BMJ Open

Infant formula supplemented with milk fat globule membrane compared with standard infant formula for the cognitive development of healthy term-born formula-fed infants: protocol for a randomised controlled trial

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To cite: Gould JF, Gibson RA, Yelland LN, *et al*. Infant formula supplemented with milk fat globule membrane compared with standard infant formula for the cognitive development of healthy term-born formula-fed infants: protocol for a randomised controlled trial. *BMJ Open* 2024;**14**:e083399. doi:10.1136/bmjopen-2023-083399

► Additional supplemental material is published online only. To view, please visit the journal online(https://doi.org/10.1136/bmjopen-2023-083399).

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (https://doi.org/10.1136/bmjopen-2023-083399).

Received 19 December 2023
Accepted 13 June 2024

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ABSTRACT

Introduction Milk fat globule membrane (MFGM) is a complex lipid–protein structure in mammalian milk and human milk that is largely absent from breastmilk substitutes. The objective of this trial is to investigate whether providing infant formula enriched with MFGM versus standard infant formula improves cognitive development at 12 months of age in exclusively formula-fed full-term infants.

Methods and analysis This is a randomised, controlled, clinician-blinded, researcher-blinded and participant-blinded trial of two parallel formula-fed groups and a breastfed reference group that were recruited in the suburban Adelaide (Australia) community by a single study centre (a medical research institute). Healthy, exclusively formula-fed, singleton, term-born infants under 8 weeks of age were randomised to either an MFGM-supplemented formula (intervention) or standard infant formula (control) from enrolment until 12 months of age. The reference group was not provided with formula. The primary outcome is the Cognitive Scale of the Bayley Scales of Infant Development, Fourth Edition (Bayley-IV) at 12 months. Secondary outcomes are the Bayley-IV Cognitive Scale at 24 months, other Bayley-IV domains (language, motor, emotional and behavioural development) at 12 and 24 months of age, infant attention at 4 and 9 months of age, parent-rated language at 12 and 24 months of age, parent-rated development at 6 and 18 months of age as well as growth, tolerance and safety of the study formula. To ensure at least 80% power to detect a 5-point difference in the mean Bayley-IV cognitive score, >200 infants were recruited in each group.

Ethics and dissemination The Women's and Children Health Network Human Research Ethics Committee reviewed and approved the study (HREC/19/WCHN/140). Caregivers gave written informed consent prior to enrolling in the trial. Findings of this study will be disseminated through peer-reviewed publications and conference presentations.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is a robust randomised controlled trial of infant formula enriched with milk fat globule membrane for exclusively formula-fed infants.
⇒ Longitudinal assessments across early development are comprehensive.
⇒ Methods were adapted to coronavirus-related restrictions prior to trial commencement.
⇒ Loss to follow-up after enrolment into the trial may contribute to risk of attrition bias.

Trial registration number ACTRN12620000552987; Australian and New Zealand Clinical Trial Registry: anzctr.org.au.

INTRODUCTION

Exclusive breast milk is considered the ideal source of nutrition for infants up to 6 months of age with suggested benefits to general health,[1] allergies,[2] infection,[2] growth and body composition,[2] for infants who are breastfed compared with infants who are formula-fed. In particular, breastmilk appears to confer benefits to general neurodevelopment,[1 3] behaviour,[3] and cognitive abilities, as captured by intelligence tests.[2–5]

Breast milk contains a range of important nutrients for health and neurodevelopment[6–8] that differs to formula and may contribute to the cognitive and other benefits seen in breastfed infants.[1 2 4 5] Recent interest has been drawn to the milk fat globule membrane (MFGM), a structurally and compositionally complex membrane of bioactive compounds. MFGM is naturally occurring as the membrane of the fat globule

in breast milk but is only present in trace amounts in most infant formula where the fat source is mainly vegetable oils.[9] It has recently become possible to produce MFGM of sufficient quality and quantity to be included in infant formulas, and multiple trials have tested the effects of giving infants MFGM.[10–17 18–21] The results from these studies indicated that supplementation of bovine milk-derived MFGM was safe and may benefit cognition, however, results were mixed partially due to heterogeneity of intervention (timing, duration and inclusion of other components in combination with MFGM), outcomes and study quality (in relation to limitations such as small samples or MFGM intake of the control group).[10–21] Most noteworthy is that technician-administered tests have suggested benefits to cognitive development at 12[10] months of age in the most robust trial of 160 formula-fed term-born infants, although by 6 years of age, there were no group differences.[22] Additionally, this benefit to cognitive development was not detected in other trials with assessments at 2, 3, 4, 6, 12 or 18 months of age.[11–13]

We report the design of a new, large, robust trial with the aim to determine the effect of MFGM-supplemented formula compared with standard formula for exclusively formula-fed full-term infants from <2 months of age until 12 months of age on their cognitive development. We hypothesise that supplementing the formula of non-breastfed infants with MFGM will improve cognitive scores at 12 months of age compared with infants receiving standard infant formula.

METHODS AND ANALYSIS

Study design

The Infant nutrition with Milk fAt Globule membrane for INfant cognition in Early life (IMAGINE) trial is a double-blind randomised controlled trial of infants that are not receiving any breastmilk, and a non-randomised breastfed reference group. The IMAGINE Trial Coordinating Centre was the South Australian Health and Medical Research Institute (SAHMRI) Women and Kids theme, based at the Women's and Children's Hospital (Adelaide, South Australia). A purpose-built web-based (Research Electronic Data Capture; REDCap) management system is used to collect and store data.

Trial status

The IMAGINE trial recruitment began on 14 May 2020. As of November 2023, randomised and enrolment is complete with a total of 620 participants. Completion of participant follow-up assessments to 24 months of age is expected by 30 June 2024.

Participants and sample selection

Infants up to 2 months (60 days) of age were eligible to enrol.

Inclusion criteria

- Singleton birth.
- Full-term birth (37^{+0} to $<41^{+6}$ weeks).
- Appropriate birth weight (>5th and <95th percentile: boys ≥2604 and ≤4215 g, girls ≥2532 and ≤4041 g).[23]
- The legal caregiver was able to provide written informed consent.
- The caregiver agreed not to enrol the infant in another interventional study that may affect infant growth or development.
- The family lived within metropolitan Adelaide or was willing to travel to appointments.
- English was the primary language spoken at home.
- Formula-fed groups only
  - Exclusively formula fed at the time of screening (anytime up to 60 days of age), where exclusively formula fed was defined as no breastmilk for at least 24 hours prior to consent, and caregivers had no plans to retry breastfeeding.
  - No medically diagnosed allergy or intolerance to lactose, soy, fish or cow's milk protein.
- Breastfed reference group only
  - Infants were at least 52 days of age at enrolment
  - Infants were exclusively fed breastmilk at enrolment (52–60 days of age).
  - Caregivers planned to feed breastmilk until the infant was at least 12 months of age.

Exclusion criteria

- Severe congenital or metabolic disease, or congenital malformation, major birth defect or any other condition likely to interfere with
  - the ability to ingest food.
  - normal growth and development.
  - evaluation of the infant
- Hypoxic ischaemic encephalopathy.
- Birth mother had pre-existing type 1 diabetes.
- Known substance or alcohol misuse during pregnancy.
- Infant was immunocompromised (according to a doctor's diagnosis of immunodeficiency such as combined immunodeficiency's, DiGeorge syndrome, Wiskott-Aldrich syndrome, severe congenital neutropenia and secondary immunodeficiencies linked to HIV infection, Down syndrome or others).
- Already participating in an intervention study that may influence development.

