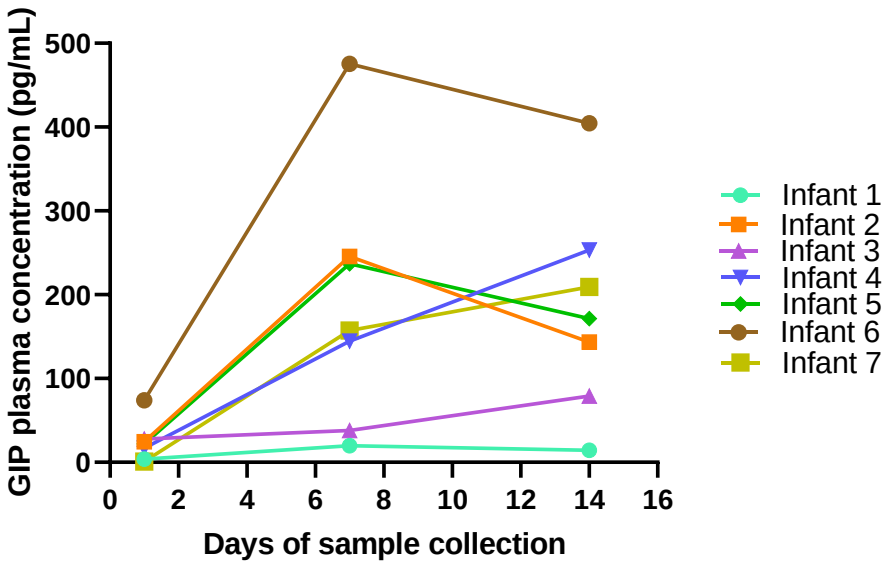


Figure 5.6 GIP plasma concentration for individual infant

Postnatal changes in plasma GIP concentrations over a two weeks period (n = 7). Each line: represents trend of GIP concentrations for individual infant; Infants 2, 6 and 7 <34 weeks gestation; Infants 1, 3, 4 and 5 > 34 weeks (All infants received OPC except infant 5). Symbols: time point (day when samples were taken): D1 (baseline), around D7 and D14); GIP: gastric inhibitory polypeptide

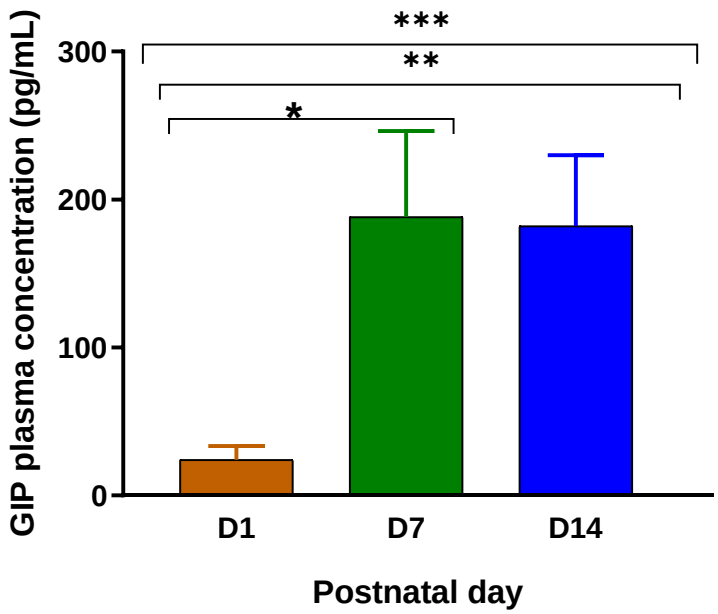


Figure 5.7 Plasma GIP concentrations over time

Postnatal changes in plasma GIP over a two weeks period (n = 7). Bar: represents GIP plasma concentrations. Blood samples were taken for analysing GIP, Brown: baseline (around first postnatal day of life); Green: around day 7 from baseline; Blue: around day 14; Values are mean ± SEM. Comparisons: repeated measure ANOVA (***: p = 0.007) and Bonferroni post hoc test (*: p = 0.02; **: p = 0.04); GIP: gastric inhibitory peptide.

5.4.2.3 Glucagon-like peptide (GLP-1)

Four (4/7) infants were included in this analysis because for three infants (one from the <34 weeks' group, and two from the >34 weeks group), only two values for plasma GLP-1 concentrations were detected (other values were below the limit of sensitivity of the assay). The four infants showed a substantial increase in plasma GLP-1 at D7, yet, by D14 there were reductions in the GLP-1 concentrations (Figure 5.8). Overall, there was a statistically significant increase in GLP-1 plasma concentrations over two postnatal weeks ($p = 0.02$). Bonferroni post hoc pairwise comparisons showed a statistically significant increase in plasma GLP-1 concentrations at D7 (adjusted $p = 0.03$) (Figure 5.9).

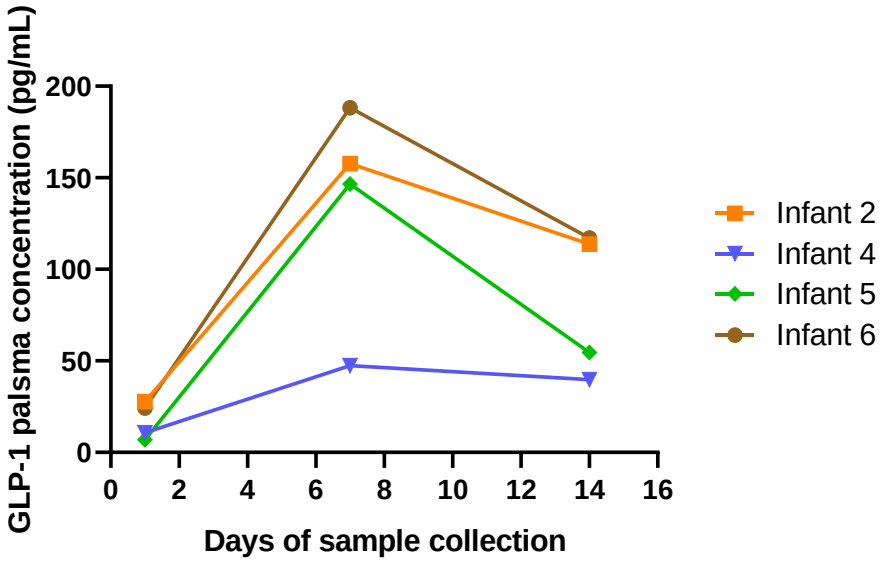


Figure 5.8 Plasma GLP-1 concentrations for individual infant

Postnatal changes in plasma GLP-1 concentrations (n = 4) over a two weeks period. Each line: represents a trend in GLP-1 concentrations for an individual infant; Infant 2 and 6 <34 weeks gestation; Infant 4 and 5 >34 weeks (All infants received OPC except infant 5). Symbols: time point (day when the sample was taken: D1 (baseline), around D7 and D14); GLP-1: glucagon-like peptide

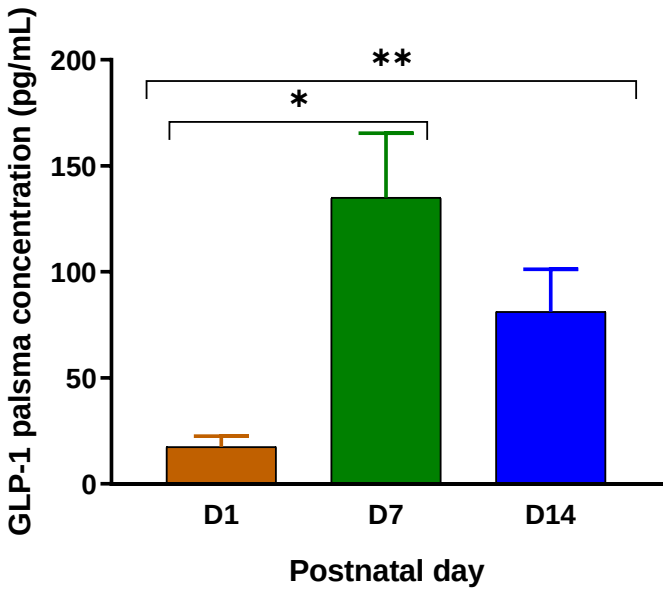


Figure 5.9 Plasma GLP-1 concentration over time

Postnatal changes in plasma GLP-1 (n = 4) over a two weeks period. Bar: represents GLP-1 plasma concentrations. Blood samples were taken for analysing GLP-1, Brown: baseline (around postnatal day 1 of life); Green: around day 7 from baseline; Blue: around day 14; Values are mean ± SEM. Comparisons: one-way ANOVA (**p = 0.02) and Bonferroni post hoc test (* p= 0.03); GLP-1: glucagon-like peptide

5.4.2.4 Ghrelin

Baseline Ghrelin plasma concentrations (D1) were below the limit of detection of the assay for five infants (5/7: 71%); therefore, Ghrelin D1 were not included in the analysis. Around postnatal D7 and D14 Ghrelin concentrations were detected in five infants. There were no significant changes in Ghrelin concentrations between D7 and D14 (p = 0.23) (Figure 5.10).

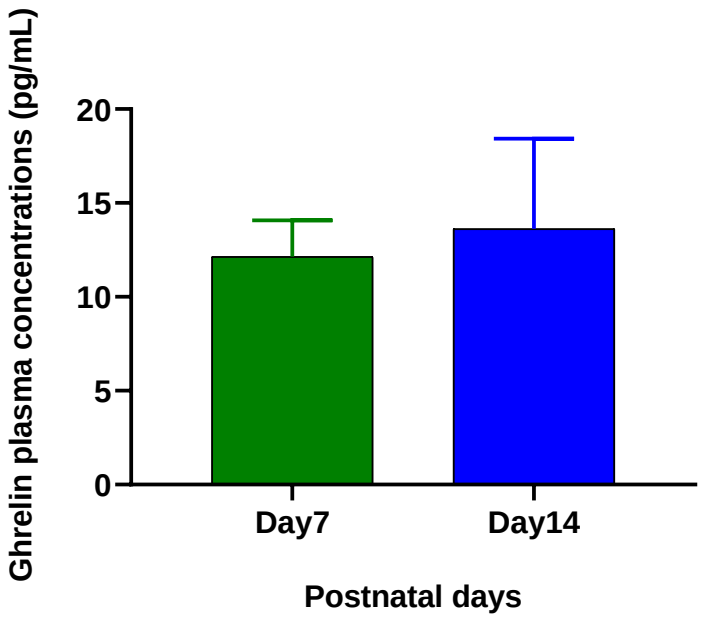


Figure 5.10 Changes in plasma Ghrelin concentrations
Postnatal age (days) when (n = 5). Bar: represents ghrelin plasma concentrations; Blood samples were taken for analysing ghrelin, Green: around day 7 from baseline; Blue: around day 14; Values are mean ± SEM. Comparisons: repeated measures one-way ANOVA

5.4.2.5 Insulin

There were no statistically significant changes in plasma insulin concentrations during the first postnatal weeks (D1: 680.58 ± 153.29 (95% CI, 305 to 1056); D7: 401.67 ± 45 (95% CI, 292 to 512); D14: 439.67 ± 286.02.59 (95% CI, 229 to 650); p = 0.19).

5.4.3 Correlation of plasma gut hormone concentrations with gestational age and birth weight

5.4.3.1 Plasma gut hormone concentrations and infant's gestational age

There were no statistically significant relationships between gestational age and the basal plasma concentrations of the gut hormones investigated in this study except for Ghrelin (Table 5.3). Ghrelin basal concentrations (D1 samples) for five infants were below the range of the detection of the assay; therefore, basal ghrelin was not included in the analysis. However, around postnatal D7 and D14 Ghrelin concentrations were detected in five infants;

there was a significant negative correlation between the mean plasma ghrelin concentrations (D7 and D14 samples) and the infant's gestational age (r = -0.7).

Table 5.3 Relationships between basal plasma gut hormone concentrations and infant's gestational age

Hormone (baseline)	Correlation coefficient r	95% confidence interval	P value
PYY (n = 7)	-0.28	-0.85 to 0.60	0.55
GIP (n = 7)	-0.48	-0.91 to 0.43	0.23
GLP-1 (n = 4)	-0.62*	0.99 to 0.63	0.16*
Insulin (n = 7)	0.02	-0.76 to 0.74	0.96

PYY: peptide tyrosine tyrosine; GIP: gastric inhibitory peptide; GLP: glucagon-like peptide; r: Pearson's correlation; * Spearman correlation; statistical significant: $p<0.05$

5.4.3.2 Basal gut hormone concentrations and infant's birth weight

There were no statistically significant relationships between basal values of the investigated gut hormones and birth weights and birth weight Z score of the infants except for ghrelin as demonstrated in Table 5.4. Ghrelin basal concentrations were below the range of the detection of the assay; therefore, basal ghrelin was not included in the analysis. As values for ghrelin concentrations were detected around D7 and D14 for five infants; the mean plasma ghrelin concentrations (D7 and D14 samples) was correlated with the infant's birth weight (r = -0.8).

Table 5.4 Relationships between basal gut hormone concentrations and infants' birth weights and birth weight Z scores

Hormone (baseline)	Birth weight		Birth WZS	
	Coefficient r (95%CI)	P value	Coefficient r (95%CI)	P value
PYY (n = 7)	-0.25 (-0.84 to 0.62)	0.58	0.06 (-0.72 to 0.78)	0.89
GIP (n = 7)	-0.28 (-0.86 to 0.59)	0.53	0.32 (-0.57 to 0.87)	0.48
GLP-1 (n = 4)	-0.80 (-0.95 to 0.68)*	0.30	-0.40 (-0.97 to 0.95)	0.75*
Insulin (n = 7)	0.02 (-0.72 to 0.78)	0.98	-0.02 (-0.76 to 0.75)	0.96

WZS: weight Z score; CI: confidence interval; PYY: peptide tyrosine tyrosine; GIP: gastric inhibitory peptide; GLP: glucagon-like peptide; r: Pearson's correlation; * Spearman correlation; statistical significant: $p<0.05$

5.4.4 Correlation of plasma gut hormone concentrations with enteral feeds

Spearman's correlation was carried out to evaluate the relationships between gut hormone levels and milk volumes received by the infants when the blood samples were taken. There were positive correlations between plasma concentrations of the investigated gut hormones and the milk volumes received by the infants except for ghrelin and insulin as illustrated in Table 5.5.