Screening and enrolment

There was no advertising for the IMAGINE trial. Potentially eligible families were not informed about a formula study unless they had already confirmed their infant was exclusively formula-fed, or exclusively fed breastmilk. Families with potentially eligible infants were screened for the study through a state-wide community nurse service that performs routine health checks for all infants between 1 and 4 weeks of age in South Australia, and other SAHMRI research activities where infants feeding practices were already being collected (see figure 1). Families with singleton term-born infants that indicated exclusive formula feeding at the health check were

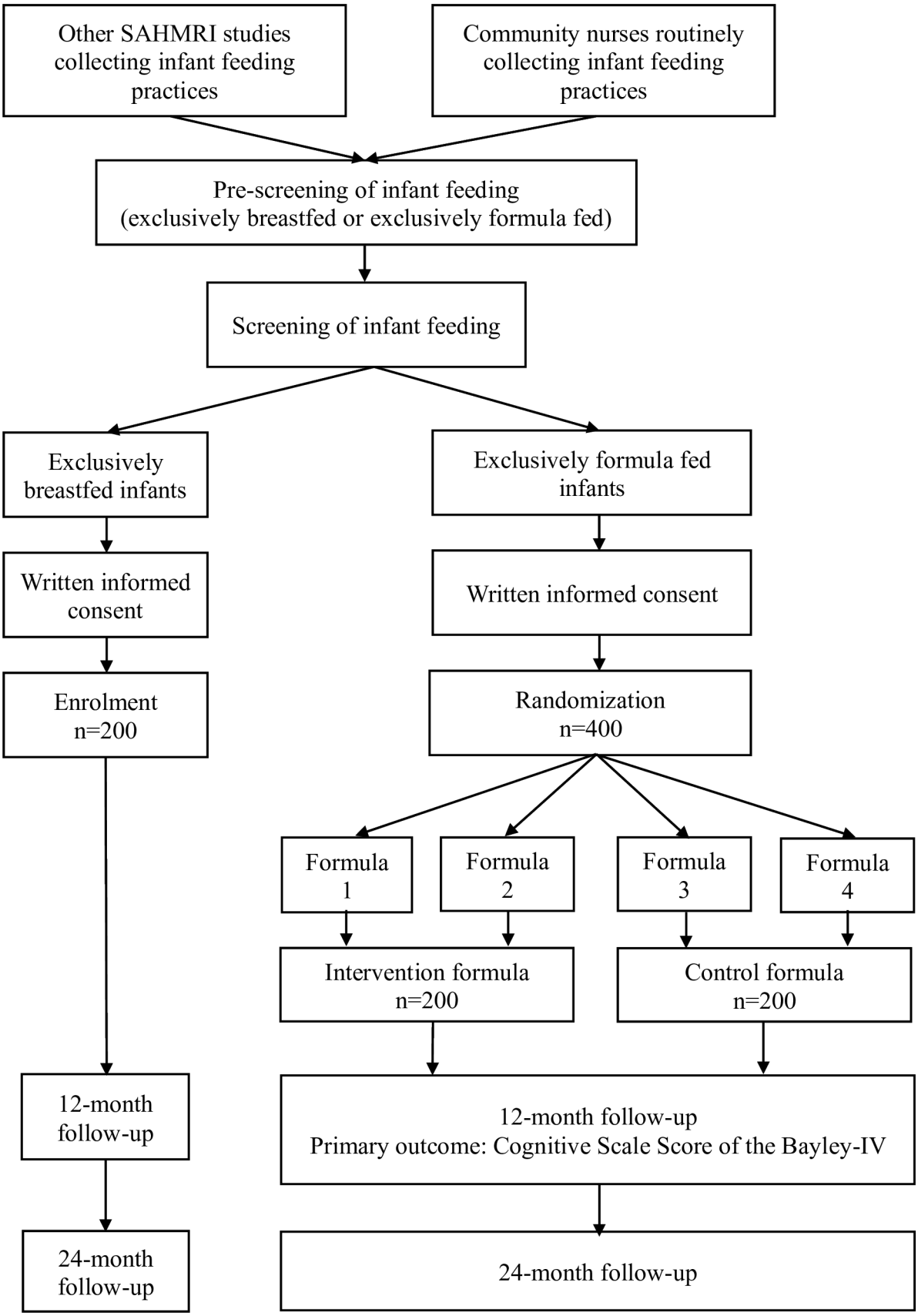

Figure 1 Participant flow from screening to enrolment and randomisation in the trial. SAHMRI, South Australian Health and Medical Research Institute.

subsequently called to confirm feeding practices, and if still exclusively formula feeing, they were asked if they would like to receive information about a SAHMRI study. Some SAHMRI Women and Kids studies collected infant feeding practices[24] and allowed screening for term-born singleton infants under 2 months of age that were either exclusively formula-fed (formula-fed groups) or exclusively fed breastmilk (reference group).

SAHMRI staff contacted potentially eligible families to determine plans for ongoing feeding practices (breastmilk and/or infant formula), complete further screening and then (if eligibility criteria were met) explain study details and send an information sheet for review (see the online supplemental file 1 for the formula-fed group and breastfed reference group Participant Information and Consent Forms). Caregivers were given time to read the study information and discuss the study with family members and healthcare providers before being contacted by SAHMRI staff to potentially book a virtual enrolment appointment. A legal caregiver provided written informed e-consent through Adobe Sign or the REDCap e-consent module, both of which could be used on a basic smartphone,[25] prior to enrolling. Infants remained in the study until they completed the 24-month assessment (study exit).

Randomisation and blinding

On enrolment, infants were allocated a unique participant identification code (study ID) in the REDCap management system. Formula-fed infants were randomised to one of four colour-coded formula groups using a computer-generated randomisation schedule using a balanced variable block design. Infant formula was provided to participants in cans with plain packaging that were identical with the exception that the colours of the labels differed (for the four coloured groups). Two of the colour groups received the intervention formula, and two of the colour groups received the control formula (total of four colour groups to ensure blinding). Randomisation was stratified by infant sex, and infant age at enrolment (<28 days or ≥28 days) with balanced variable blocks of size 4 and 8. Both participants, their caregivers and all study staff were blinded to randomised allocation. The randomisation list was held by an independent statistician who could see which colour formula the infant was assigned to. Two custodians that did not have any contact with the study participants or have access to the data collected for the study held the randomisation list, with the unblinded group allocation. In the event of a medical or other emergency in which knowledge of group allocation was necessary, the custodians were available to provide emergency unblinding to the treating physician and the family.

The reference group of breastfed infants was not randomised or blinded to being the reference group.

Study intervention

Randomised infants received study formula manufactured and provided by Fonterra Co-Operative Group Limited (New Zealand) from within a week of enrolment (≤2 months of age) until 12 months of age. The study formulas were cow's milk based and were matched for energy, fats, proteins, carbohydrates and key nutrients, except for MFGM (see table 1). The intervention formula contained a minimum ganglioside concentration of 17.9 mg/100 g, manufactured using bovine MFGM-rich ingredient as a source of gangliosides and milk phospholipids (MFGM Lipid 100; NZMP, Fonterra) from anhydrous milk fat production. The control formula was manufactured with the same macro and micronutrient composition but without the MFGM-enriched ingredient. Intervention and control study formulas were in powdered form to be reconstituted to deliver 66 kcal per 100 mL.

Study formulas were delivered to participants' residences packed in 900 g cans labelled with clear instructions for preparing the formula. Frequency and dose of study formula feeding were ad libitum at the discretion of the caregiver. Caregivers could request regular deliveries of formula as needed and study staff enquired about formula delivery requirements at each study contact before 12 months. Caregivers were instructed to feed study formula as the sole source of infant formula through to 12 months of age, unless otherwise instructed by their physician. As per the Australian National Health and Medical Research Council guidelines for infant feeding,[26] caregivers of infants in the breastfed reference group were encouraged to feed breastmilk as the sole source of milk until 6 months of age and to continue breastfeeding until the infant was 12 months. Reference group families were offered appointments with a lactation consultant as needed prior to the infants reaching 6 months of age but were not provided with study product, regardless of timing of breastfeeding cessation. As per Australian guidelines, caregivers of all infants (randomised and reference groups) were advised to introduce complementary feeding around 6 months of age,[26] unless otherwise instructed by their physician.