Table 5.5 Relationships between gut hormone concentrations and mean milk volumes received by the infants

Hormone	Correlation coefficient (r)	95% confidence interval	P value
PYY (n = 7)	0.57	0.17 to 0.80	0.007
GIP (n = 7)	0.65	0.29 to 0.85	0.002
GLP-1 (n = 4)	0.55	0.03 to 0.80	0.03
Ghrelin (n = 4)	-0.28	-0.74 to 0.36	0.36
Insulin (n = 7)	-0.41	-0.72 to -0.05	.07

PYY: peptide tyrosine tyrosine; GIP: gastric inhibitory peptide; GLP: glucagon-like peptide; r: Spearman's correlation; n= number of infants; statistical significant: $p<0.05$

5.4.5 Changes in gut hormone concentrations by gestational age group

No statistically significant differences were observed in the changes of gut hormone plasma concentrations among the groups at baseline, D7 and D14 in either gestational age (<34 weeks and >34 weeks of gestation) expect for plasma GLP-1 ($p = 0.01$) (Figure 5.11).

As most of the included infants (6/7 (86%) had received OPC during the first week of life; therefore, comparison of infants who received OPC with those who did not receive was not possible (lack of a control group).

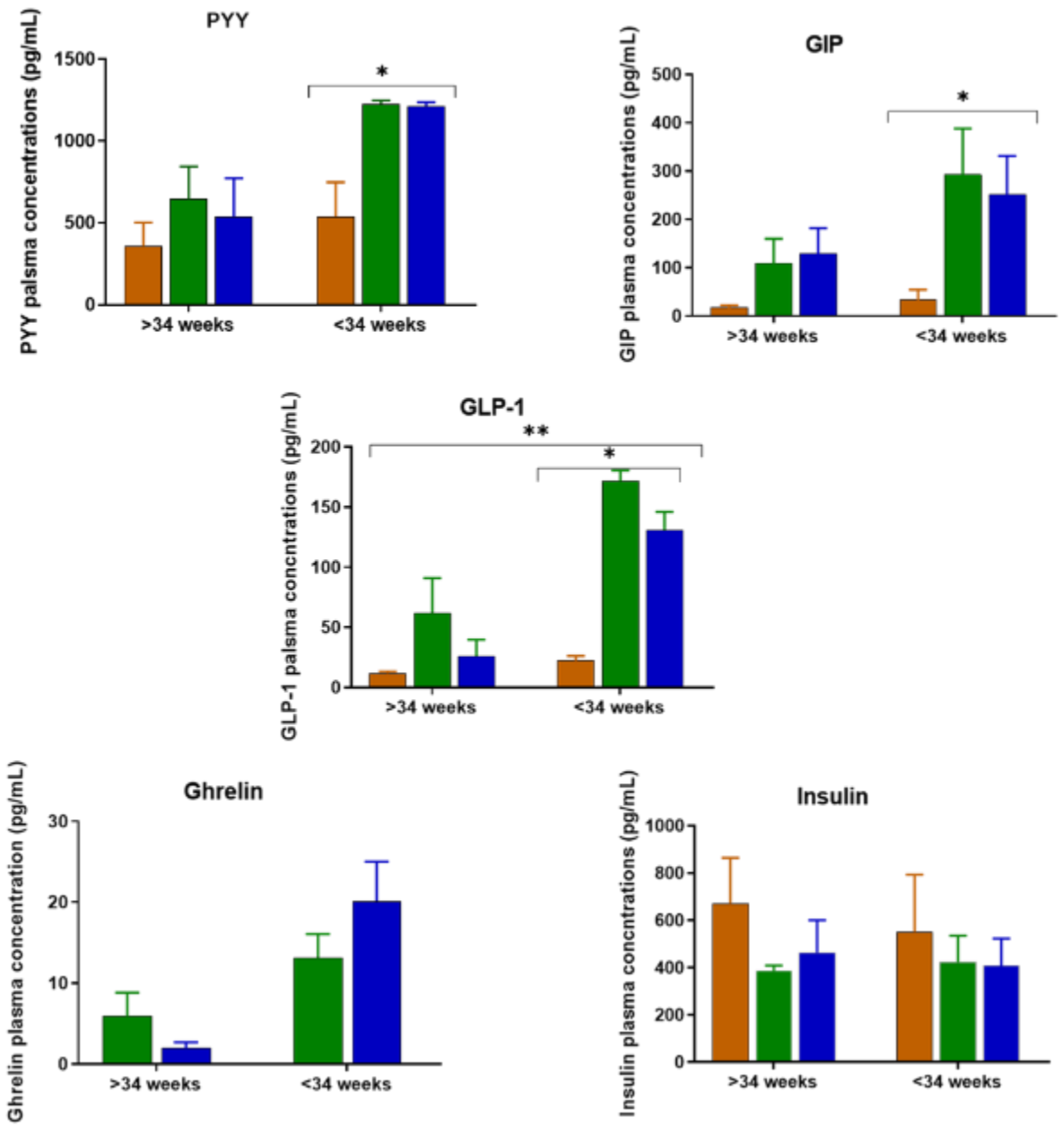


Figure 5.11 Changes in gut hormone concentrations by gestational age group

Comparisons of changes in plasma gut hormone concentrations among the study groups; >34 weeks of gestation (n = 4); <34 weeks of gestation (n = 3). Brown: basal sample (D1); Green: second sample (D7: around 7 days from D1); Blue: third sample (D14 around 14 from D1). Values: mean ± SEM; **: p = 0.01 (two-way repeated measures ANOVA). PYY: peptide tyrosine tyrosine; GIP: gastric inhibitory peptide; GLP: glucagon-like peptide.

5.5 Discussion

5.5.1 Key findings

These preliminary data showed that there was a trend for an increase in the plasma concentrations of different gut hormones during the first postnatal week in preterm and term infants. Each hormone had a specific pattern of plasma concentrations soon after birth and over the early postnatal weeks. The changes in gut hormone concentrations are likely to be related to the infant's feeds rather than the gestational age or birth weight.

5.5.2 Gut hormone concentrations during early postnatal weeks

Gut peptides, which were measured in the current study, showed a significant increase in their plasma concentrations during the first postnatal week except for ghrelin and insulin. The mean plasma PYY, GIP and GLP-1 concentrations rapidly increased during the first week and sustained high in the second week when compared with the baseline fasting concentrations. This selective rising of these three hormones may suggest that their actions may be what is required for this period of life. A significant rise in the levels of plasma PYY, GIP and GLP-1 between birth, and postnatal day 7 were observed by a study that investigated the relationship between enteral feeds and gut hormones among preterm infants <33 weeks gestation (581). However, participants were retrospectively selected, and the serum samples were not primarily collected for the study. Another study also reported a peak in serum PYY concentration at day 12 postnatal in preterm infants and day 18 in full-term infants (584). The fasting levels of PYY, GIP and GLP-1 were higher than adult fasting levels, this finding was built on previous studies, which have found in preterm and term infants higher levels of these hormones compared with older children and adults (584, 598, 645). The postnatal surge of plasma PYY, GIP and GLP-1 concentrations were linked to postnatal adaptation to prepare the GIT for enteral feeding. High plasma PYY and GIP concentrations during the first week of life have been associated with quicker attainment of full enteral feeds in preterm infants (< 33 weeks) adjusted for potential confounders (581); this may indicate functioning enterocytes that may reflect intact gut hormone axes (581, 590, 593). Therefore,

their plasma concentrations may be possible indicators that could identify high-risk infants for feeding intolerance (581).

In the current study, the median fasting plasma PYY, GIP and GLP-1 concentrations were higher compared to a previous study (581) that investigated gut hormone concentrations during the first postnatal week among preterm infants (PYY: 356.08 versus 14.0; GIP: 21.8700 versus 9.0; GLP-1: 6.94 versus 0.6 pg/mL). Although the previous study used the same immunoassay methods (Milliplex Map Human Gut Hormone Panel, Millipore, Billerica, MA); however, it included a lower gestational age. Notably, the higher level of plasma GLP-1 could be related to the use of DPP-IV inhibitor to counteract the physiological degradation of GLP-1 by the enzyme DDP-IV (628) while the previous study did not state if DPP-IV inhibitor has been added to the blood samples. In the present study, plasma PYY, GIP and GLP-1 concentrations were also higher compared to another previous study, which found high PYY, GIP and GLP-1 at birth associated with a significant postnatal increase among preterm infants (< 37 weeks gestation) (590). This previous study also used the same multiplex immunoassay, and DDP-IV inhibitor was added to the blood samples (590). The observed higher plasma PYY, GIP and GLP-1 concentrations in the current study might be suggested to the use of OPC in the study cohort (86% of the infants received OPC during fasting status and when the basal samples were collected). Colostral growth factors such as epidermal growth factor and insulin-like growth factors (234, 236) may travel to the gut if not absorbed by the buccal mucosa, and stimulate gut maturation. Moreover, gastrointestinal peptides such as GIP has been found in human colostrum and milk during the first postnatal weeks may also be important factors for using OPC (646, 647). However, the sample size was not sufficient to detect this relationship, lacked a control group, and the potential confounders might have influenced the results. Additionally, using different methodology and sitting might limit comparison with previous studies.

In this study as well as other studies (581, 590), fasting plasma Ghrelin concentrations were very low or undetectable and, plasma ghrelin did not show significant changes during the study period. Plasma ghrelin concentrations had been reported to increase by 2 to 3 weeks

after birth and peak after four weeks continuing up to 24 months (597, 598). Nevertheless, measurements of ghrelin taken during the first two weeks, with further serial measurements of plasma ghrelin may provide more data on the changes of plasma ghrelin. Delayed postnatal increase in ghrelin levels compared to other gut hormones may be related to the period of maturation of the stomach, which is the main site for ghrelin secretion (594), by other gut peptides that increased earlier such as PYY and GIP. Ghrelin stimulates growth hormone secretion and appetite, reduces utilisation of fat and maintains blood glucose levels (648, 649). Consequently, it may reflect a physiological requirement, at this stage of life, when growth hormone commences to exert its effects, and changes in feeding occur.

Postnatal plasma insulin concentrations also did not show a significant increase; this might be related to the physiological fall in the blood glucose concentrations after birth, that suppresses insulin secretion as a part of the regulatory mechanisms for postnatal glucose homeostasis (91). Plasma insulin levels also did not show a consistent trend over two weeks; this could be attributed to variability in the supplemental parenteral nutrition had been received by the infant while insulin blood samples were being taken. Moreover, differences in the clinical conditions of the participant infants may have a role in determining circulating insulin concentration. For instance, there was one participant who had hypoglycaemia during the first postnatal day, and he received intravenous bolus glucose. This infant had a higher basal plasma insulin level compared to the other infants. Rapid correction of hypoglycaemia is a probable reason for the high insulin in this infant. Another infant (27 weeks gestation) was receiving total parenteral nutrition and intravenous insulin during the period of sample collection.

The trend of plasma insulin was variable after birth. Whilst one study found no significant changes in insulin concentrations during the early postnatal weeks (590), another study reported a significant increase in plasma insulin concentrations during the first week of life (581) in preterm term infants <33 weeks with a mean gestational age of 28.1 ± 1.3 weeks, and most of the infants were receiving parenteral nutrition. However, variable insulin response to blood glucose concentrations has been demonstrated during the neonatal

period, particularly for extremely preterm infants (605). Early postnatal hypoglycaemia resulting in the reduction of insulin levels was thought to be an essential part of adaptation to extra-uterine life (650). Because blood samples were only obtained at times when blood sampling was required for clinical reasons, exact blood sugar values were not available, especially when the second and third samples for gut hormones were taken. Therefore, the correlation of insulin concentrations with the corresponding blood sugar was not achievable.

5.5.3 Correlation of plasma gut hormones with gestational age and birth weight

There were no correlations between the basal (fasting) concentrations of the different gut hormones investigated in the present study and the infant's gestational age or birth weight. Previous studies were conflicting for correlation of circulating PYY and GIP with gestational age. Whilst some studies found that PYY concentrations negatively correlated to gestational age (585, 598, 645) others found no relationships between the levels of circulating PYY and gestational age (581, 584); this conflict could be attributed to differences in the methodology between the studies. The basal GLP-1 levels were negatively correlated ($r = -0.84$) to the gestational age but this did not reach statistical significance that might be related to the sample size for baseline GLP-1 ($n = 4$) because three infants had low values that were below the sensitivity of the assay. Likewise, some studies showed no relationship between GLP-1 and gestational age (592, 593, 616) and a negative correlation was reported by others (590). Some studies reported higher concentrations in preterm infants compared to full term, (585, 597, 598); this difference was explained to be due to increased synthesis and decreased hepatic and renal clearance as a consequence of prematurity (648).

There was a significant negative correlation between the mean plasma ghrelin concentration and the infant's birth weight ($r = -0.8$); this finding was consistent with previous studies investigating ghrelin during the early life (585, 598, 651). High ghrelin levels have been reported in the umbilical cord blood and at one week of life in SGA infants (651). In preterm infants, at birth, high plasma ghrelin reflects the nutritional status and represents a negative

energy balance (585). Increased plasma ghrelin concentrations have also been associated with statuses of negative energy-weight balance such as anorexia nervosa and malignancy cachexia as a compensatory mechanism through its orexigenic and adipogenic effects (652). Therefore, Ghrelin has been suggested to be related to postnatal growth and its plasma concentrations could be considered as a predictor for postnatal catch-up growth, particularly in preterm infants (585, 597).

5.5.4 Correlations of plasma gut hormones with enteral feeds

The observed postnatal increase in plasma concentrations of gut hormones was likely related to the initiation of enteral feeding. Plasma concentrations of PYY, GIP and GLP-1 during the early postnatal weeks were in direct relationship with the milk volumes received by the infants. The rapid increase in plasma gut hormones with trophic and enteral feeding has been previously reported (580, 590). This relationship depends on the milk increment and the cumulative milk taken by the infants (580) and what time the blood sample was obtained around the feed (before or after). In the current study, although 86% of the participant infants received OPC during the first week of life, a control group was not available for comparison. Relating the timing of sample collection to the timing of the feeds was also not feasible because the blood samples were only obtained when clinically indicated blood sampling; this limitation is less likely relevant especially in preterm infants who are often fed more frequently and over longer periods in comparison to older infants and children (579). Furthermore, potential factors, such as receiving a blood transfusion, which is expected especially in an infant requiring NIC, may have confounded the results.

Though, the data suggested a significant rise in gut hormones in response to enteral feeding, but the small sample size and the heterogeneous cohort could have affected the findings. As enteral feeding in newborn infants is influenced by many factors such as gestational age and the infant’s clinical status, it may be difficult to elucidate the relationship of enteral feeds to the plasma gut hormones in a small cohort during a short postnatal period. Additionally, the type of milk and the pattern of enteral feeding play a role in the pathway of gut hormone

secretions and maturation of the gut (581, 653). Nutritional compositions of the milk, such as protein, fat and carbohydrates, and their proportions that were received by the infants, influence the release of certain gut peptides (581). For example, GIP is secreted mainly in response to carbohydrates in the intestinal lumen to stimulate insulin secretion (654), while, PYY release has been related to the enteral intake of protein, fat and also carbohydrates (581). Higher GIP concentrations (induce insulin secretion and fat deposition) were reported in formula-fed infants compared to breastfed infants at 1, 3 and 6 months of age (655); this might in part explain the protective role of breastfeeding against obesity.

Notably, plasma ghrelin concentrations did not correlate to enteral feeds in this study, as well as in previous studies (590, 597). This finding supports the requirement of ghrelin in a later postnatal life as discussed previously. However, as feeding preterm infants is not determined by appetite and the milk volume is influenced by the infants' clinical conditions, evaluating the effects and relationship of ghrelin with enteral feeds in preterm infants might be a complex issue during the early postnatal period.

Although gut hormones have been studied since the1970s (653, 656), most studies investigating different gut hormones during the neonatal period were observational, with small sample size and some were retrospective. Many studies were conducted in animal models, which may not have represented the conditions of preterm infants (657-659). Furthermore, experimental data have to be translated into the clinical setting. Evidence underpinning the pattern of gut hormone responses and their potential implications in clinical practices during early life appears to be insufficient.

5.5.5 Strengths and limitations

This is the first study that highlighted the potential effects of OPC administration on gut hormone release in preterm and sick infants, to the best of my knowledge. Moreover, most of the previous research studied OPC in EXP and ELBW and VLBW infants; this study included all preterm infants' categories and other sick infants requiring NIC. The study followed open access published protocol (623). In advance publication of the protocol can enhance

transparency of the research, provides detailed information and more opportunities for peer reviews of the methods and collaboration (571). It also minimises potential reporting bias (463).

I have also used multiplex immunoassay an advanced technique that involves small sample volumes. Preterm and sick infants have high haematocrits (61% ± 7.4% (660)) during the first few postnatal days. The haematocrit is the percentage of red blood cells in the blood. When the haematocrit is high; there is a less plasma or serum obtainable from a blood sample. Therefore, using a technique, which requires a small plasma volume was very helpful. This technique also saves time and was cost-effective as multiple hormones could be analysed simultaneously in one sample.

This current study faced many limitations; it was not completed because of the Chief Investigator left the university necessitating a new ethical approval that took time more than expected.

The small sample size and heterogeneity of the participant infants created an important limitation for the study. Recruitment of participants was limited by the busy NICUs where clinical needs take priority over activities that are entirely research orientated; this was compounded by the lack of a clinical staff member who had GCP training; therefore, some eligible infants could not be recruited. Additionally, another a clinical trial was ongoing during this study period had some influence on the recruitment. For instance, the Study of Preterm Infant and Neurodevelopment Genes (SPRING) (661), a prospective cohort study that investigating the relationship between neuropsychiatric disorders, genetic risks and preterm birth (delivery < 32 weeks gestation), was also collecting blood sample (1ml) from each participant and parents were concerned about this.

Some infants were transferred to another hospital before enrolment in the study although their parent agreed. This issue created a threat to the progress of the study particularly when infants were transferred after enrolment, and the basal blood samples had been taken.

However, this is a challenge for any research recruiting neonates, whose clinical care often necessitates transfer to other hospitals.

Blood samples were obtained only when routine blood sampling of the infant was needed for clinical indications; therefore, it was difficult, taking the blood samples at the exact scheduled time; however, generally, most of the samples were taken around the planned days. The different techniques of sample collection, such as heel prick or using an indwelling central catheter, may have affected the quality of the samples for immunoassay of the hormones. Moreover, some values were below the range of the range of the standard curves; however, using higher sample volumes and saturation of the standard curves at lower ends may broaden the detection especially for hormone such as ghrelin, which is expected to be low at this stage of life (581, 590).

5.5.6 Conclusion

Preliminary data from this pilot study demonstrated that there was a trend for postnatal increase in plasma gut hormone concentrations, which may be due to enteral feeding, a physiological trend or another explanation. Despite, the heterogeneity of the study cohort, and variability in the feeding of the participants, most of the gut hormones of interest in this study showed a similar postnatal trend. Alhough most of the participant infants received early OPC, the small sample and lack of comparison group limited demonstrating a potential effect of OPC administration on plasma gut hormone concentrations. However, the study did not complete and recruiting further participants are needed to report a final conclusion. Further research is needed to establish whether early OPC administration could stimulate gut hormone secretions and promote maturation of the gut.

5.5.6.1 Implications for future research

Given the current lack of studies investigating the effects of OPC administration on the response of gut hormones in preterm and sick neonates, there is scope for the development of further research. Some likely areas for future research are suggested below:

- Large, well-designed RCTs that attempt to control for potential confounders, such as gestational age, birth weight and clinical status of the infants.
- Future research may consider the effect of different feeding strategies on the different gut hormones (e.g. continuous versus bolus feeding).
- Gut hormone concentrations might be assessed more precisely if the collection of blood samples are obtained more frequently during the first few postnatal weeks. The samples could be best taken at a fixed time to feeds (before and after) as secretion of the gut peptides is related to food intake (579, 593). The type of milk feeds should also be taken into consideration to minimise potential variability and may produce better results.
- Comparing sick newborn infants requiring NIC with healthy infants could be an option for future research. This comparison may identify a possible physiological pattern of hormonal change from that influenced by the OPC.

Chapter 6. Conclusion

This thesis was undertaken to determine whether early (within the first seven days of life) oropharyngeal administration of mother's colostrum (OPC) to preterm and sick infants prevents prematurity-related complications and improves health outcomes for the infants.

This chapter summarises the main findings and limitations of this thesis and overviews the implications for clinical practice and research.

6.1 Summary of findings

6.1.1 Oropharyngeal administration of mother's own colostrum to preterm infants: a survey of practice

This study aimed to gain an overview of the current use and practice of OPC in the UK. This aim was achieved by surveying neonatal professionals across UK neonatal units to determine the practice of OPC administration, and their knowledge and perceptions toward OPC application in the care of preterm infants, using an online questionnaire. Surveys are well-recognised in healthcare research as a method for gathering data on healthcare practices, knowledge and attitudes among professionals and providers (380). This survey showed that OPC use had been introduced into UK neonatal practice despite a lack of evidence concerning its use. This survey also revealed an inconsistency in the practices of OPC administration amongst UK neonatal units. Whilst OPC appears to be an easy and feasible procedure that is well tolerated by preterm infants; it was often provided without written guidelines or policy indicating the need for evidence-based clinical guidelines and policies to practice OPC.

6.1.2 Oropharyngeal colostrum in preventing mortality and morbidity in preterm infants: Cochrane systematic review

This Cochrane systematic review was conducted to appraise the available randomised controlled trials (RCTs) to assess the use of OPC in reducing mortality and morbidities for

preterm infants. OPC administration shortens the time to achieve full enteral feeds. The available evidence was insufficient and of low to very low quality to establish if early OPC can reduce the duration of hospital stay, the risk of necrotising enterocolitis (NEC), late-onset infection (LOI), or death in preterm infants. Adequately powered RCTs that also evaluate potential harm would be needed for a more precise assessment of OPC effects on health outcomes of preterm infants.

6.1.3 The impact of oropharyngeal administration of mother's colostrum on the clinical outcomes of preterm infants: a case-control study

Although OPC has been increasingly adopted by the UK neonatal units, to the best of my knowledge, there is no published study investigating the use and effects of OPC in preterm infants in the UK. This study aimed to evaluate the effects of OPC on the clinical outcomes for preterm infants in the UK. This aim was achieved by conducting a matched case-control study that compared clinical outcomes during the hospital stay of preterm (≤ 32 weeks) infants who were admitted to the Nottingham neonatal units after the implementation of OPC in the care of preterm infants, with those who were admitted before the use of OPC in the units. Preterm infants who received OPC started feeding and attained full enteral feeds (150ml/Kg/day for consecutive 72 hours) earlier than those infants who did not receive OPC. They also received more breast milk at discharge home. However, the two groups had a similar length of hospital stay, weight Z-score at hospital discharge, and incidence of NEC, LOI and death.

6.1.4 Gut hormones response to oropharyngeal administration of mother's colostrum to infants in neonatal intensive care

The Cochrane review (Chapter 3) and the case-control study (Chapter 4), as well as previous studies (353, 417), showed that infants who received OPC reached full enteral feeds faster than controls. Gut hormones are peptides secreted by the gastrointestinal tract (GIT) in

response to nutrients intake (306). Enteral feeds are vital for the growth and development of the GIT to adapt to extra-uterine life (579). After birth, there is substantial secretion of different gut hormones in response to the introduction of trophic and enteral feedings (580). The postnatal rise in gut hormones is absent in those infants who do not receive enteral feeds (306, 574). This study was, therefore, conducted to investigate the effect of colostrum administered by the oropharyngeal route on the secretion of gut hormones to gain insight into the possible mechanisms responsible for quicker attainment of full enteral feeding in response to OPC use observed in this thesis and previous studies. To achieve this, I commenced a non-randomised observational study in the Nottingham neonatal intensive care units to evaluate the impact of OPC administration on postnatal changes in a set of gut hormones in preterm and ill infants requiring neonatal intensive care (NIC).

To the best of my knowledge, no published study has investigated gut hormone secretion in response to OPC administration. Moreover, most of the previous research has studied OPC in extremely preterm, extremely low birth weight and very low birth weight infants; this study included all categories of preterm infants and other sick infants requiring NIC. This study did not complete due to unforeseen obstacles. However, preliminary data showed that there were significant changes in the plasma gut hormone concentrations over two weeks in preterm and term infants who received OPC. Each hormone had a specific pattern of plasma concentrations soon after birth and over the early postnatal weeks. The changes in gut hormone concentrations are likely to be related to the infant's feeds rather than the gestational age or birth weight. Recruitment of more participants may confirm or amend these findings. Further research is needed to establish whether and if so, how early OPC administration stimulates gut hormone secretion and promotes maturation of the gut.

6.2 Strengths and Limitations

This thesis has provided more insight into the use of OPC in the care of preterm infants. This work contributes to the existing knowledge about the oropharyngeal administration of the mother's colostrum to preterm infants during the early neonatal period by presenting data

from the UK. Additionally, I believe this is the first research investigating the secretion of gut hormones in response to OPC in preterm and ill full-term infants. Although a relatively limited sample with ongoing recruitment, this project will add to the growing body of research that indicates the potential benefits of administering OPC in the care of preterm infants. This was achieved using a range of different research methodologies.

The survey of neonatal professionals found that OPC use was adopted by the UK neonatal units and highlighted that its current practice of OPC administration was not evidence-based. The documentation of uncertainty in this area of care and a gap in the available evidence may result in the development of high-quality RCTs and evidence-based guidelines.

The Cochrane systematic review appraised the currently available evidence to evaluate the effects of OPC in preventing mortalities and morbidities of preterm infants. This review reported the lack of high-quality evidence, to support the proposed effects of OPC on preterm infants, and concluded that large well-designed RCTs are needed. This review was the first Cochrane review focusing on the effects of OPC (Cochrane database for systematic reviews of intervention (429)).

This thesis also underlined the variability of OPC use and its practice nationally (as presented in the survey study) and internationally (as demonstrated in the Cochrane review). These findings indicate the requirement of a standardised protocol for using OPC that may enable more infants to benefit from this intervention, and may also facilitate and enable more precise comparisons in future research. Standardisation of clinical feeding protocols for preterm term infants have been linked with a reduction in practice variation (662, 663); for example, differences in clinical practice were proposed as one of the iatrogenic factors for NEC, and standardised feeding protocols have been reported as one of the modifiable factors that might reduce and prevent NEC in preterm infants (151, 662).

The case-control study (Chapter 4) found that although a guideline supported the practice of OPC administration, compliance with the unit’s guideline for OPC administration was low.

This finding highlighted the need for regular reviewing of clinical guidelines and more education, especially for newly adopted guidelines, for effective practice changes (663).

This thesis faced some limitations as expected in any research. For instance, the survey of the UK neonatal professionals may reflect the performance of those who responded and may not reflect the actual practice that could limit generalisability to all UK neonatal units. However, this study surveyed both doctors and nurses and included all levels of neonatal units which may minimise this limitation.

Due to the relatively small number of studies identified, the Cochrane review could be affected by publication bias, however, to minimise this bias, additional search sources such as the reference lists of included trials and abstracts and proceedings of major perinatal conferences were screened. Despite much effort to ensure that the search was comprehensive, some relevant studies have been missed should also be considered. Another potential bias was incomplete reporting, to minimise this bias, study authors were contacted as needed. Additional information that was provided by study authors were included in the analysis. However, most data entered in the analyses were obtained from the study reports. Inclusion of very or extremely low birth weight infants may limit the applicability of the review findings to these sub-groups of preterm infants.

The case-controlled study (Chapter 4) was susceptible to information bias due to the use of secondary data that was collected from the participants' medical records. These data were routinely collected health records, collected without predefined specific research questions as they were not documented intentionally for research purpose. However, to minimise this limitation, the Badger neonatal database was used for data collection. Badger neonatal is regularly assessed by the National Neonatal Audit Programme (414) and also monitored by the performing and publications of research that evaluate the accuracy, validity, and quality of these data. Furthermore, additional sources such as the local NHS Trust's Digital Health Records were also used as needed. Using a historical cohort before and after the introduction of OPC might also bias the results (442, 520); however, this potential bias was

minimised by using a matched case-control design. Cases were matched with controls using sex, gestational age and birth weight, which are well-known potential confounders that influence the outcomes of preterm infants. Another possible limitation for this study is that other interventions may have been occurring in the Nottingham neonatal units during the same period as the new OPC guideline was being introduced, which may have influenced the results. However, efforts were taken to identify whether any other intervention were implemented during OPC adoption. In particular, guidelines for feeding preterm infants and breastfeeding support did not change throughout the study period. Data included in the analysis were only from the Nottingham neonatal units; therefore, the generalisability of the findings might be limited.