Outcomes and measures

At enrolment, caregivers underwent a virtual appointment, and then received phone calls 4, 7, 14 and 21 days after enrolment, completed surveys and were invited to attend study visits (see figure 2 for ages and timelines of study contact points). Families were invited to attend face-to-face appointments when the infant was 4, 9, 12 and 24 months of age. As the face-to-face assessment at 4 and 9 months of age was conducted to collect an exploratory, secondary outcome, appointments were conducted virtually (without the face-to-face component of the assessment) if caregivers preferred. Online questionnaires were emailed or texted (dependent on caregiver preference) to families when the infant turned 3, 5, 6, 12, 18 and 24 months of age. See online supplemental table for a summary of study milestones and information collected. Infants in the formula-fed groups and the reference group underwent all assessments, with the exception that formula-fed groups were not asked about breastfeeding,

Table 1 Nutrient composition of study formulae

		Intervention formula		Control formula	
		Per 100 g	Per 100 mL	Per 100 g	Per 100 mL
Energy	kJ	2091.0	276.0	2091.0	276.0
Energy**	cal	500	66.0	500	66.0
Protein	g	10.9	1.4	10.9	1.4
Fat, total	g	26.52	3.5	26.52	3.5
Linoleic acid	mg	4807	635	4807	635
α-linolenic acid	mg	427	56	427	56
Docosahexaenoic acid (DHA)	mg	60	8	60	8
Arachidonic acid (ARA)	mg	76	10	76	10
Carbohydrate	g	54.4	7.2	54.4	7.2
Calcium	mg	370	49	370	49
Phosphorus	mg	210	28	210	28
Sodium	mg	142	19	142	19
Potassium	mg	560	74	560	74
Chloride	mg	350	46	350	46
Magnesium	mg	50	6.6	50	6.6
Iron	mg	6	0.8	6	0.8
Zinc	mg	4.6	0.61	4.6	0.61
Copper	µg	410	54	410	54
Manganese	µg	80	11	80	11
Iodine	µg	90	12	90	12
Selenium	µg	15	2	15	2
Vitamin A	µg-RE	500	66	500	66
Vitamin D3	µg	7.1	0.94	7.1	0.94
Vitamin E (α-Tocopherol acetate)	mg α-TE	9	1.2	9	1.2
Vitamin K1	µg	50	6.6	50	6.6
Thiamin (vitamin B1)	µg	500	66	500	66
Riboflavin (vitamin B2)	µg	1000	132	1000	132
Niacin (vitamin B3)	µg	4000	528	4000	528
Pantothenic acid (vitamin B5)	µg	3000	396	3000	396
Vitamin B6	µg	350	46	350	46
Biotin	µg	15	2	15	2
Folic acid	µg	85	11	85	11
Vitamin B12	µg	1.7	0.23	1.7	0.23
Vitamin C	mg	81.2	10.7	81.2	10.7
Choline	mg	100	13	100	13
Taurine	mg	40	5.3	40	5.3
L-carnitine	mg	10	1.3	10	1.3
Inositol	mg	51.7	6.8	51.7	6.8
Nucleotides	mg	19.7	2.6	19.7	2.6
Galacto-oligosaccharide (GOS)	g	4.1	0.54	4.1	0.54

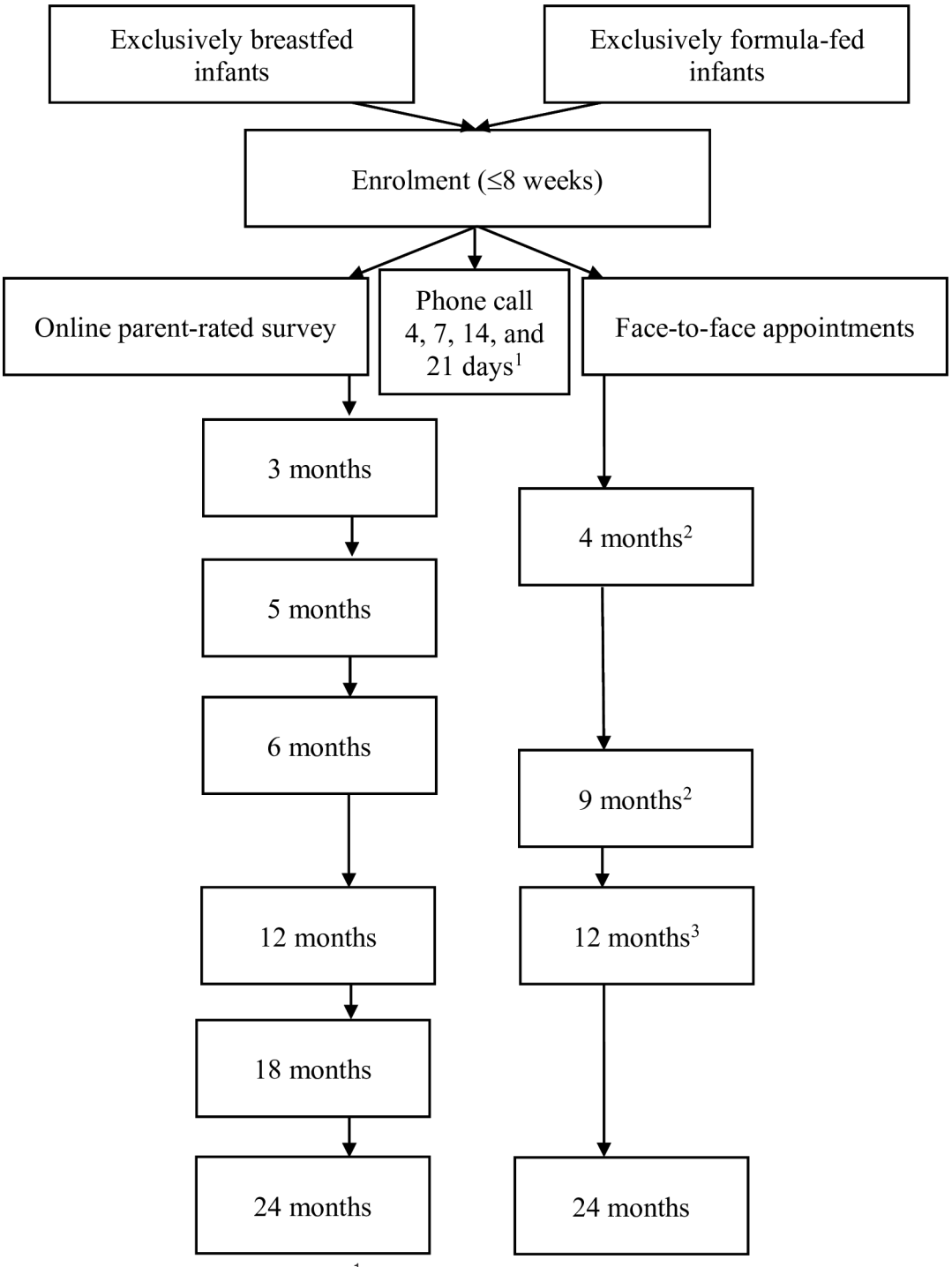

Figure 2 Age of participants at study milestones. [1]Timing of phone calls are based on days since enrolment, therefore infant age at phone calls varied. [2]Optional face-to-face visit or virtual appointment. [3]Primary outcome collected at 12-month appointment.

and the reference group was not asked about use of study formula.

Primary outcome: cognition at 12 months

The primary outcome is infant cognition at 12 months of age, as assessed with the Cognitive Scale score of the Bayley Scales of Infant and Toddler Development, Fourth Edition, Australian and New Zealand (Bayley-IV). The Cognitive Scale score evaluates sensorimotor development, exploration, manipulation, object relatedness, concept formation, memory and simple problem-solving.[27] The Cognitive Scale score was age standardised to a mean of 100 and SD of 15 and can be considered an early indicator of IQ. Scores range from 45 to 155 where higher scores reflect more advanced performance, and development can be classified as normal (85–115), impaired (<85) or accelerated (>115). The Cognitive

Scale is administered at a face-to-face appointment by a psychologist, trained health professional or trained technician. Appointments were conducted at clinic rooms at the Women's and Children's Hospital, SAHMRI or the family home, depending on the preference of the family.

Secondary outcomes assessed by study staff

Other Bayley-IV Subscales

The Bayley-IV includes assessor-administered Language and Motor Scales Scores, in addition to the Cognitive Scale.[27] The Language Scale is a composite of receptive communication (verbal comprehension, vocabulary) and expressive communication (babbling, gesturing and utterances) abilities. The Motor Scale evaluates both gross (big, large movements such as rolling over or walking) and fine (small movements and manipulations using small muscles, such as the fingers or lips) motor functioning. All scales are administered when children reached 12 and 24 months of age and scores are age standardised.

Attention

The attention assessments required a face-to-face appointment at the hospital clinic room due to the equipment needed. The assessments were an exploratory outcome (to be published separately from the primary results paper) and, therefore, optional for family's willing and able to attend, ideally within ±3 days of the infant turning 4 months of age and within ±7 days of the infant reaching 9 months of age. Assessments were conducted in a plain quiet clinic room according to a standard operating procedure.[28–30] Infants were seated on a caregiver's lap or in a highchair in front of a computer monitor while their face was video recorded and heart rate was monitored.

The first task assessed visual habituation to repeatedly presented stimuli. Habituation is a longstanding and widely accepted measure of non-associative learning, and the visual habituation task assessed the relative speed with which infants encoded a visual stimulus. Infants were presented with a single stimulus (a woman's face[30 31] over multiple trials interspersed with a blank screen while their attention to the stimulus was coded live).[28] Infant fixation (looking) declines with repeated exposure due to information processing or encoding (ie, learning). Once looking had declined to a predetermined threshold, the infant was considered 'habituated' to the stimulus. Subsequently, the infant was presented with the habituated stimulus and a novel stimulus (another woman's face[30] simultaneously paired). Infants who had successfully encoded the initial (ie, habituated) stimulus look preferentially at the novel stimulus. Along with assessing the learning that has occurred during habituation, the preference for the novel stimulus serves as an indication of recognition memory. The heart rate recording was synchronised with presentation of the stimulus and the video recording of infant looks to and from the stimulus to parse specific heart rate-defined phases of attention; orienting (initial look at the stimulus, prior to stable deceleration of the heart rate), sustained attention (heart rate deceleration, reflecting encoding or processing of the stimulus) and attention termination (looking at the stimulus but disengaging, heart rate deceleration has ended).[28 32–34] Outcomes for comparison were fixation duration, novelty preference, heart rate and proportion of looking spent in the different phases of attention.[28]

The second task assessed ability to disengage attention or visual reaction time.[35–37] The infant was presented with a stimulus (small colourful shape) in the middle of the monitor until their attention had been drawn to the centre of the screen. Another similar stimulus is then presented in the periphery of the infant's visual field either after the withdrawal of centre stimulus (ie, there was a 'gap' between the central stimulus' disappearance and the presentation of the peripheral stimulus) or prior to the withdrawal of the centre stimulus (ie, the two stimuli 'overlap'). The main outcome for comparison was the latency for the infant to initiate a correct shift in gaze from the initial stimulus location to the peripheral stimulus in the gap and overlap conditions.

Growth

Length (or height), weight and head circumference are assessed at 4, 9, 12 and 24 months of age. Where study staff are unable to conduct assessments, measurements taken by healthcare professionals (from routine check-ups or vaccination clinics) are obtained. Measurements will be converted to Z (SD) scores appropriate for age and sex.[38] Growth measurements at each time point as well as trajectory of growth across the study will be compared between the groups.

Secondary outcomes reported by parent-completed questionnaires or interview

Bayley-IV

The Bayley-IV Social-Emotional and Adaptive Behaviour Scales are administered when the infant is 12 and 24 months of age. The Social-Emotional Scale assesses milestones such as engagement and use of a range of emotions, experiences and expressions. The Adaptive Behaviour Scale captures self-direction abilities such as learning to feed oneself, crawling, walking and toileting, and communicating for basic needs.[27]

Ages and Stages Questionnaire, Third Edition

The Ages and Stages Questionnaire, Third Edition (ASQ-3) ASQ-3 is a screen of early developmental milestones in five domains: communication skills, gross motor skills, fine motor skills, problem-solving skills and personal-social development.[39] The ASQ-3 was administered as infants turned 6 and 18 months of age.

MacArthur-Bates Communicative Development Inventory, Third Edition

The MacArthur-Bates Communicative Development Inventory, Third Edition (MCDI-3) is a well-validated and standardised parent-reported measure of early verbal and non-verbal communication.[40] The Words and

Gestures form was administered at 12 months to capture words understood and spoken as well as gestures used to communicate. The Words and Sentences form is used at 24 months of age to assess words produced and how they were used, early grammar and words understood. The MCDI-3 includes an online scoring programme with age-standardised norms. We modified some words for use in an Australian sample (eg, 'coin' rather than 'penny' and 'mummy' rather than 'mommy').[40]

Feeding information and compliance

Feeding practices were collected at each call, survey and visit up to and including the visit at 12 months of age to determine compliance and identify group imbalances. Ongoing use of study formula, non-study formula, introduction of complementary feeds and use of dietary supplements were collected. Caregivers were asked how many bottles of study formula their infant had been provided in the previous 3 days, how much made-up formula was generally in each bottle and how much of the bottle was typically discarded (left over-after feeding). Reasons for and number of days of providing non-study formula (to the formula-fed or breastfed groups) during the intervention period were asked, as was the date and reason for ceasing study formula or breastfeeding. If non-study formula was provided to an infant from the formula-fed groups, or if any formula was fed to infants in the breastfed group, the formula was recorded as being either cows' milk based, soy based or hypoallergenic.

Infants in both the formula-fed groups and breastfed reference group were considered compliant if the number of non-compliant days (days where one or more feeds are non-study formula, respectively) was <10% between study commencement (7 days after enrolment) and 6 months of age.

Illness and serious adverse events

At each visit and survey, caregivers are asked to list consultations (visits or phone calls) with a healthcare professional (eg, a doctor, community nurse, hospital or parent helpline), symptoms that prompted the consultation, and any medications prescribed as well as any fevers (not in relation to a vaccination). At 24 months, caregivers provide neurological or chronic medical conditions the infant had been diagnosed with (for example, cerebral palsy or allergies). Potential serious adverse events (SAEs) are confirmed through review of infant medical records, and were defined as any one of the following

- Requirement of inpatient hospitalisation for 6 or more hours or prolongation of existing hospitalisation.
- Persistent or significant disability/incapacity.
- Life-threatening condition.
- Death.

Tolerance

At each visit and survey during the intervention, caregivers were asked about spilling and vomiting after feeding, fussiness and crying, faecal characteristics (rated with a Bristol Stool Chart,[41] and sleeping patterns to indicate tolerance). Formula-fed group caregivers were also asked about use of thickeners or other additives when preparing the study formula.

Background information and characteristics

Characteristics were collected to describe the sample, identify possible randomisation group imbalances and for inclusion of prespecified covariates in analysis models. At enrolment, a range of parental and infant sociodemographic and health information was collected through scripted interview. Where possible, caregivers provided their infants' health and development booklet (a hard copy booklet given to infants for health professionals to record health information) for verification. Recorded maternal information included age, country of birth, cultural ethnicity, education, working status in the previous 12 months, height, weight and prepregnancy weight. If the primary carer was not the biological mother, we collected information about education and working status in the last 12 months. Pregnancy and neonatal characteristics collected included prenatal smoking or alcohol use, mode of delivery, parity, anthropometrics at birth, neonatal illness or medical conditions, type of first feed, feeds while in hospital, any exclusive breastfeeding, alcohol use while breastfeeding and use of infant dietary supplements or thickeners prior to enrolment.

Stimulation within the home environment has an important role in the cognitive, social and emotional developmental outcomes of infants and is measured with a revised version of the Home Screening Questionnaire[42] at 12 and 24 months of age. Childcare and number of adults and children living with the infant may be considered when comparing neurodevelopment outcomes or communicable illnesses.

Sample size

A sample size of 143 infants per formula-fed group (total 286) provides 80% power (two-tailed alpha 0.05) to detect a 5-point mean difference in the primary outcome of 12-month Bayley-IV Cognitive Scale between the randomised formula-fed groups. A 5-point difference was chosen as the minimum clinically important difference as it is similar in magnitude to the effect sizes that led to iron fortification of infant cereals,[43] and the removal of lead from petrol and the environment.[44] The sample size calculation is based on the Bayley-IV scales having a mean of 100 and SD of 15 in the general population at any time point. In the term-born, healthy population eligible for this study, the SD is likely to be lower (closer to 13,[45] which would mean a sample size of 144 per formula-fed group confers 90% power to detect a 5-point mean difference in the Cognitive Scale). A sample size target of 200 infants randomised per group allows up to 28% loss to follow-up.

As a secondary analysis, there will be a three-way comparison between the two randomised formula-fed groups and the breastfed reference group. Pairwise comparisons involving the reference group are powered

to detect a similar difference (90% power for 5 points on the Bayley-IV Cognitive Scale, SD of 13, two-tailed α=0.05 for each comparison) to the primary outcome comparison.

Data management and analysis plan

Data are entered into REDCap and hosted on SAHMRI's secure servers. REDCap uses a MySQL database via a secure web interface with data checks during data entry to ensure quality. REDCap includes a complete suite of features to support compliance with the Health Insurance Portability and Accountability Act of 1996, including a full audit trail, user-based privileges and integration with the institutional LDAP server.

All analyses will be conducted according to a prespecified statistical analysis plan[46] approved by the IMAGINE Steering Committee. Analyses will not commence until completion of data collection and blinded data cleaning, and the database is locked. All participants will be analysed according to the group into which they were randomised (intention-to-treat principle) in the primary analyses, regardless of compliance. Blinded treatment group codes (eg, group A or B) will be made available to the trial statistician and analyses will be performed using these group codes to allow analyses to be performed blinded to treatment group. Blinding will be broken after the results have been reviewed by the investigators.