My thesis also faced unforeseen limitation as the study that was investigating gut hormones response to OPC in preterm infants did not complete. The study has been paused due to the move of the Chief Investigator to a different institution outside the UK; this necessitated getting another ethical approval, which took a drastically longer time than expected. Therefore, I could not complete data collection for this study while being constrained with my PhD timeframe. However, preliminary analysis of the available data may help in generating hypothesis and calculating sample size for future studies. The study is ongoing, and completing recruitment of the planned sample may confirm or alter the findings.

6.3 Implications for practice

Rates of preterm birth are rising (2, 58) and surviving preterm infants are at high risk of short and long-term morbidities (12) such as NEC and LOI which are the main causes of deaths among preterm infants (59). Consequently, preterm infants represent a major challenge to public health worldwide; therefore, preventive interventions are highly needed. Currently, there is a progressive use of OPC among neonatal units to prevent sepsis and NEC. OPC appears to be a beneficial intervention, its effectiveness remains uncertain. Although it did, however, appear safe in the short-term so the current adoption into practice across the UK may be warranted research to confirm or refute this would be helpful to clinical practice.

However, available evidence does not allow firm recommendations for or against the use of OPC to be made. An ongoing RCT (359) aims to recruit 498 extremely preterm infants with birth weight < 1250 grams, within the first 96 hours of life; the results of this trial may support or against the use of OPC in the care of preterm infants.

Through the survey study (Chapter 2), lack of knowledge was the main reason for those units that did not use OPC; though, many responders were enthusiastic for the introduction of OPC practice in their units. OPC was also often not in keeping with the unit's guideline for OPC administration, as demonstrated in the case-control study (Chapter 4). Therefore, more education and regular audit of practice may be valuable for the implementations of new practice and guidelines such as OPC. Moreover, the ideal dosage and frequency and the best timing of initiation and duration of OPC administration need to be optimised. Further RCTs should provide more definitive evidence. Ongoing RCTs evaluating the use of OPC in preterm infants (359, 503, 619, 620) may present more data to inform the future development of evidence-based clinical guidelines on OPC use.

6.4 Implications for future research

Prematurity-related complications and the continued increase in preterm births necessitate more interventions that may improve outcomes for preterm infants. Therefore, the well-known advantages of breast milk (185, 186, 664) and the proposed benefits and cost-effectiveness of OPC (351-353) warranted research in this thesis and other studies. The increase of OPC use in the care of preterm infants despite the lack of high-quality studies indicates a definite need for further research to evaluate whether OPC administration to preterm and sick infants during the early neonatal period is safe and could have a positive impact on this specific population. The future studies should be high-quality research including, adequately powered RCTs, systematic reviews such as follow up of the published Cochrane review (Chapter 3).

Several suggestions have emerged as a consequence of this work as potential themes for future research:

- As described in this thesis there is variation in OPC procedures between different settings (Chapter 2) and among neonatal units within the UK (Chapter 3). Therefore, further research to optimise the practice of using OPC, regarding the dose, frequency, duration and procedure for administration of OPC is needed.
- Validation of clinically relevant outcome measures is needed for more reliable and precise research.
- This thesis has shown that the safety and adverse effects of OPC administration were not adequately addressed in currently available studies. Therefore, well-designed longitudinal studies that focus on safety is worthy.
- A further study to assess long-term outcomes is also needed.
- In neonatal practice, providing feeds to preterm infants and oral care including OPC procedure are primarily a nurse prerogative. Thus nurses may have a unique role in contributing to new knowledge regarding this intervention. Involving nurses in the design of clinical guidelines and further research might be helpful.
- Parent-related outcomes such as parental anxiety and depression may also be of interest. Involving the parents might be informative in future research as they may provide evidence from their perspective that may improve the quality of the research.
- Current studies have focused on the administration of colostrum by the oropharyngeal route, however, administrating transitional and mature milk by this route might also be of benefit particularly to ELBW infants who are tube feed for extended period.
- Owing to the substantial burden of prematurity on the health care system, families and societies, evaluation of the cost-effectiveness of OPC use in the standard care of preterm and ill infants could be a practical consideration.
- It is important to note that most of the previous research has come from high-income countries. Therefore, more focus on low-resource settings, where approximately 60% of preterm births and the highest mortality occur (12), could provide important findings.

6.4.1 Ongoing studies

Despite research assessing OPC administration continuing, it appears that ongoing studies are still insufficient, they include:

- "Oropharyngeal administration of mother's colostrum, health outcomes of premature infants: study protocol for a randomized controlled trial" (359)

  A randomised double-blind, placebo-controlled (ClinicalTrials.gov: NCT02116699)

  Target sample: 498 extremely preterm infants from five NICUs within the USA

  Primary outcome: incidence of late-onset sepsis, necrotising enterocolitis and death

- "The effect of oropharyngeal colostrum administration in preterm infants"

  Randomised double-blind- placebo-controlled (who.int/trialsearch: JPRN UMIN000022923), Okayama University Hospital, Japan

  Target sample: 30 infants (<32 weeks)

  Primary outcome: longitudinal change of reactive oxygen metabolite and biological anti-oxygen potential of preterm infants with oropharyngeal colostrum administration.

- "Impact of Oropharyngeal Administration of Mother's Milk Prior to Gavage Feeding on Hospital Acquired Neonatal Infection"

  Randomised single-blind (assessor blinded) (clinicaltial.gov: NCT03513146), Mansoura University Children Hospital, Egypt

  Target sample: 100 infants <32 weeks' gestation

  Primary outcome: culture-proven neonatal sepsis acquired during neonatal care admission

- "Efficacy of oropharyngeal administration of colostrum in reducing morbidity and mortality in very preterm infants: A randomized controlled trial –Colostrum"

  Double-blinded, placebo-controlled (who.int/trialsearch: CTRI/2017/11/010396)

  Neonatology department, Paediatrics division, Women and Child Hospital, India

  Target sample: 260 preterm infants 26-31 completed weeks (recruiting)

  Primary outcome: incidence of death, late-onset sepsis or necrotising enterocolitis.

6.5 Personal reflections

Throughout my PhD study, I benefitted from having the opportunity to gain new skills and develop competencies that enriched me both as a researcher and as a clinician. The approach of my thesis required different methodologies; critical appraising of evidence using systematic review, descriptive study, case-control study and non-randomised observational study, every study had its challenges to overcome. In particular, the study which seeks to deal with patients allowed experiencing writing a study protocol for regulatory approvals and to manage biological samples. Additionally, I also learned that it is imperative to conduct this process at an early stage to facilitate as smooth a passage as possible.

A great lesson I have learned that sometimes we have to take a more flexible attitude and be broadminded when looking for alternative ways to meet challenges, however, by patience and perseverance these challenges can be overcome.

Should this research contribute to improving the outcomes and quality of life for any of these vulnerable infants, then the difficulties and challenges I faced during my study would be worthwhile.

6.6 Conclusive remarks

Preterm infants deserve to have a good quality of life and to receive appropriate management supported by high-quality evidence. OPC administration is a biologically plausible intervention that can reduce the mortality and morbidities of preterm infants. However, the evidence base for this intervention is currently inadequate. The work in this thesis should in part help to expand the current knowledge about OPC use informing guidelines and future clinical decision by providing data needed to efficiently plan further high-quality research.

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Appendices

Appendix 1 Survey study-Ethical approval letter

The University of Nottingham

Faculty of Medicine and Health Sciences

Research Ethics Committee
School of Medicine Education Centre
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24th April 2015

Dr Amna Widad Ahmed Nasuf
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Dear Dr Nasuf

Ethics Reference No: R16042015 SoM CHOG – please always quote
Study Title: Oropharyngeal administration of mother's own colostrum to preterm infants: a Survey of Neonatal Professionals.
Chief Researcher/Academic Supervisors: Professor Helen Budge, Professor of Neonatal Medicine, Dr Jon Dorling, Clinical Associate Professor in Neonatology, Dr Shalini Ojha, Clinical Lecturer inChild Health Academic Child Health, School of Medicine
Lead Researcher/Student: Dr Amna Widad Ahmed Nasuf, PhD Student Academic Child Health, School of Medicine.
Duration of Study: 1/4/15-31/5/16 12mths **No of Subjects:** 400 (18+ yrs)

Thank you for submitting the above application which has been reviewed and the following documents were received:

- FMHS Research Ethics Application Form dated 27/3/15
- Protocol version dated 3/12/2015
- Invitation E-mail dated 3/12/2015
- Information Sheet dated 3/12/2015
- Questionnaire dated 3/12/2015

These have been reviewed and are satisfactory and the study is approved.

Approval is given on the understanding that the Conditions of Approval set out below are followed.

1. You must follow the protocol agreed and inform the Committee of any changes using a notification of amendment form (please request a form).

2. You must notify the Chair of any serious or unexpected event.

3. This study is approved for the period of active recruitment requested. The Committee also provides a further 5 year approval for any necessary work to be performed on the study which may arise in the process of publication and peer review.

4. An End of Project Progress Report is completed and returned when the study has finished (Please request a form).

Yours sincerely

Dr Clodagh Dugdale
Chair, Faculty of Medicine & Health Sciences Research Ethics Committee

Appendix 2 Survey questionnaire

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Pilot: Oropharyngeal administration of mother's own colostrum to preterm infants: A Survey of Neonatal Professionals

Oropharyngeal administration of mother's own colostrum to preterm infants

Thank you for agreeing to participate in this study. We would appreciate you taking the time to complete the following survey. It should take about ten minutes of your time.

Colostrum, the milk produced within the first few days after birth, is rich in nutrient and immune-protective factors, and it is widely accepted that administration of colostrum to preterm infants is beneficial. However, the best method to deliver colostrum to preterm infants is less clear. Oropharyngeal administration of colostrum is a recent intervention during which a small amount of colostrum is placed in the buccal cavity of the infant. It is hoped that it will stimulate the infant's immune system via absorption by the buccal mucosa, without the need to be swallowed.

In this survey, we would like to ask you a few questions about your practice regarding the administration of colostrum to preterm infants. The survey results will provide a better understanding of the current practice in the U.K. and help to design a potential trial to study of the benefits and risks of administering colostrum via the oropharyngeal route.

Your responses are voluntary and will be confidential. Responses will not be identified by individual. All responses will be compiled and will not be identifiable.

1 Do you administer colostrum to preterm infants in your unit?

- ○ Yes
- ○ No

Colostrum user

2 Whichtype of colostrum do you use? Please, tick all that apply.

- ☐ Mother's own colostrum
- ☐ Donor human milk
- ☐ Animal colostrum

3 How do you administer colostrum to preterm infants? Please, tick all that apply.

- ☐ By nasogastric tube (NGT)
- ☐ By orogastric tube (OGT)
- ☐ In the mouth
- ☐ By bottle

4 Do you administer colostrum by oropharyngeal route to preterm infants (placing few drops in the mouth or swabbing the buccal cavity with colostrum without the need to be swallowed by the infant) in you unit?

- ○ Yes
- ○ No

Oropharyngeal Colostrum user 1

5 At what age of the baby do you commence administration of oropharyngeal colostrum? Please, tick all that apply.

- ☐ Within 24 hours of birth
- ☐ 24- 48 hours of birth
- ☐ 49- 72 hours of birth
- ☐ 73-96 hours of birth
- ☐ Any age

6 Based on weeks of gestation in which group of preterm infants (compleled weeks of gestation at birth) do you use oropharyngeal colostrum?

- ☐ < 28 weeks.
- ☐ 28 to < 32 weeks
- ☐ 32 to < 37 weeks
- ☐ Any gestational age
- ☐ Other

6.a If you select Other, please specify:

7 Based on birth weight, for which weight range do you use oropharyngeal colostrum?

- ☐ < 1000 g
- ☐ 1000- 1500 g
- ☐ 1500- 2000 g
- ☐ 2000- 2500 g
- ☐ Any weight
- ☐ Other

7.a If other range, please specify:

8 Do you give oropharyngeal colostrum to infants with the following feeding regimens? Please, tick all that apply.

- ☐ Nil per orally (NPO)
- ☐ Trophic feeding (<1 ml/kg/hr of milk)
- ☐ Enteral feeding (>1 ml/kg/hr of milk)
- ☐ Parenteral nutrition
- ☐ Nil via OGT/NGT

9 Do you give oropharyngeal colostrum to infants receiving the following enteral feeds? Please, tick all that apply.

- ☐ Mother's own milk
- ☐ Donor breast milk
- ☐ Mixed (mother & donor)
- ☐ Formula
- ☐ Breast and formula

10 In which situation would oropharyngeal colostrum not be given? Please, tick all that apply.

- ☐ Intensive care
- ☐ Mechanical ventilation
- ☐ Inotropes for hypotension
- ☐ None of these, it will always be given regardless of the infant's condition
- ☐ Other reasons

10.a If you selected Other, please specify:

Oropharyngeal colostrum user 2

11 How easy is it to administer colostrum by the oropharyngeal route?

- Extremely easy
- Very easy
- Moderately easy
- Very difficult
- Quite difficult

12 How long has it been since oropharyngeal colostrum administration was introduced in your unit?

- <6 months
- 6 months- 1 year
- 1 year- 2 year
- 2 years- 4years
- >4 years

12.a If you selected >4 years please specify how many years?

13 Do you document oropharyngeal administration of colostrum (and not simply just gastric EBM) on the infant's record charts?

- Yes
- No

14 Have you experienced any adverse effects with the use of oropharyngeal colostrum?

- ○ Yes
- ○ No

14.a If yes, what adverse effects have you experienced? Please, tick all that apply.

- ☐ Aspiration pneumonia
- ☐ Decrease in oxygen saturation (Spo2 < 85%)
- ☐ Bradycardia (heart rate < 100/min)
- ☐ Tachycardia (heart rate > 180/min)
- ☐ Tachypnea (respiratory rate > 80/min)
- ☐ Other

14.a.i If you selected Other, please list these adverse effects?

15 Do you have written guidelines on the use of oropharyngeal colostrum? Please, tick all that apply

- ☐ Yes, a guideline for all professional staff
- ☐ Yes, a policy for nursing staff
- ☐ Yes, a policy for all medical staff
- ☐ No

15.a If you have a written policy, we would be very greatfull if you could please provide us with a copy?

16 Would you recommend oropharyngeal colostrum as part of the standard care of preterm infants?

- Very likely
- Quite likely
- Likely
- Unlikely
- Very unlikely (would not recommend)

16.a If you would not recommend, please tell us why?