Differences between the two randomised groups on outcomes measured at a single time point will be assessed using linear regression models for continuous outcomes, and log binomial regression models for binary outcomes and negative binomial regression for count outcomes. Differences between the two randomised groups on outcomes measured over time will be assessed using linear mixed effects models for continuous outcomes and log binomial models with generalised estimating equations for binary outcomes. The models will include terms for randomised group, time (treated as categorical due to measurements being collected at a small number of specific timepoints) and the interaction between group and time, with adjustment for randomisation strata (infant sex and infant age at enrolment) in adjusted analyses. Both adjusted and unadjusted analyses will be performed, with the adjusted results used to draw conclusions about the effect of the intervention on the outcomes of interest. Results will be presented as differences in means for continuous outcomes, or relative risks for binary outcomes, or incidence rate ratios for count outcomes at each time point where applicable, with 95% CIs and two-sided p values. Statistical significance will be assessed at the 5% level and no adjustment will be made for the number of analyses planned, as a single primary outcome has been pre-specified for the study. No interim analyses are planned.

Secondary per-protocol analyses will be conducted for the primary outcome and secondary outcomes from the Bayley-IV assessments and will include all participants consented into the study with no major protocol violations and adequate compliance (<10% non-compliant days, with additional secondary analyses performed for <15% and <25% non-compliant days).

Comparisons involving the breastfed reference group will be based on the same statistical models as described above, but with additional adjustment for prespecified potential confounders. A global test will be performed for the effect of group (intervention, control or reference) followed by post hoc pairwise comparisons with no adjustment for multiple comparisons due to the exploratory nature of these analyses. Only pairwise comparisons involving the reference group (ie, intervention vs reference group, and control vs reference group) will be reported from these models.

Data collected on participants up to the point of withdrawal or loss to follow-up will be included in the analysis. Where data are missing, multiple imputation will be used to create 100 complete datasets for analysis of the primary and secondary Bayley-IV related outcomes using the fully conditional specification method, performed separately by treatment group.[47] Imputed datasets will include all surviving infants. Infants who were missing scores on psychological assessments because they were unable to complete the assessment for neurological reasons will be reviewed by the Chief Investigator and the Clinical Psychologist (blinded to treatment group) to determine whether assigning the lowest possible score is appropriate. All analyses will be performed blinded on both the raw and imputed data, with conclusions to be drawn based on the results using the imputed data.

Data monitoring and safety

The Steering Committee met monthly to monitor participants screened, enrolled/randomised, visits due/overdue/missed, adverse and SAEs), tolerance and compliance, product inventory and participant communication logs. An external monitor reviewed and verified all study documents and procedures as well as all signed consent forms, and a random selection of full participant records according to a predefined monitoring plan. A neonatologist reviewed all SAEs as well as monthly reports of illnesses and tolerance throughout the trial.

A Data Safety and Monitoring Committee comprising a paediatrician, a clinical researcher, and a biostatistician that were not otherwise involved in the study followed a formal charter to safeguard the interests of participants. The committee met quarterly to review adverse event data, SAEs as well as the indicators of dietary tolerance, growth and general well-being of the study groups to determine likelihood that involvement in the trial could have contributed to an event, and recommend whether or not to continue the trial.

Patient and public involvement

Neither patients nor the public were directly involved in the development of the research question or design of this study. Prior to the commencement of study recruitment, there was a COVID-19 outbreak, that necessitated

reducing face-to-face contact to minimise opportunities to spread the virus. A Community Board advisory group, comprising parents as well as clinicians and researchers specialising in paediatrics, was consulted on the revision of planned study procedures to safely conduct research activities during the COVID-19 outbreak.[25]

Ethics and dissemination

The IMAGINE trial is being conducted in accordance with the Australian National Statement on Ethical Conduct in Research Involving Humans which builds on the ethical codes of the Declaration of Helsinki and the Principles of International Conference on Harmonisation Good Clinical Practice (as adopted in Australia). The study product and protocol were reviewed and approved by the Drug and Therapeutics Committee Clinical Trials Group (subcommittee of the WCHN Drug and Therapeutics Committee, approval date 17/10/2019). All study procedures and materials were reviewed and approved by the Women's and Children's Health Network Human Research Ethics Committee (approval No. HREC/19/WCHN/140, approval date: 22/11/2019) as well as the research governance officer at the Women's and Children's Hospital prior to commencement. Any change to the protocol, informed consent form or other study materials or procedures were considered amendments and were submitted to the governing ethics committee for approval prior to becoming effective. Any protocol deviations were noted in participant's records and were discussed by the steering committee.

The IMAGINE Trial is registered on the Australia and New Zealand Clinical Trial Registry (ANZCTR: ACTRN12620000552987) and the World Health Organisation International Clinical Trials Registry Platform (Universal Trial Number U1111-1250-1917).

Caregivers were provided with a participant information sheet about the IMAGINE trial and provided written informed consent for their infant's involvement prior to enrolment. Caregivers were free to renegotiate consent for each procedure in the study and could decline any part of the study, pause involvement in the study or withdraw their infants from the study at any time either verbally or in writing. If participants were non-compliant (in either the formula groups or the breastfeeding group), caregivers were encouraged and supported to remain in the study.

Caregivers were offered AUD$40 (6 and 9 months) or AUD$60 (12 and 24 months) to reimburse travel costs for attending clinical appointments. Families that relocated away from the study site were reimbursed for travelling from their residence to the clinic as per the concurrent Australian Government Tax Office cents per kilometre rate,[48] and in some circumstances, a night of accommodation or return flights were provided to allow attendance at the 12 and 24-month assessments.

This protocol and the results of this study will be published in peer-reviewed journals. Primary and key secondary outcomes related to neurodevelopment, tolerance and safety will form the main results paper. Publication of information related to this study in formats including, but not limited to, conference abstracts, posters or presentations, seminars, journal articles, public reports, social media and internet postings. No participants will be identified in the dissemination of study results and data collected are treated with confidence. Participating families who have not withdrawn will receive a lay report of the study findings.

Access to data

Individual participant data, including data dictionaries, may be shared after deidentification on reasonable request. Proposals to access the data must be scientifically and methodologically sound and must be reviewed and approved by the IMAGINE trial Steering Committee, the Study Sponsor and the Women's and Children's Human Research Ethics Committee. To gain access, data requestors need to sign a data access agreement. Proposals should be directed to Chair of the Steering Committee, Jacqueline Gould through email (Jacqueline.gould@sahmri.com).

DISCUSSION

This protocol details a trial of MFGM-enriched formula compared with a standard infant formula without added MFGM for formula-fed infants to determine the effect on cognition. As is the gold standard for formula trials, we included an exclusively breastfed reference group that received no study formula even if breastfeeding ceased.

We carefully selected a comprehensive series of global and specialised assessments of early cognitive development. Our primary outcome is assessed with the Bayley Scales of Infant Development (now fourth edition),[27] a well-established test of early development and the most widely used assessment of early development internationally. Specialised assessments have more recently become of interest to nutrition researchers as they target specific abilities during infancy and toddlerhood that may be more sensitive to a nutrient intervention in a generally healthy, nutritionally replete population.[49–51] Additional outcomes included health, growth and tolerance, as per guidelines set out by European Society for Paediatric Gastroenterology Hepatology and Nutrition for collecting core data in nutrition trials in infants.[52] All assessments were conducted by trained research staff according to standard operating procedures. Study procedures were documented and published on a trial register in line with the recommendations for conducting and reporting clinical trials.[53–59]

The sample size for this trial is large, based on the power calculation for the primary outcome and allowing for some loss to follow-up, although loss of contact with study families will still contribute to attrition bias. While the formula-fed groups were double-blinded to group allocation, the breastfed reference

group was not blinded to their group status or to the study aim of investigating the effect of formula with MFGM. At the time of designing the IMAGINE trial, face-to-face appointments were planned for enrolment, 4, 9, 12 and 24 months of age. However, prior to commencing, the COVID-19 became a worldwide pandemic and the Australian government mandated restrictions on non-essential face-to-face contact, particularly in the healthcare setting, from February 2020 for >7 months. Appointments were adapted to be conducted virtually, requiring adoption of e-consent, interviews via phone and postage of study formula to avoid the potential to spread the virus. Due to the need for remote data collection, infant growth measurements that were intended to be conducted at each appointment by trained study staff using calibrated equipment[25] were not possible. Instead, growth measurements conducted by healthcare professionals at routine check-ups (where available) were reported by caregivers and may not be performed as consistently as planned or may be missing if the infant has not attended a routine health check. Outcomes that necessitated a face-to-face appointment required face masks were occasionally missed (attention assessments) or postponed (Bayley-IV). Although the attention assessments were an optional outcome, the impact of the COVID-19 restrictions increased the amount of missing data for this assessment and reduced the available sample size. Overall, some adaptations were advantageous by allowing remote data collection and preferred by participants, while others may have contributed to a larger amount of missing data or wider age range at outcome assessments.[25] Importantly, IMAGINE commenced after the COVID-19 outbreak, so that study procedures were consistent throughout the study.

Australian guidelines recommended that infants are exclusively breastfed until 6 months, with ongoing breastfeeding to 12 months or beyond.[26 60] Although breastfeeding initiation is high in Australia, exclusive breastfeeding and ongoing breastfeeding drops rapidly, so that by 6 months more than half of infants are fed formula, and by 12 months more than 80% are.[61] It is important that breastmilk substitutes are nutritionally replete, and results of this trial will provide robust evidence for the effect of including MFGM in infant formula.

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Acknowledgements We would like to thank the families who are generously participating in the IMAGINE trial, the IMAGINE Trial Data Safety and Monitoring Committee (paediatrician Prof Cameron Grant, clinical researcher Lynley Drummond, and biostatistician Barbara Kuhn-Sherlock), as well as biostatistician Dr Thomas Sullivan, the IMAGINE Trial Steering Committee (Gould, Makrides, Yelland, McPhee), Investigative Team (Gould, Makrides, Yelland, McPhee, Gibson, Colombo, Roberts, Shaddy, Bednarz), and research staff, particularly co-ordinators Kimberly Hamlyn, Lauren Williams, Aya Gharram, Eleanor Gardner and Beth Kean.

Contributors JFG, MM, AJM, JC, SG and RAG conceived the study and proposed the trial design. JFG, MM, JC, SG and RAG obtained funding. LNY and JB designed the analyses and performed the sample size calculations. JFG, MM, LNY, DJS and JC drafted the protocol. JFG, MM, AJM, JC, SG, LNY, JB, RMR, DJS and RAG contributed to the refinement of the study protocol manuscript and approved the final manuscript. JFG is the guarantor of the manuscript.

Funding The project was funded by the sponsor, Fonterra Co-Operative Group Limited, Auckland, New Zealand, and funding is paid to the South Australian Health and Medical Research Institute. MM is supported by an Australian National Health and Medical Research Council Principal Research Fellowship ID: GNT 2016756. Study products are provided by Fonterra Co-Operative Group Limited. The Australian National Health and Medical Research Council has no role in the study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication and has no authority over any of these activities.

Competing interests The project was funded by the sponsor, Fonterra Co-Operative Group Limited, Auckland, New Zealand, and funding was paid to the South Australian Health and Medical Research Institute. MM is supported by an Australian National Health and Medical Research Council Principal Research Fellowship ID: GNT 2016756. Study products were provided by Fonterra Co-Operative Group Limited. The Australian National Health and Medical Research Council has no role in the study design; collection, management, analysis and interpretation of data; writing of the report; and the decision to submit the report for publication and have no authority over any of these activities. Honoraria have been paid to Dr Gould's institution to support conference travel by NuMega Ingredients. No other competing interests are declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval All study procedures and materials were reviewed and approved by the Women's and Children's Health Network Human Research Ethics Committee (approval No. HREC/19/WCHN/140, approval date: 22/11/2019) as well as the research governance officer at the Women's and Children's Hospital prior to commencement.

Provenance and peer review Not commissioned; externally peer-reviewed.

Data availability statement Individual participant data, including data dictionaries, may be shared after de-identification upon reasonable request. Proposals to access the data must be scientifically and methodologically sound and must be reviewed and approved by the IMAGINE trial Steering Committee, the Study Sponsor, and the Women's and Children's Human Research Ethics Committee. To gain access, data requestors need to sign a data access agreement. Proposals should be directed to Chair of the Steering Committee, Jacqueline Gould through email (Jacqueline.gould@sahmri.com).

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work; Participant Information and Consent Form for (1.) the Formula-feeding group and (2.) the Breastfeeding group

Supplement to:

Infant formula supplemented with milk-fat-globule membrane compared with standard infant formula for the cognitive development of healthy term-born formula-fed infants; a protocol for a randomised controlled trial

Participant Information Sheet/Consent Form – Parent/Guardian

Formula Feeding Group

Women's & Children's Hospital, North Adelaide, South Australia

Lay Title	Infant nutrition with milk fat globule membrane for infant cognition in early life
Short Title	Infant Feeding Study
Protocol Number	3.0
Project Sponsor	Fonterra Cooperative Group Limited
Principal Investigator	Professor Maria Makrides (SAHMRI Women and Kids)
Local Principal Investigator	Dr Andrew McPhee and Dr Jacqueline Gould (SAHMRI Women and Kids) (Chief Investigator)
Associate Investigator(s)	Professor Robert Gibson, Dr Lisa Yelland (SAHMRI Women and Kids) and Professor John Colombo (University of Kansas, USA)

1. Introduction

The first year of life is an important time for the way a baby's brain grows and develops. This growth and development are partly influenced by a nurturing environment and partly by the quality of nutrition. The nutrition needed for optimal development are met via breastmilk, baby formula, or a mixture of both. One of the complex nutrients found in breastmilk is a mix of protein and fat called the milk fat globule membrane (MFGM). Although standard baby formula is a good substitute for breastmilk, it contains only trace amounts of MFGM.

The purpose of this study is to determine whether supplementing baby formula with additional MFGM is beneficial to the way healthy babies develop. To do this, we plan to compare the growth and development of babies that are fed breastmilk, standard baby formula or a formula supplemented with additional MFGM.

Approximately 600 babies will be enrolled in this study; 200 in the standard infant formula group, 200 in the MFGM-supplemented infant formula group and 200 in the breast-fed reference group. This study is being conducted by SAHMRI in South Australian Hospitals and in the community through the Child and Family Health Service.

This Participant Information Sheet/Consent Form tells you about the study. Please read this information carefully. Ask questions about anything that you don't understand or would like to know more about.

2. What is the purpose of this research?

Sometimes we don't know which treatment is best and to find out, we need to compare different treatments. It is known that MFGM is present in breastmilk and may play a role in both brain and immune development. We would like to look at whether supplementing standard baby formula with additional MFGM can improve health and development in formula fed babies.

We are also interested in looking at infant feeding practices among Australian mothers of term born babies.

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3. What does participation in this research involve?

Your baby will be randomly assigned (like tossing a coin) to either the 'standard formula' or a 'standard formula with additional MFGM' group. Your baby will have one in two chances of receiving either the 'standard formula' or the 'standard formula with additional MFGM' to feed to your baby from enrolment into the study (less than or equal to 60 days of age) until he or she is 12 months old.

Neither you, nor the research team, will be able to choose which group you are in or know which type of formula you have been assigned. All 'study formula' will be provided to you free of charge until your baby is 12 months of age. At the end of the study the results will be compared to see if one treatment is better than the other. We have also included a breastfed group to help us compare the formula fed groups.

If you have chosen to take part in this study we ask that you do not offer any solids or other drinks for at least 4 months following birth. If you choose to start breastfeeding your baby, please contact the research team.

We will ask you to attend some study visits and do some surveys, so we can assess the effects of the two study formulas.

Study Contact

All study contact is outlined in the flow chart on the next page. When you enrol into the study, we will ask you some questions about education, race and details about your pregnancy. We will also review your baby's blue book to determine information about your baby's growth, birth and health history. You will also receive a few phone calls in the first few weeks following enrolment to discuss formula commencement and current feeding practices.

The study visits involve answering some questions about how you are feeding your baby, your baby's health and development and doing some assessments with your baby. You will be reimbursed for your travel costs to attend these study visits. At each study visit we will collect your baby's measurements, ask some questions about feeding your baby, how windy or gassy your baby has been, your baby's bowel movements, any illnesses and childcare attendance.

You have the option of attending two study visits when your baby is 4 and 9 months of age where we can assess your baby's attention and heart rate at two of the study visits. These optional visits would involve showing your baby pictures of faces, while they are sitting on your lap (or in a highchair) and assessing your baby's attention to these faces (and how your baby's attention may change when seeing the same face or new different face). This will be done by measuring your baby's heart rate through a monitor on their chest and videorecording their eye movements whilst they look at the pictures. This takes between 5-15 minutes and is generally interesting for babies. If you prefer, the 4 and 9 month milestones can be done over the phone.