17 Do you have any other comments that you would like to add?

Oropharyngeal colostrum non-user

18 Why are you currently not using oropharyngeal colostrum for preterm infants (placing few drops in the mouth or swabbing the buccal cavity with colostrum without the need to be swallowed by the infant) in your unit?

- ☐ Not knowledgeable about this practice
- ☐ There is little evidence for its use
- ☐ No guidelines in the unit
- ☐ Nurses do not support its use
- ☐ Doctors do not support its use
- ☐ Other

18.a If you selected Other, please tell us why?

19 Do you give colostrum down a gastric tube?

- ○ Always
- ○ Mostly
- ○ Sometimes
- ○ Occasionally
- ○ Never

20 Do you administer colostrum via a gastric tube with the following feeding regimens? Please, tick all that apply.

- ☐ Nil by mouth (NBM) or Nil per oral (NPO)
- ☐ Trophic feeding (<1ml/kg/hr of milk)
- ☐ Enteral feeding
- ☐ Parenteral nutrition

21 Do you administer mother's own milk in the order that it is expressed ?

- Always
- Mostly
- Sometimes
- Occasionally
- Never

22 If you are not currently giving oropharyngeal colostrum, how likely are you to introduce it in the future?

- Extremely likely
- Quite likely
- Moderately likely
- Slightly likely
- Not at all likely

Professional information

We will be very grateful if you can provide us with the following information which will be used only for follow-up or any further enquires we may need.

23 In which neonatal unit do you work?

24 What is the level of your neonatal Unit?

- Special care unit (SCU)
- Local Neonatal Unit (LNU)
- Neonatal Intensive Care Unit (NICU)

25 What is your Job title?

- Consultant
- Senior Trainee Doctor(ST6 and above)
- Junior Doctor (ST5 or below)
- Senior Nurse (Band 6 and above)
- Nurse (Band 5 or below)

26 Please state approximately how many years you have been working in neonatal care?

- 0-5 years
- 5-10 years
- 10-20 years
- > 20 years

27 Please indicate if you would be interested in taking part in a research study to assess bucal colostrum administration.

- Yes
- No

27.a If you would like to join a study research to assess bucal colostrum administration, please could you provide your contact details, if possible. *Optional*

28 Do you have any other comments that you would like to add?

Thank you

Many thanks for taking the time to complete our questionnaire.

If you have any questions about the survey you are welcome.
Please, contact:

Dr Jon Dorling

Clinical Associate Professor in Neonatology

School of Medicine

University of Nottingham

Phone: 0115 9249924 (Switchboard).

Email: mszjsd1@exmail.nottingham.ac.uk

www.nottingham.ac.uk/medicine/about/childhealthobsgyn/people/jon.dorling

Appendix 3 Survey study-Invitation email to neonatal network staff

Email sent to neonatal network staff

Dear Network Lead clinician / Lead Nurse / Administrator

My name is Amna Widad Ahmed Nasuf, I am planning to conduct a survey research as part of my PhD at Child Health Division, The University of Nottingham titled

"Oropharyngeal Administration of Mother's own Colostrum to Preterm Infants: a Survey of Neonatal Professionals" under the supervision of Dr Jon Dorling, Clinical Associate Professor in Neonatology.

The oropharyngeal administration of colostrum (the milk produced within the first few days after birth) is a new intervention which involves the instillation of a mother's colostrum into her infant's mouth to provide a local protective effect and to be absorbed by the mucosa of the mouth to stimulate the immune system of the infant. This may protect the preterm infant against infections and other conditions like necrotising enterocolitis. This intervention does not involve swallowing of the colostrum by the infant.

This survey will be conducted to determine the knowledge, attitudes and practices of UK neonatal professionals regarding the oropharyngeal administration of colostrum to preterm infants.

We would therefore greatly appreciate it if you would provide us with the name and email address of the lead neonatal doctor and lead neonatal nurse of each neonatal unit within your neonatal network. This will enable us to contact them to complete a short survey which will hopefully help in the development of a trial to assess this intervention. The study participant's identification will be anonymous.

We are happy to provide any further information you may require and would happily discuss this over the telephone if that will be helpful.

Contact Dr Jon Dorling,

Division of Child Health, Obstetrics & Gynaecology- School of medicine
Phone: 0115 9249924, ask for 'neonatal secretaries'
Emails: msxawn@exmail.nottingham.ac.uk, Jon.Dorling@nottingham.ac.uk

Thank you very much
Yours Sincerely

Appendix 4 Survey invitation and reminder emails

Survey email

Dear Neonatal Clinical Lead

Hello

"Oropharyngeal Administration of Mother's own Colostrum to Preterm Infants: a Survey of Neonatal Professionals"

This survey is conducted to determine the knowledge, attitudes and practices of UK neonatal professionals regarding the oropharyngeal administration of colostrum to preterm infants.

As you are a neonatal professional, you have been invited to the survey and if you agree to contribute in this survey, please could you complete the online questionnaire.

The questionnaire will take approximately 5-10 minutes. Your participation is entirely voluntary and completion of the survey will be considered as consent.

This study has been reviewed and approved by the Faculty of Medicine & Health Sciences Research Ethics Committee, University of Nottingham.

The survey link:

https://nottingham.onlinesurveys.ac.uk/opc-last-version-2

Best wishes
Amna Nasuf
PhD Student
Division of Child Health, Obstetrics & Gynaecology- School of medicine
University of Nottingham,
E floor, East Block,
Queen's Medical Centre,
Nottingham,
NG7 2UH
Phone: 01158230607
Email: msxawn@nottingham.ac.uk

Reminder email

Dear clinical leads and nurse lead of neonatal unit

Hello

I understand you have a busy diary but this is just a gentle reminder to participate in the online survey

"Oropharyngeal administration of mother's own colostrum to preterm infants: a Survey of Neonatal Professionals."

We previously sent you an email containing a link for you to complete the survey. If you have completed the survey, thank you and your time is greatly appreciated.

If you have not completed the survey yet, could you please do so before the deadline of October 30 2015 so that we can use your opinion?

As noted in the invitation email, your participation will be anonymous, and the results from this survey will help in the development of a trial to assess this intervention.

To access the survey, please follow this link:

https://nottingham.onlinesurveys.ac.uk/opc-last-version-2

Kind regards

Amna Widad Ahmed Nasuf
Division of Child Health, Obstetrics & Gynaecology- School of medicine
University of Nottingham
Phone: 01158230607
Email: msxawn@nottingham.ac.uk

Appendix 5 Cochrane review search strategy

Search Terms: (colostrum) OR (oropharyngeal*colostrum0) OR (oral*care)

Plus, the following database-specific terms:

PubMed: ((infant, newborn[MeSH] OR newborn OR neonate OR neonatal OR premature OR low birth weight OR VLBW OR LBW or infan* or neonat*) AND (randomized controlled trial [pt] OR controlled clinical trial [pt] OR Clinical Trial[ptyp] OR randomized [tiab] OR placebo [tiab] OR clinical trials as topic [mesh: noexp] OR randomly [tiab] OR trial [ti]) NOT (animals [mh] NOT humans [mh]))

Embase: (infant, newborn or newborn or neonate or neonatal or premature or very low birth weight or low birth weight or VLBW or LBW or Newborn or infan* or neonat*) AND (human not animal) AND (randomized controlled trial or controlled clinical trial or randomized or placebo or clinical trials as topic or randomly or trial or clinical trial)

CINAHL: (infant, newborn OR newborn OR neonate OR neonatal OR premature OR low birth weight OR VLBW OR LBW or Newborn or infan* or neonat*) AND (randomized controlled trial OR controlled clinical trial OR randomized OR placebo OR clinical trials as topic OR randomly OR trial OR PT clinical trial)

Cochrane Library: (infant or newborn or neonate or neonatal or premature or very low birth weight or low birth weight or VLBW or LBW)

Clinical Trials: (colostrum AND infant) OR (oropharyngeal*colostrum AND infant)

Appendix 6 Case-control study-Favourable opinion

The University of Nottingham

Direct line/e-mail
+44 (0) 115 8232561
Louise.Sabir@nottingham.ac.uk

Faculty of Medicine and Health Sciences

Research Ethics Committee
C/o Faculty PVC Office
School of Medicine Education Centre
B Floor, Medical School
Queen's Medical Centre Campus
Nottingham University Hospitals
Nottingham
NG7 2UH

10th November 2016

Professor Helen Budge
Professor of Neonatal Medicine
Co-Director of the Clinical Academic Training Programme
Child Health, Obstetrics and Gynaecology
School of Medicine
QMC Campus
Nottingham University Hospitals
NG7 2UH

Dear Professor Budge

Ethics Reference No: A09102016 Audit 16-086C **– please always quote**	
Study Title: To evaluate the impact of oropharyngeal administration of mother's own colostrum to preterm infants.	
Chief Investigator/Supervisor: Professor Helen Budge, Professor of Neonatal Medicine, Child Health, Obstetrics and Gynaecology, School of Medicine	
Lead investigator/Student: Amna Nasuf, PhD Student, School of Medicine	
Other Key Investigators: Dr Jon Dorling, Clinical Associate Professor & Honorary Consultant Neonatologist/Chair of Nottingham Neonatal Clinical Services Unit. Dr Stephen Wardle, Consultant, Nottingham University Hospitals Trust NHS Dr Shalini Ojha, School of Medicine.	
Type of Study: Clinical Audit, PhD	
Proposed Start Date: 1/10/2016	**Proposed End Date:** 30/09/2017 12mths
Sample Size: 111	
School: SoM	

Thank you for your letter dated 29th September 2016 seeking an opinion on the above clinical audit study to be conducted by a PhD student in Nottingham University Hospitals NHS Trust to inform one of her research studies and the results will be included in the final PhD thesis.

On review of the information provided this study does not require full Research Ethics Committee review or approval because it is a clinical audit of practice against current guidelines which has been registered and signed off by the Clinical Business Unit at Nottingham University Hospitals Trust.

Yours sincerely

pp Lomiszewski

Professor Ravi Mahajan
Chair, Faculty of Medicine & Health Sciences Research Ethics Committee

Appendix 7 Nottingham neonatal service guideline for OPC administration

Nottingham Children's Hospital

Title of Guideline (must include the word "Guideline" (not protocol, policy, procedure etc)	**Administration of Oropharyngeal Colostrum to Infants in the Neonatal Intensive Care Unit guideline (D3a)**
Author: Contact Name and Job Title	Amna Nasuf (Child Health Academic) Dr Shalini Ojha (Neonatal Consultant) Chris Jarvis (Clinical Paediatric Dietician) Dr Jon Dorling (Neonatal Lead Consultant)
Directorate & Speciality	Family Health: Neonatal Unit
Date of submission	10.2.2017
Explicit definition of patient group to which it applies (e.g. inclusion and exclusion criteria, diagnosis)	Patients of the Nottingham Neonatal Service of the Nottingham University Hospitals NHS Trust who fit the inclusion criteria of the guideline.
Version	1
If this version supersedes another clinical guideline please be explicit about which guideline it replaces including version number.	N/A
Key Words	Oropharyngeal colostrum, Late onset sepsis, Necrotising enterocolitis, Breast feeding

Statement of the evidence base of the guideline – has the guideline been peer reviewed by colleagues?

1	NICE Guidance, Royal College Guideline, SIGN (please state which source).	
2a	meta-analysis of randomised controlled trials	
2b	at least one randomised controlled trial	
3a	at least one well-designed controlled study without randomisation	
3b	at least one other type of well-designed quasi-experimental study	
4	well –designed non-experimental descriptive studies (ie comparative / correlation and case studies)	x
5	expert committee reports or opinions and / or clinical experiences of respected authorities	x
6	recommended best practise based on the clinical experience of the guideline developer	x

Consultation Process	Nottingham Neonatal Service Staff and Clinical Guideline Meeting, Midwifery Services
Ratified by: Date:	Nottingham Neonatal Service Staff and Neonatal Task & Finish Guideline group. February 2017
Target audience	Staff of the Nottingham Neonatal Service, Delivery Suites and Postnatal Wards
Review Date: (to be applied by the Integrated Governance Team) A review date of 5 years will be applied by the Trust. Directorates can choose to apply a shorter review date, however this must be managed through Directorate Governance processes.	March 2022

This guideline has been registered with the trust. However, clinical guidelines are guidelines only. The interpretation and application of clinical guidelines will remain the responsibility of the individual clinician. If in doubt contact a senior colleague or expert. Caution is advised when using guidelines after the review date.

1 Background

Colostrum is milk produced in the first few days after delivery. It is rich in immuno-protective and growth factors such as immunoglobulin A, lactoferrin, and epidermal growth factor and has the potential to modulate the infant's immune system, especially in preterm infants. Colostrum produced by mothers who deliver preterm infants has a higher concentration of immunologically active factors compared to colostrum of mothers who deliver at term gestation.

Preterm infants are at a particularly high risk of late onset sepsis (LOS) and necrotising enterocolitis (NEC) which can lead to death, increased morbidities, prolonged hospital stay, increased cost of care, and worse long term outcomes among survivors. Some studies have demonstrated that giving colostrum by the oropharyngeal route to preterm infants improves their immunity and may help to reduce the risk of LOS and NEC.

Administration of oropharyngeal colostrum (OPC) is a new practice used to provide the benefits of colostrum to infants and can be used even in the critically-ill, fragile preterm infants who cannot yet tolerate enteral feeding. A small volume of colostrum is placed in the buccal cavity by a syringe or swab. It does not require swallowing of colostrum by the infant but allows it to act locally and to be absorbed by the buccal mucosa. Preliminary studies suggest that this is a safe practice, even in the sickest preterm infants.

This guideline suggests a safe and practical procedure for administration of OPC to infants. It should be used in conjunction with other infant feeding and oral care guidelines.

2 Aim

To enable preterm and/or sick infants to receive mother's own colostrum via oropharyngeal administration.

Patient Group

- Preterm infants (born <34 weeks gestation) admitted to the NICU

or

- Any infant who is not receiving feeds such as infants on respiratory support and/or inotropes or with a surgical condition contraindicating feeding (e.g. gastroschisis, oesophageal atresia +/- fistula, duodenal atresia).