At 12 and 24 months of age we will assess your baby's development with the Bayley Scales of Infant and Toddler Development, fourth edition (Bayley-IV). This usually takes 1 hour with a research assistant and involves a series of games-like activities with toys and picture books that children generally enjoy. We also have some questionnaires for you to complete about your child's general development (Ages and Stages Questionnaire), language skills (MacArthur-Bates Communicative Development Inventories), and emotion and behaviour. We will give you the results of this assessment and if you have any concerns, we can offer you a referral to a GP.

We will also send you some questions via an online survey to your phone or email (or via telephone if you prefer) in between the study visits. There will be four online surveys to complete over a two-year period. They will take between 5-30 minutes and will include questions about feeding your baby, how windy or gassy your baby has been, your baby's bowel movements, any illnesses and childcare attendance. We will also collect some information about your baby's sleeping habits. Please note that we may contact you

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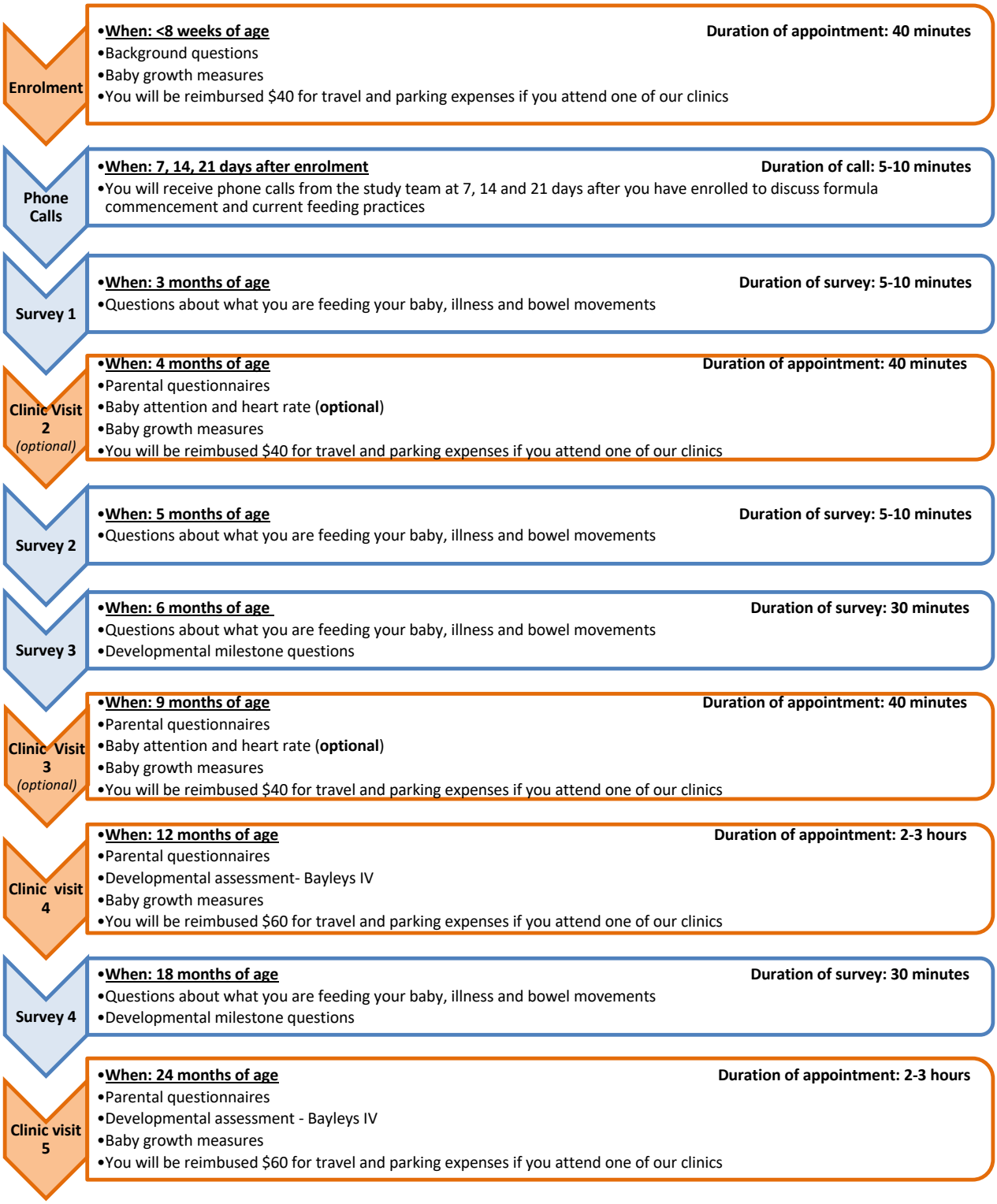
with some additional information that you might find helpful (e.g., safe sleeping practices), depending on how you respond to your surveys. Two of the surveys will also include questions about your child's general development (like whether they have started crawling or walking or speaking yet, or whether they are able to hold a crayon).

Outline of study contact



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4. What does my baby have to do?

We ask that you provide the study formula to your baby until 12 months of age.

We ask that you provide us with information about any use of any antibiotics or other medications prescribed or given to your baby during the study.

Whilst your baby is participating in this study, it is important to tell the study team about any treatments or medications you may give your baby, including over-the-counter medications, vitamins or herbal remedies. You should also tell the study team about any changes to these during your baby's participation in the research study.

We will be collecting information about the general health and development of your baby throughout the study and may access your medical records, if your baby has been admitted to hospital.

5. Other relevant information about the study

The standard formula and MFGM-supplemented formula are similar to infant formula that is currently sold within Australia. The formula used in this study are not currently sold in Australia or internationally.

Both the standard formula and MFGM-supplemented formula comply with all the requirements of the Australian and New Zealand Food Standards Code for infant formula and international regulations.

We will collect you and your partners contact details to enable us to communicate with you throughout the study. We will also collect details of up to four alternate contacts. We may contact one or more of the alternate contacts that you have provided to us in the event we have difficulties in reaching you throughout the study.

6. Does my baby have to take part in this study?

Participation in any study is voluntary. If you decide that your baby can take part and later change your mind, you are free to withdraw your baby from the project at any stage.

Your decision that your baby can or cannot take part, or that they can take part and then be withdrawn, will not affect their routine treatment, relationship with those treating them, their hospital or relationship with SAHMRI.

7. What are the possible benefits of taking part?

We cannot promise that your baby will receive any benefits from this research. The study assessments may assist in detecting a problem or delay with your baby's health or development. You will be supplied with formula to feed your baby until they are 12 months of age and reimbursed for attending clinic visits.

This study will lead to an improved understanding of whether supplementing infant formula with additional MFGM will benefit infant health and development.

8. What are the possible risks and disadvantages of taking part?

There are minimal risks for taking part in the study. As with any cow's milk formula, there may be rare occurrences of intolerance. Attending the clinic visits and participating in this study will involve a time commitment for you and your baby.

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9. What if I withdraw my baby from this study?

If you decide to withdraw your baby from the project, please notify a member of the research team before you withdraw them. This notice will allow that person or the research supervisor to further discuss any special requirements linked to withdrawing.

If you do withdraw your baby during the study, study staff will not collect additional personal information, although personal information already collected will be retained to ensure that the results of the study can be measured properly and to comply with law.

10. What happens when the study ends?

Following completion of this study, all records identifying you and your baby will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

The results of this study may be published in medical journals or presented at professional meetings, but you or your baby will not be identified in any way. We will send you a newsletter with the results of the study.

We may contact you following the completion of this study to see if you are interested in participating in any potential follow up studies, pending approval by the Human Research Ethics Committee.

11. What will happen to information about you and your baby?

You have a right to privacy, and all information that is collected because of this study is confidential. Your and your baby's data may be viewed by governments or ethics committees for auditing purposes, or by an external party for the purposes of monitoring data quality and adherence to the study protocol. To deliver study formula to you, your contact information, including your address will be provided to a warehouse. If you decide to take part in the attention and heart rate clinic assessment at 4 and 9 months, the video files of your baby will be sent to our international collaborators at the University of Kansas in the U.S.A. The video files are sent and stored and are destroyed at the completion of the study. All external parties involved in this project have signed agreements to ensure that your and your baby's information will be kept secure and confidential at all times.

In the case of a legal requirement, we may need to pass on personal information to authorised third parties. This requirement is standard and applies to information collected both in research and non-research situations. Such requests to access information are rare however, we have an obligation to inform you of this possibility.