3 Contraindications

- Any contraindication for receiving mother's own milk, such as maternal HIV infection
- Breast feeding

4 Procedure

- All mothers anticipating delivery of an eligible infant should be informed about the benefits of colostrum and advised to express breast milk soon after delivery. This information must also be included in antenatal counselling, wherever possible.
- Administration of OPC should ideally be initiated within 6 hours of birth but otherwise as soon as colostrum is available
- Only the mother's own colostrum should be used
- Fresh colostrum should be used whenever possible but stored colostrum can be used (as per the guidelines for breast milk storage)

4.1 Steps of administration

1. Provide mother with labelled sterile containers for colostrum collection. Labels should identify mother's name, infant's hospital number, and the date and time of colostrum expression. Colostrum can be collected in appropriately labelled 1 or 2 ml enteral syringes.

2. When mother's colostrum is available, separate two samples of 1.5 ml of fresh colostrum and label them for use within the first 48 hours of birth/initiation of OPC.
3. Using clean gloves put 0.2 ml of mother's colostrum in a 1ml enteral syringe, cap and label it. At the infant's bedside, verify that the medical records on the colostrum container match those on the infant's record chart.
4. Perform mouth care as routine.
5. Remove the cap of the syringe and gently insert the tip of the syringe into the infant's mouth along the right side and directed posteriorly towards the oropharynx. Administer 0.1 ml of colostrum slowly. Place the syringe along the left side and another 0.1 ml of colostrum is delivered by the same procedure.
6. Avoid oral suction for 30 min
7. Monitor the vital signs of the infant throughout the procedure.
8. Repeat the procedure **every four hours for 3 days.**
9. Record the procedure on the infant feeding record chart.
10. If feeds are commenced, the oral colostrum should be given first and then the NG feed. Oral volumes should be recorded separately and not included as part of the feed volume.
11. Record any adverse effects on the chart and in the medical notes.
12. Parental involvement in the administration of OPC is recommended. Nursing staff may teach and supervise them to give colostrum by this route.

5 Information for parents

Parents should be given information about the benefits of mother's colostrum/milk, preferably antenatally, and the mother advised to start expressing milk as soon after delivery as possible. Encourage mothers to express colostrum within 6 hours of delivery or as soon as possible and a minimum of 4 hourly. Inform the parents that colostrum is initially expressed in small volume.

6 For further information please contact:

jon.dorling@nuh.nhs.uk; chris.jarvis@nuh.nhs.uk;

7 References

BREASTFEEDING, S. O. 2012. Breastfeeding and the Use of Human Milk. *Pediatrics,* 129, e827-e841.

DIANE L. SPATZ, P. R. F., AND TARYN M. EDWARDS, BSN RNC 2009. The Use of Colostrum and Human Milk for Oral Care in the Neonatal Intensive Care Unit. *National Association of Neonatal Nurses E-News.*

RODRIGUEZ, N. A., MEIER, P. P., GROER, M. W. & ZELLER, J. M. 2009. Oropharyngeal administration of colostrum to extremely low birth weight infants: theoretical perspectives. *Journal of Perinatology,* 29, 1-7.

RODRIGUEZ, N. A., MEIER, P. P., GROER, M. W., ZELLER, J. M., ENGSTROM, J. L. & FOGG, L. 2010. A pilot study to determine the safety and feasibility of oropharyngeal administration of own mother's colostrum to extremely low-birth-weight infants. *Adv Neonatal Care,* 10, 206-12.

SEIGEL, J. K., SMITH, P. B., ASHLEY, P. L., COTTEN, C. M., HERBERT, C. C., KING, B. A., MAYNOR, A. R., NEILL, S., WYNN, J. & BIDEGAIN, M. 2013. Early administration of oropharyngeal colostrum to extremely low birth weight infants. *Breastfeeding Medicine: The Official Journal of the Academy of Breastfeeding Medicine,* 8, 491-5.

Appendix 8 Oropharyngeal administration of colostrum (OPC) data collection form

Feeding Status	Start/day	End/day	Volume	Remarks
TPN				
Trophic F				
Enteral				>2 ml/kg/hr of milk

Feeding	Date (DOL)	EBM	Formula	Route	Volume/kg/24	
Full enteral (120ml/kg)						
Full enteral (150ml/kg/day)						
Breast feeding: Yes ☐ No ☐						
Withholding feeds >4 hours: No ☐ Yes ☐	Reasons: Duration:					

OPC administration

Received OPC	Yes ☐ No ☐ Postnatal day:	Start:	Stop:
Frequency (hourly)	2 ☐ 3 ☐ 4 ☐ other ☐ specify:		Total doses received:
Duration	24 hours ☐ 48 hours ☐ 72 hours ☐ Other ☐ Specify:		Total volume received: ml
Colostrum	Fresh ☐ Refrigerated ☐ Frozen ☐		Type:
OPC doses/day	D1: doses volume= D2: doses volume= D3: doses volume= D4: doses volume= D5: doses volume=		Trophic feeds: Yes ☐ No ☐ Milk volume: type: Enteral feed: Yes ☐ No ☐ Milk volume: type:

Adverse effects	Yes	Date/time	Frequency	No	comments
HR < 100					
HR > 100					
RR > 80					
Apnoea >20 sec					
SpO_2 <80%					
Aspiration/Chocking Milk in mouth X-ray changes					

Appendix 9 Blinded Endpoint Review for incidence of NEC (Chapter 4)

The impact of oropharyngeal administration of mother's colostrum on the clinical outcomes of preterm infants

Blinded Endpoint Reviews are an important part of clinical research for validating the data process.

The reviews are carried out by clinicians who are experts in the field of conditions for which study information is being collected for analysis.

Blinded Endpoint Review for the outcome Incidences of Necrotising Enterocolitis (NEC)

Infants who had a possible diagnosis of NEC during a stay in the neonatal units have been reviewed by two clinicians (neonatal registrars). If a consensus cannot be reached, cases will be discussed with a third reviewer (consultant neonatologist).

Based on the final decisions of the reviewers, NEC has been confirmed or not occurred.

An anonymous spreadsheet that included all the cases with a diagnosis of NEC according to the infants' records in the Badger neonatal database of the Nottingham neonatal units had been provided to the reviewers.

NEC was defined according to the followings:

- Modified Bell's criteria ≥ II (1).
- Suspected NEC, as per the Nottingham neonatal service guideline; treated by cessation of feeding and receiving triple antibiotic therapy for ≥7days.

Please complete the included table.

Please confirm whether an episode of NEC had occurred or not fulfilling the criteria as such by clicking one box in the column (Reviewer diagnosis).

Please refer to the included table (page 13) for NEC definitions (Bell' stage ≥ II).

SID	Infant's Criteria for diagnosing NEC						Reviewer diagnosis
S245	Infant's baseline data DOB: DOA: GA: Bwt: High risk:	Clinical signs	X-ray signs	Management	Histopathology	Diagnosis	**Confirm NEC:** **Yes** ☐ **No** ☐ **Unclear** ☐ **Further information** ☐
W158	Infant's baseline data DOB: DOA: GA: Bwt: High risk:	Clinical signs	X-ray signs	Management	Histopathology	Diagnosis	**Confirm NEC:** **Yes** ☐ **No** ☐ **Unclear** ☐ **Further information** ☐

SID: infant's study identification number; NEC: necrotising enterocolitis; DOB: date of birth; DOA: date of admission to the Nottingham neonatal unit; GA: gestational age; Bwt: birth weight

Comments:	
Name: **Signature:**	**Job title:** **Date:**

Bell stage	Systemic signs	Gut signs	Radiographic findings	Treatment
Suspected NEC **Stage I**	History of risk factors Non-specific/Reluctant to feed Lethargy/apnoea/bradycardia Temperature instability	High gastric residual (pre-feed) Vomiting ± bilious Mild abdominal distension Occult blood in the stool	Normal/mild intestinal distension	NBM, antibiotics for three days
Definite NEC **Stage II** - **A**: mildly ill	Increased desaturations and/or bradycardia Temperature instability Lethargy	High gastric residual (pre-feed) Definite abdominal distension abdominal tenderness Possibly bloody stools Absent bowel sounds	Ileus, fixed dilated bowel loops, pneumatosis intestinalis	NBM, antibiotics 7-10 days
- **B**: moderately ill	As **IIA** with thrombocytopenia and/or mild metabolic acidosis	Same as previous Abdominal distension with definite tenderness Possible abdominal wall oedema and/or discolouration Mass at the lower right abdomen	As **IIA** and portal vein gas, ascites (possible)	NBM, antibiotics 14 days
Advanced NEC **Stage III (severely ill)** - **A**: bowel intact	As **IIB** plus hypotension, bradycardia, apnoea, severe metabolic acidosis, respiratory acidosis Disseminated intravascular coagulopathy, neutropenia	Same as previous, plus severe abdominal distension and tenderness with abdominal wall induration (sings of peritonitis)	As **IIB** with definite ascites	NBM, antibiotics 14 days, inotropes, fluid resuscitation, ventilator support, paracentesis
Stage IIIB	As **IIIA**	As **IIIA**	As **IIIA** and Perforation, Pneumoperitoneum	Same as **IIIA** with laparotomy

NBM: nil by mouth

1. Kliegman RM, Walsh MC. Neonatal necrotizing enterocolitis: Pathogenesis, classification, and spectrum of illness. Current problems in pediatrics. 1987;17(4):219-88.

Appendix 10 Case-control study- Neonatal consultants 'letter

The University of Nottingham

UNITED KINGDOM · CHINA · MALAYSIA

Division of Child Health,
Obstetrics & Gynaecology
University of Nottingham
E Floor, East Block
Queens Medical Centre
Nottingham
NG7 2UH

Tel 0115 8230635

Dear Neonatal Consultant of the Week

We would greatly appreciate your support in encouraging the adoption of the guideline for the administration of oropharyngeal colostrum to infants on the Neonatal Intensive Care Unit (D3a). This is intended to aid the implementation of OPC as standard practice in the Nottingham neonatal units.

Please find enclosed a pack of information leaflets for the nursing staff along with the guideline which are provided for you during your on-service week. We would be very grateful if you could use these to remind the nursing staff to initiate and maintain the use of OPC 4 hourly for 72 hours.

Please distribute these to the clipboards of eligible babies as a reminder and guide to the nurses.

Thank you for your support

Dr Jon Dorling

Dr Amna Nasuf

Prof Helen Budge

Appendix 11 Case-control study- Nurse Information Sheet

Nottingham University Hospitals NHS
NHS Trust

Hospital sticker

Oropharyngeal colostrum (OPC)

A baby is eligible to start for OPC when they:

- have been born before 34 weeks of gestation ☐ and/or
- are, or were, not initially receiving feeds ☐

A baby is eligible to have OPC, given as described in Guideline D3, even if they are on inotropes or have a surgical condition contraindicating enteral feeding (e.g. gastroschisis, oesophageal atresia, duodenal atresia).

This baby is eligible to start oral colostrum (OPC) as soon as mother's colostrum available (within 96 hours of birth)

Guideline number: D3a

1. Insert the tip of the oral syringe into the infant's mouth along the right side and directed posteriorly towards the oropharynx. Administer 0.1 ml of colostrum slowly. Place the oral syringe along the left side and give another 0.1 ml of colostrum by the same procedure.
2. Avoid oral suction for 30 min (if possible).
3. Monitor the infant's heart rate and oxygen saturations throughout the procedure.
4. Record OPC separately on the infant feeding record chart (example below)
5. Repeat OPC administration **every four hours for 3 days, even if NGT or OGT feeds are commenced and even if the infant becomes NBM.**
6. When NGT/OGT feeds are being given, give the oral colostrum first and record it separately from the NG/OG feed on the chart (example below).

ENTERAL NUTRITION																								
Type of Milk EBM Formula Special	C				C		EBM		C / EBM		EBM		C / EBM		EBM		C / EBM		EBM		C / EBM		EBM	
Method of feed	OP				OP		NGT		OP / NGT		NGT		OP / NGT		NGT		OP / NGT		NGT		OP / NGT		NGT	
Volume of Milk Given	0·2				0·2		1		0·2 / 1		1		0·2 / 1		1		0·2 / 1		1		0·2 / 1		1	

Colostrum(C) is mother's milk produced in the first few days after delivery and is rich in immuno-protective and growth factors. For more information: see Guideline

Appendix 12 Research Ethics Committee favourable opinion (Chapter 5)

NHS
Health Research Authority
East Midlands - Leicester South Research Ethics Committe
The Old Chap
Royal Standard Plac
Nottinghar
NG1 6F

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

12 October 2017

Dr Jon/JD Dorling
Division of Child Health, Obstetrics & Gynaecology
Queen's Medical Centre, University Hospital Nottingham
Nottingham
NG7 2UH

Dear Dr Dorling,

Study title:	**Gut hormone response to oropharyngeal administration of mother's colostrum to infants in neonatal intensive care**
REC reference:	**17/EM/0323**
Protocol number:	**17055**
IRAS project ID:	**223294**

Thank you for your letter of 04 October 2017, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact hra.studyregistration@nhs.net outlining the reasons for your request.

Appendix 13 Health Research Authority Approval (Chapter 5)

NHS
Health Research Authority

Dr Jon Dorling
Division of Child Health, Obstetrics & Gynaecology
Queen's Medical Centre, University Hospital Nottingham
Nottingham
NG7 2UH

Email: hra.approval@nhs.net

23 October 2017

Dear Dr Dorling

Letter of HRA Approval

Study title:	**Gut hormone response to oropharyngeal administration of mother's colostrum to infants in neonatal intensive care**
IRAS project ID:	**223294**
Protocol number:	**17055**
REC reference:	**17/EM/0323**
Sponsor	**University of Nottingham**

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

Your IRAS project ID is **223294**. Please quote this on all correspondence.

Yours sincerely

Juliana Araujo
Assessor
Email: hra.approval@nhs.net

Appendix 14 Gut hormone response to OPC-Parent Information Sheet

The University of Nottingham

UNITED KINGDOM · CHINA · MALAYSIA

Gut hormone response to oropharyngeal administration of mother's colostrum to infants in neonatal intensive care

Parent Information Sheet

Final version 1.2: 28 September 2017

IRAS project ID: 223294

Researchers

Dr Amna Nasuf, Prof Helen Budge, Dr Jon Dorling

Welcome

We appreciate that your baby being in the Neonatal Intensive Care Unit (NICU) can be a difficult and worrying time for you and your family. We would like to inform you of a study that could help to improve health outcomes for babies which we would like to invite you to consider agreeing to take part in.

Please take your time to read this information leaflet. Before you decide we would like you to understand why the research is being done and what it would involve for you and your baby. Our team will go through the information sheet with you and answer any questions you may have. Please talk to others about the study if you wish and do ask us if there is anything that is not clear.

What is the purpose of the study?

Feeding is a major challenge in babies requiring intensive care after birth. It is an important factor that can influence nutrition, growth, and health outcomes. Delay in giving milk feeds after birth is associated with increased risk of infection, longer hospital stays, and other complications.
Colostrum is the first breast milk produced by a mother in the first few days after birth. It is rich in nutrients and protective factors that can help unwell babies fight infections. After birth if a baby is critically ill and cannot be fed by his mother, bottle or tube, mother's own colostrum can be given as a few drops into the baby's mouth to provide the benefits of mother's colostrum to those infants. This route has been called oropharyngeal route (OPC).

In this study, we want to know whether giving colostrum into the mouth stimulates the secretion of gut hormones which might help babies to reach full milk feeding earlier, leading to a better weight gain and potentially helping babies go home sooner. It may also help you to continue breast feeding for longer or more effectively, things that have been linked with improved health outcomes for babies and mothers.

This study is taking place in the Nottingham Neonatal Units at Queen's Medical Centre and City Hospital and will form part of a PhD thesis.

Why have I and my baby been invited?

You have been invited to take part in this study because your baby is receiving care in a Nottingham Neonatal Unit and their care needs that they are not receiving milk into the stomach. Your baby is eligible to be given your colostrum by the oropharyngeal route according to the Nottingham Neonatal Service Guideline. We are inviting 40 parents with their babies to participate in our study.

Do I have to take part?

It is up to you to decide whether or not to agree that your baby will take part in this study. Participation is entirely voluntary. If you do decide to participate, you will be asked to sign a consent form, a copy of which you will be given to keep along with this information sheet. If you decide to take part you are still free to withdraw your baby at any time and without giving a reason. This would not affect the health care of you or your baby.

What will happen to me if I and my baby take part?

If you agree to enrol your baby in this study you will be asked to sign a consent form. There will be no extra procedures, nor medicines given above the normal care of the neonatal unit where your baby is receiving care.
Your baby will be enrolled in this study from admission until discharge from the neonatal unit.
Should your baby not receive OPC, we would still like to collect information.
In this study, we will collect the following:

- Information on the clinical progress of your baby during the stay at the neonatal unit. All of these data are routinely collected for any baby receiving care in a neonatal unit.
- For the purpose of this study, we will take 4-6 drops of blood from your baby

before giving colostrum by the oropharyngeal route and another two samples after 7 and 14 days. This is to measure gut hormone levels in the blood to see the response of the gut to colostrum. This may help in future to assess when babies can receive milk feeds.

- The blood samples will be collected when routine blood tests are required for clinical reasons so there will be no additional discomfort or disturbance to your baby. The blood sample tubes will be labelled with a unique study identification number, ensuring anonymous analysis. We will use a technique that permits simultaneous measurement of multiple proteins using a very small amount of blood. Any remaining samples will be discarded in accordance to the UK regulations.
- If your baby is transferred to another neonatal unit before discharge home, we would like to retain your baby in the study, and relevant information about your baby's medical care will be collected.

What are the possible disadvantages and risks of taking part?

We believe that there are no disadvantages for your baby in taking part in this study, there will be no additional procedures nor medications beyond the standard care of the neonatal unit. No extra visits to the hospital will be needed over the routine follow-up visits. As normal, all aspects of care will be at the discretion of the clinical team.

What are the possible benefits of taking part?

There are no direct benefits for you or your baby from taking part in this study but the information we get from this study may help in the future to improve feeding tolerance of babies in neonatal intensive care, shortening hospital stay, decreasing infections, and enhancing health outcomes.

What if there is a problem?

As there will be no additional procedures or treatments over the routine care of your baby in the neonatal unit, we expect no direct problems related to participation in the study. However, if you have a concern about any aspect of this study, please ask to speak to the research team who will do their best to answer your questions. Our contact details are given at the end of this information sheet. If, despite this you are considering a formal complaint, you can do this by contacting the Patient Advice and Liaisons Services (phone number 0800 1830204; email pals@nuh.nhs.uk).

Will my taking part in the study be kept confidential?

Yes, your taking part (and your baby) in the study will be kept confidential. We will follow good ethical and legal practices and all information about you and your baby will be handled in confidence. However, if information is disclosed during the study that could pose a risk of harm to you, your baby or others, the research group will discuss this and where appropriate report accordingly.

If you agree that your baby will participate in the study, some parts of you and your baby's medical records and the data collected for the study will be looked at by authorised persons from the University of Nottingham who are organising the research to check that the study is being carried out correctly. All will have a duty of confidentiality to you and your baby as a research participant and we will do our best to meet this duty. All information which is collected about your baby during the course of the research will be kept strictly confidential, stored in a secure and locked office, on a password protected database. These will be retained for at least 7 years in accordance with the University of Nottingham Code of Research Conduct and Research Ethics.

What will happen if I don't want to carry on with the study?

Participation of your baby is voluntary and you are free to withdraw your baby at any time, without giving any reason, and without

your legal rights being affected. If you withdraw your baby then the information collected so far cannot be erased and this information may still be used in the project analysis but no further information will be collected.

What will happen to any samples taken from my baby?

The samples will be securely stored with a code unique to your baby at the University of Nottingham under the University's Human Tissue Research Licence (no 12265). Any samples or data used will be anonymised, and your baby will not be identified in any way. After completion of the study all samples will be disposed of in accordance with the Human Tissue Authority's code of practice.

What will happen to the results of the research study?

The results of the study will be published in a medical journal and submitted in a PhD thesis to the University of Nottingham. Outcomes of the study will be available to parents/guardians through relevant parent support networks and the website of the University of Nottingham. You and your baby will not be identifiable in any report or publications.

Who is organising and funding the research?

This research is being organised and funded by the University of Nottingham.

Who has reviewed the study?

All research in the NHS is assessed by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by the Leicester South Research Ethics Committee (17/EM/0323).

Further information and contact details

Thank you for reading this information sheet and if you would like any further information about the study, please do not hesitate to contact the study team.

Chief Investigator: Dr Jon Dorling

Phone: 0115 9249924
Email: jon.dorling@nottingham.ac.uk

Co-Investigators: Professor Helen Budge

Phone: 0115 8230611
Email: helen.budge@nottingham.ac.uk

Dr Amna Widad Ahmed Nasuf

Phone: 01159249924
Email: msxawn@nottingham.ac.uk

Appendix 15 Gut hormone response to OPC-Consent Form

University of Nottingham
UK | CHINA | MALAYSIA

Nottingham University Hospitals NHS
NHS Trust

CONSENT FORM FOR PARENTS
(Final version 1.2: 18.05.2018)

Title of Study: Gut hormones response to oropharyngeal administration of mother's colostrum to infants in neonatal intensive care

IRAS Project ID: 223294

Name of Researcher: Dr Jon Dorling, Prof Helen Budge, Dr Amna Widad Ahmed Nasuf

Name of Parent:

Name of Participant (Infant): **Please initial box**

1. I confirm that I have read and understand the Parent information sheet version number 1.3 dated 18Th May 2018 for the above study and have had the opportunity to ask questions and have had satisfactory answers. ☐
2. I understand that my baby's participation is voluntary and that they are free to withdraw at any time, without giving any reason, and without their medical care or legal rights being affected. I understand that should they withdraw from the study then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐
3. I understand that relevant sections of both my baby's and my medical notes and data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group, and regulatory authorities where it is relevant to our taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my baby's participation in this study. I understand that my and my baby's personal details will be kept confidential. ☐
4. I understand and agree that blood samples will be taken for analysis of gut hormones. ☐
5. I understand that if my baby is transferred to another neonatal unit before discharge home, relevant information about my baby's medical care may be collected for the purpose of the study if regulatory approvals are in place. I agree for this information to be collected. ☐
6. I understand that if information is disclosed during the study that could pose a risk of harm to my baby or others, the research group will discuss this and where appropriate report accordingly. ☐
7. I agree for my baby (named above) to take part in the above study. ☐

Name of Parent	Date	Signature
Name of Person taking consent	Date	Signature

3 copies: 1 for participant, 1 for the project notes and 1 for the medical notes

Gut hormones and oropharyngeal colostrum in NICU. Parent Consent Form. Final Version 1.2 18.May 2018

Appendix 16 Gut hormone response to OPC- Sample Collection Information Sheet

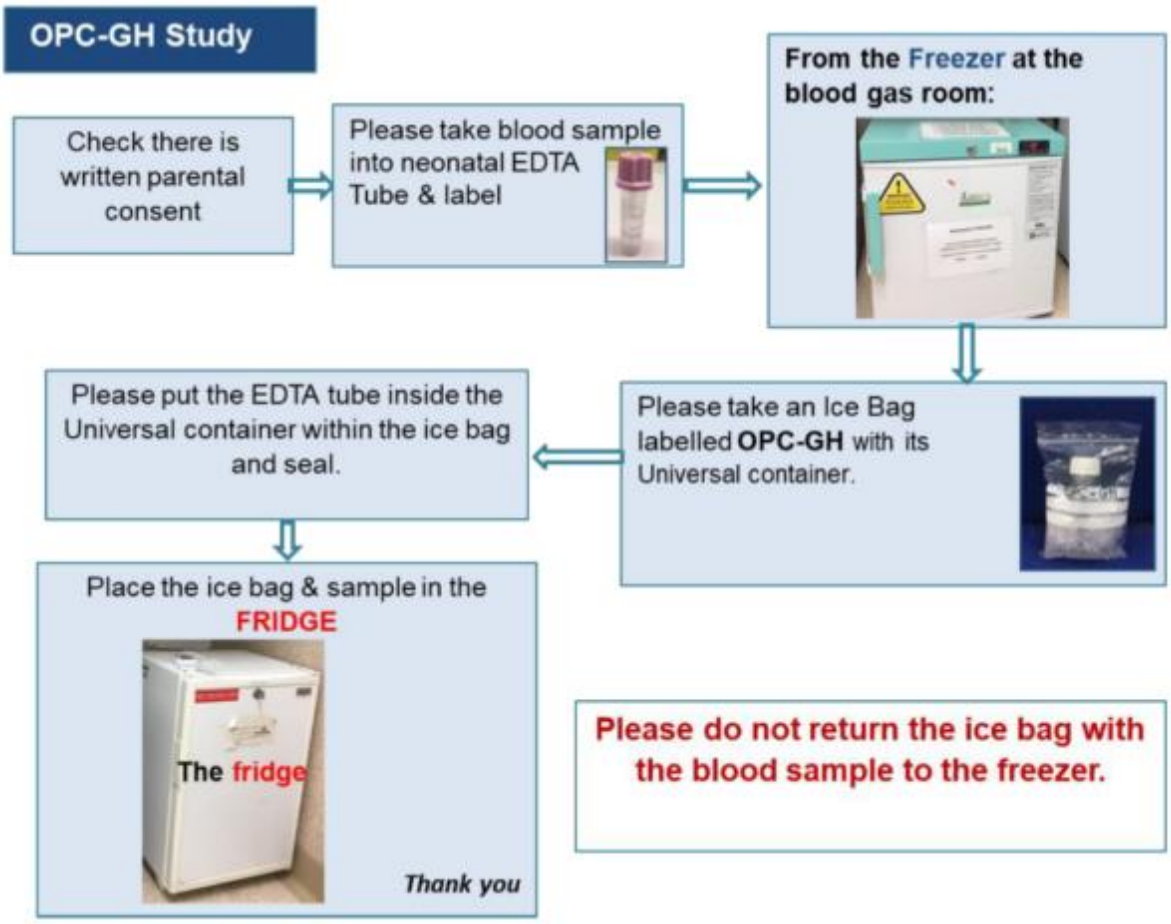
Gut hormone response to oropharyngeal administration of mother's colostrum to infants in neonatal intensive care

(OPC-GH)

Sample collection information

Blood samples collection and handling should be collected according to the local safety regulations of the unit.

- With informed written parental consent.
- Please collect the study blood sample into neonatal EDTA tube with routine bloods. Label the tube with the baby's ID.
- In the blood gas room, from the **freezer**, please take an Ice Bag with Universal container. Ice bag labelled **OPC-GH**.
- Please put the blood sample tube inside the Universal container within the ice bag.
- Please seal the container with the ice bag carefully and put the ice bag with the sample in the **fridge** in the blood gas room.
- **Please do not return the ice bag with the blood sample to the freezer.**



Appendix 17 Multiplex immunoassay laboratory procedure

MILLIPLEX® MAP: Human Metabolic Hormone Magnetic Bead Panel
96-Well Plate Assay- HMHEMAG-34K

Preparation of Plasma Samples:

- Plasma collection using EDTA as an anti-coagulant is recommended.
- After collecting blood sample, invert tube serval times to mix, immediately add DPPIV inhibitor (for GLP-1 measurement), and serine protease inhibitor (for Active Ghrelin measurement). These should be used following manufacturers' instructions.
- Centrifuge for 10 minutes at 1000xg within 30 minutes of blood collection. Remove plasma and assay immediately or aliquot and store samples at <-20°C.
- Avoid multiple >2 freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well **by vortexing and centrifuge prior to use to remove particulates**

All samples must be stored in polypropylene tubes. A maximum of 25 µL per well of neat plasma can be used.

Preparation of reagents for immunoassay

A. Preparation of Antibody-Immobilized Beads

For individual vials of beads, sonicate each antibody-bead vial for 30 seconds; vortex for 1 minute. Add 150 µL from each antibody-bead vial to the Mixing Bottle and bring final volume to 3.0 mL with Bead Diluent. Vortex the mixed beads well. Unused portion may be stored at 2-8°C for up to one month. (Note: Due to the composition of magnetic beads, you may notice a slight colour in the bead solution. This does not affect the performance of the beads or the kit.)

Example 1: When using 13 antibody-immobilized beads, add 150 µL from each of the 13 bead vials to the Mixing Bottle. Then add 1.05 mL Bead Diluent.
Example 2: When using 3 antibody-immobilized beads, add 150 µL from each of the 3 bead vials to the Mixing Bottle. Then add 2.55 mL Bead Diluent.

B. Preparation of Quality Controls

Before use, reconstitute Quality Control 1 and Quality Control 2 with 250 µL deionized water. Invert the vial several times to mix and vortex. Allow the vial to sit for 5-10 minutes. Unused portion may be stored at 2-8°C for up to one month.

C. Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 60 mL of 10X Wash Buffer with 540 mL deionized water. Store the unused portion at 2-8°C for up to one month.

D. Preparation of Serum Matrix

Add 1 mL deionized water to the bottle containing lyophilized Serum Matrix. Mix well. Allow at least 10 minutes for complete reconstitution. Leftover reconstituted Serum Matrix should be stored at □ 20°C for up to one month.

E. Preparation of Human Metabolic Hormone Standard

1. Prior to use, reconstitute the Human Metabolic Hormone Standard with 250 µL deionized water. Refer to table below for analyte concentrations. Invert the vial several times to mix. Vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes. This will be used as Standard #7; the unused portion may be stored at < 20°C for up to one month.

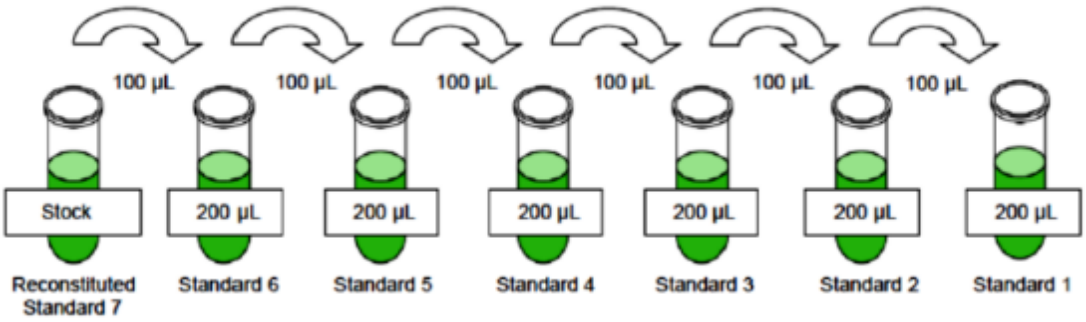
2. Preparation of Working Standards

Label 6 polypropylene microfuge tubes 1-6. Add 200 µL of Assay Buffer to each of the 6 tubes. Prepare serial dilutions by adding 100 µL of the #7 reconstituted standard to the #6 standard tube, mix well and transfer 100 µL of the #6 standard to the #5 standard tube, mix well and transfer 100 µL of the #5 standard to the #4 standard tube, mix well and transfer 100 µL of the #4 standard to the #3 standard tube, mix well and transfer 100 µL of the #3 standard to the #2 standard tube and mix well, and transfer 100 µL of the #2 standard to the #1 standard tube and mix well. The 0 pg/mL standard (Background) will be Assay Buffer.

Standard #	Volume of Deionized Water to Add	Volume of Standard to Add
Standard #7	250 µL	0

Standard #	Volume of Assay Buffer to Add	Volume of Standard to Add
Standard #6	200 µL	100 µL of Standard #7
Standard #5	200 µL	100 µL of Standard #6
Standard #4	200 µL	100 µL of Standard #5
Standard #3	200 µL	100 µL of Standard #4
Standard #2	200 µL	100 µL of Standard #3
Standard #1	200 µL	100 µL of Standard #2

Preparation of Standards



Standard	PYY (pg/mL)	GIP (pg/mL)	GLP-1 (pg/mL)	Ghrelin (pg/mL)	Insulin (pg/mL)
Standard 1	13.7	1.4	2.7	13.7	137
Standard 2	41.2	4.1	8.2	41.2	412
Standard 3	123	12.3	24.7	123	1,235
Standard 4	370	37.0	74.1	370	3,704
Standard 5	1,111	111	222	1,111	11,111
Standard 6	3,333	333	667	3,333	33,333
Standard 7	10,000	500	2,000	10,000	100,000

PYY: Peptide tyrosine tyrosine GIP: Gastric inhibitory polypeptide; GLP-1: Glucagon-like peptide; pg: picograms per milliliter

Immunoassay procedure

- Allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Diagram the placement of Standards [0 (Background), Standards 1-7, Controls 1 and 2, and Samples on Well Map Worksheet in a vertical configuration. (Note: Most instruments will only read the 96-well plate vertically by default.) It is recommended to run the assay in duplicate.
- set the filter plate on a plate holder at all times during reagent dispensing and incubation steps so that the bottom of the plate does not touch any surface.

- Proceed according to the following steps (Figure 5.4):

1. Add 200 µL of Assay Buffer into each well of the plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25°C).

2. Decant Assay Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.

3. Add 25 µL of each Standard or Control into the appropriate wells. Assay Buffer should be used for 0 pg/mL standard (Background).

4. Add 25 µL of Assay Buffer to the sample wells.

5. Add 25 µL of appropriate matrix solution to the background, standards, and control wells. Use the Serum Matrix provided in the kit.

6. Add 25 µL of Sample (neat) into the appropriate wells.

7. Vortex Mixing Bottle and add 25 µL of the Mixed Beads to each well. (Note: During addition of Beads, shake bead bottle intermittently to avoid settling.)

8. Seal the plate with a plate sealer. Wrap the plate with foil and incubate with agitation on a plate shaker for overnight incubation (16-18 hours) at 4°C.

9. Gently remove well contents and wash plate 3 times following instructions listed in the **Plate washing** section.

10. Add 50 µL of Detection Antibodies into each well. (Note: Allow the Detection Antibodies to warm to room temperature prior to addition.)

11. Seal, cover with foil and incubate with agitation on a plate shaker for 1 hour at room temperature (20-25°C). **DO NOT ASPIRATE AFTER INCUBATION.**

12. Add 50 µL Streptavidin-Phycoerythrin to each well containing the 50 µL of Detection Antibodies.

13. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).

14. Gently remove well contents and wash plate 3 times following instructions listed in the **Plate washing** section.

15. Add 100 µL of Sheath Fluid (or Drive Fluid if using MAGPIX®) to all wells. Resuspend the beads on a plate shaker for 5 minutes.

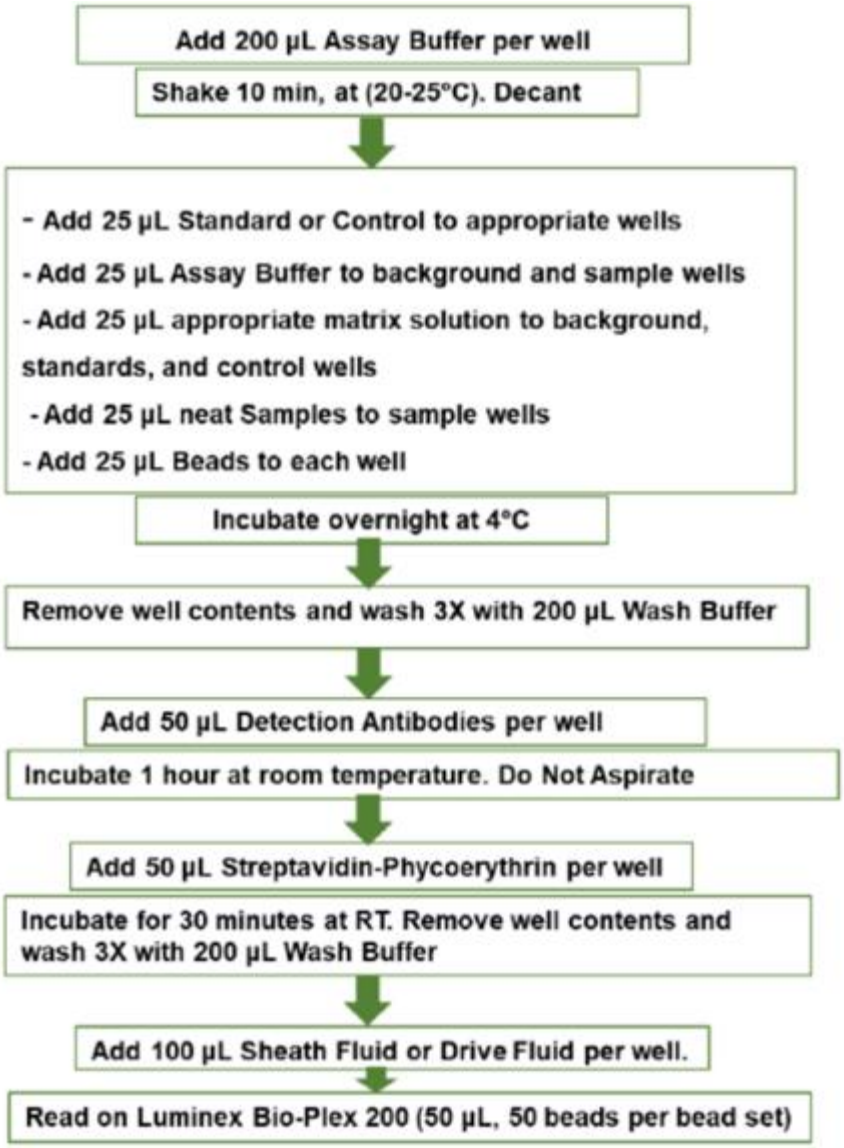
16. Run plate on Luminex® 200™, HTS, FLEXMAP 3D® or MAGPIX® with xPONENT® software.

17. Save and analyse the Median Fluorescent Intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method for calculating analyte concentrations in samples. (Note: For diluted samples, multiply the calculated concentration by the dilution factor.)

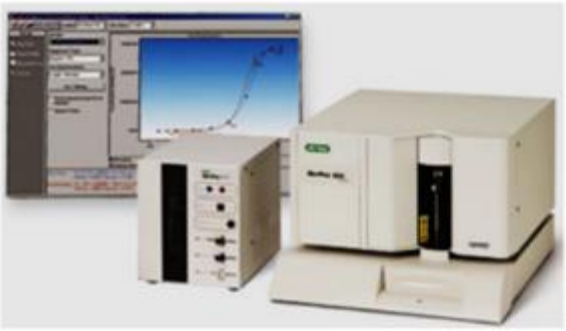
Plate washing

For a solid plate, use either a handheld magnet or magnetic plate washer.

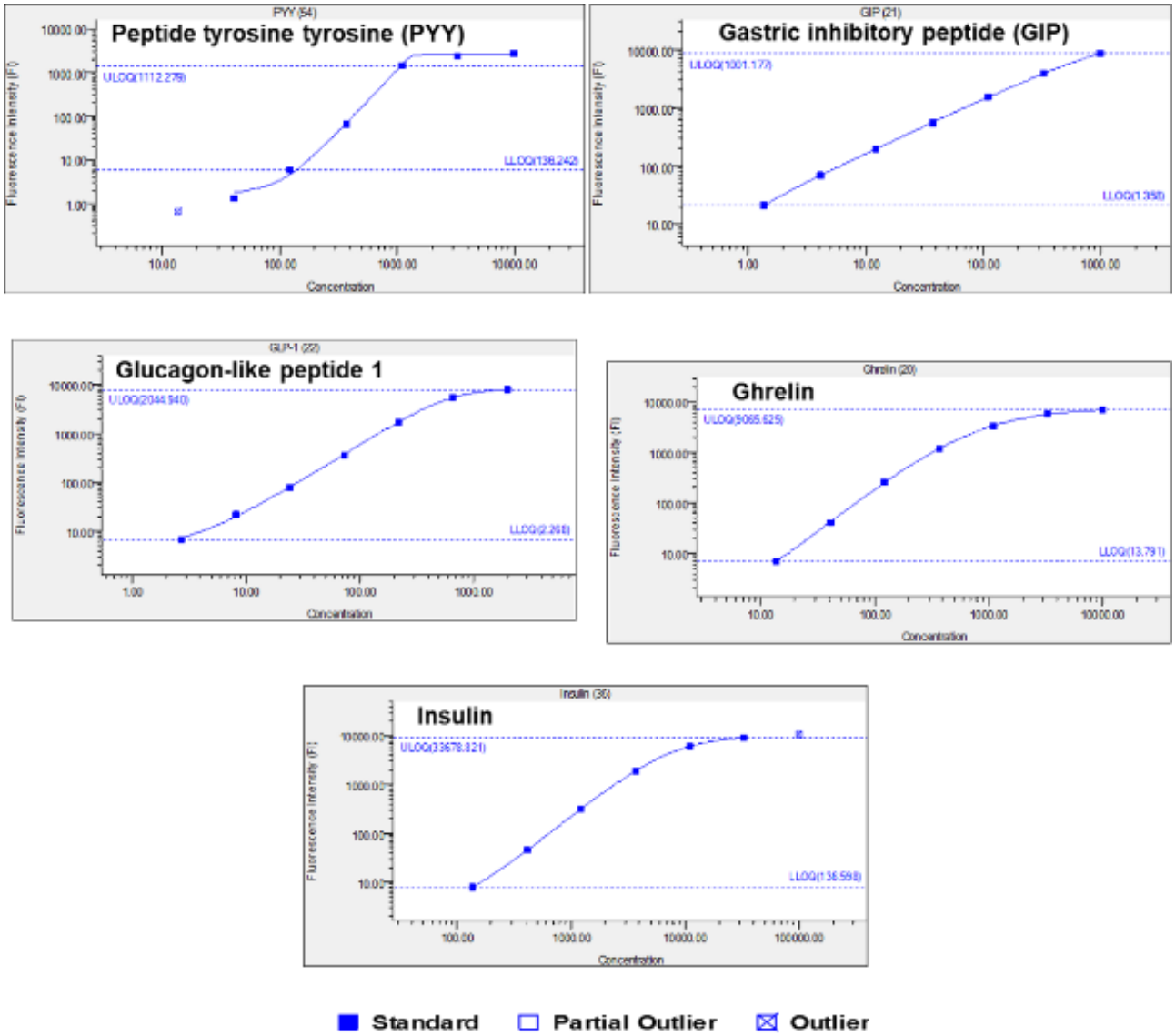
Handheld magnet (EMD Millipore Catalog # 40-285) - Rest plate on magnet for 60 seconds to allow complete settling of magnetic beads. Remove well contents by gently decanting the plate in an appropriate waste receptacle and gently tapping on absorbent pads to remove residual liquid. Wash plate with 200 µL of Wash Buffer by removing plate from magnet, adding Wash Buffer, shaking for 30 seconds, reattaching to magnet, letting beads settle for 60 seconds and removing well contents as previously described after each wash. Repeat wash steps as recommended in Assay Procedure.



Bio-Plex 200 Luminex system



Source: BIO-RAD http://www.bio-rad.com/en-uk/product/bio-plex-200-systems

Bio-Plex Manager™ version 6.1 software — controls the instrument, data acquisition, and data analysis for superior curve fitting, statistics reporting, and charting features.

Appendix 18 Standard curve for the investigated gut hormones