In any publication and/or presentation, information will be provided in such a way that no participant can be identified. All data collected in the study may be stored for use in future research studies that may or may not be related to the original study. For example, to explore how baby's grow and develop over the first 24 months of life and patterns for the introduction of the first solid foods. Any stored data will be identified by a unique study number only, so that you and your baby cannot be identified and use of the data for research purposes would only occur if the research has been approved by the trial steering committee and the Human Research Ethics committee.

12. Complaints and Compensation

If your baby requires medical attention as a result of effects attributable to the consumption of any of the study formulas, you will be reimbursed for such medical costs by Fonterra Cooperative Group Limited. Such compensation shall be in accordance with the Australian Pharmaceutical Manufacturers Association (APMA) Clinical Trials Compensation Guidelines.

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SAHMRI will receive payment from Fonterra Cooperative Group Limited to undertake this study. No member of the research team will receive a personal financial benefit from this study, or your baby's involvement in this study (other than their ordinary wages).

14. Who has reviewed the study?

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this study have been approved by the HREC of the Women's & Children's Health Network.

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.

15. Further information and who to contact

If you would like to contact us, the person you may need to contact will depend on the nature of your query. If you want any further information concerning this project or if the baby has any medical problems which may be related to their involvement in the project (for example, any side effects), you can contact a member of the study team on 8128 4436 or any of the following people:

Study related matters

Name	Dr Jacqueline Gould
Position	Chief Investigator
Telephone	
Email	

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Reviewing HREC approving this research and HREC Executive Officer details

Reviewing HREC name	
HREC Executive Officer	
Telephone	
Email	

Local HREC Office contact (Single Site - Research Governance Officer)

Name	
Position	
Telephone	
Email	

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Infant Feeding Study CONSENT FORM

LAY TITLE: Infant Feeding Study

SCIENTIFIC TITLE: Infant nutrition with milk fat globule membrane for infant cognition in early life.

I _____

hereby consent to my child's involvement in the research study described above:

1. The nature and purpose of the study described on the attached Information Sheet has been explained to me. I understand it and agree to my child taking part.
2. I understand that my child may not directly benefit by taking part in this study.
3. I acknowledge that the possible risks and/or side effects, discomforts, and inconveniences, as outlined in the Information Sheet, have been explained to me.
4. I understand that I can withdraw my child from the study at any stage and that this will not affect medical care or any other aspects of my / my child's relationship with any healthcare service.
5. I understand that there will be no direct payment to me or my child for taking part in this study. I understand I will be reimbursed $40 for the first three study visits, and $60 for the final two study visits should they require travel to the hospital clinics. All visits completed in the home will not be reimbursed.
6. I have had the opportunity to discuss taking part in this study with a family member or friend, and/or have had the opportunity to have a family member or friend present whilst the researcher was explaining the study.
7. I am aware that I should retain a copy of the Consent Form, when completed, and the Information Sheet.
8. I agree to the accessing my child’s medical records, if they are hospitalised during the study period.
9. I understand that my and my child’s information will be kept confidential as explained in the Information Sheet except where there is a requirement for it to be shared with external parties for the purposes of monitoring, delivery of study formula, or when required by law.
10. I understand that the alternate contacts I have provided may be used to contact me as explained in the Information Sheet for study related purposes.
11. I understand that I may be contacted following the completion of this study to see if I am interested in participating in a follow-up of this study.

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Infant Feeding Study CONSENT FORM

14. I consent to my baby's de-identified data being used in other studies, provided the project has the approval of the Women's & Children's Hospital Research Ethics Committee.

Full name of Parent/Guardian: .....

Signature of Parent/Guardian: .....

Relationship to baby: .....

Full name of baby: .....

Dated: .....

I certify that I have explained the study to the parent / guardians and consider that he/she understands what is involved.

Researcher Name: .....

Researcher Signature: .....

Title: .....

Dated: .....

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Participant Information Sheet/Consent Form – Parent/Guardian

Breastfeeding Group

Women's & Children's Hospital, North Adelaide, South Australia

Lay Title	Infant nutrition with milk fat globule membrane for infant cognition in early life
Short Title	Infant Feeding Study
Protocol Number	3.0
Project Sponsor	Fonterra Cooperative Group Limited
Principal Investigator	Professor Maria Makrides (SAHMRI Women and Kids)
Local Principal Investigator	Dr Andrew McPhee, Dr Jacqueline Gould (SAHMRI Women and Kids) (Chief Investigator)
Associate Investigator(s)	Professor Robert Gibson, Dr Lisa Yelland (SAHMRI Women and Kids) and Professor John Columbo (University of Kansas, USA)

1. Introduction

The first year of life is an important time for the way a baby's brain grows and develops. This growth and development are partly influenced by a nurturing environment and partly by the quality of nutrition. The nutrition needed for optimal development are met via breastmilk, baby formula, or a mixture of both. One of the complex nutrients found in breastmilk is a mix of protein and fat called the milk fat globule membrane (MFGM). Although standard baby formula is a good substitute for breastmilk, it contains only trace amounts of MFGM.

The purpose of this study is to determine whether supplementing baby formula with additional MFGM is beneficial to the way healthy babies develop. To do this, we plan to compare the growth and development of babies that are fed breastmilk, standard baby formula or a formula supplemented with additional MFGM.

Approximately 600 babies will be enrolled in this study; 200 in the standard infant formula group, 200 in the MFGM-supplemented infant formula and 200 in the breast-fed reference group. This study is being conducted by SAHMRI in South Australian Hospitals and in the community through the Child and Family Health Service.

This Participant Information Sheet/Consent Form tells you about the study. Please read this information carefully. Ask questions about anything that you don't understand or would like to know more about.

2. What is the purpose of this research?

Sometimes we don't know which treatment is best and to find out, we need to compare different treatments. It is known that MFGM is present in breastmilk and may play a role in both brain and immune development. We would like to look at whether supplementing standard baby formula with additional MFGM can improve health and development in formula fed babies.

We are also interested in looking at infant feeding practices among Australian mothers of term born babies.

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3. What does participation in this research involve?

We want to work out whether we can improve on our current feeding practices for infants who are formula-fed. To do this, we will compare two groups of babies being fed two different infant formulas with a group of breastfed babies, the 'breastfeeding reference group'.

At the end of the study we will compare our results to see if one formula is better than the other at fulfilling the nutritional requirements of babies. We will also compare results with the breastfeeding baby group as this is the gold standard feeding method.

If you have chosen to take part in our breastfeeding group, we ask that you do not offer any solids or other drinks for at least 4 months following birth.

If you have difficulties with breastfeeding, we will offer the assistance of a lactation consultant whose services will be provided to you free of charge until your baby is 6 months of age.

Study Contact

All study contact is outlined in the flow chart on the next page. When you enrol into the study, we will ask you some questions about occupation, education, race and details about your pregnancy. We will also review your baby's blue book to determine information about your baby's growth, birth and health history. You will also receive a few phone calls in the first few weeks following enrolment to discuss your current feeding practices.

The study visits involve answering some questions about how you are feeding your baby, your baby's health and development and doing some assessments with your baby. You will be reimbursed for your travel costs to attend these study visits.

At each study visit we will collect your baby's measurements, ask some questions about feeding your baby, how windy or gassy your baby has been, your baby's bowel movements, any illnesses and childcare attendance.

You have the option of attending two study visits when your baby is 4 and 9 months of age where we can assess your baby's attention and heart rate at two of the study visits. These optional visits would involve showing your baby pictures of faces while they are sitting on your lap (or in a highchair) and assessing your baby's attention to these faces (and how your baby's attention may change when seeing the same face or new different face). This will be done by measuring your baby's heart rate through a monitor on their chest and videorecording their eye movements whilst they look at the pictures. This takes between 5-15 minutes and is generally interesting for babies. If you prefer, the 4 and 9 month milestones can be done over the phone.

At 12 and 24 months of age we will assess your baby's development with the Bayley Scales of Infant and Toddler Development, fourth edition (Bayley-IV). This usually takes 1 hour with a research assistant and involves a series of games-like activities with toys and picture books that children generally enjoy. We also have some questionnaires for you to complete about your child's general development (Ages and Stages Questionnaire), language skills (MacArthur-Bates Communicative Development Inventories), and emotion and behaviour. We will give you the results of this assessment and if you have any concerns, we can offer you a referral to a GP.

We will also send you some questions via an online survey to your phone or email (or via telephone if you prefer) in between the study visits. There will be four online surveys to complete over a two-year period. They will take between 5-30 minutes and will include questions about feeding your baby, how windy or gassy your baby has been, your baby's bowel movements, any illnesses and childcare attendance. We will also collect some information about your baby's sleeping habits. Please note that

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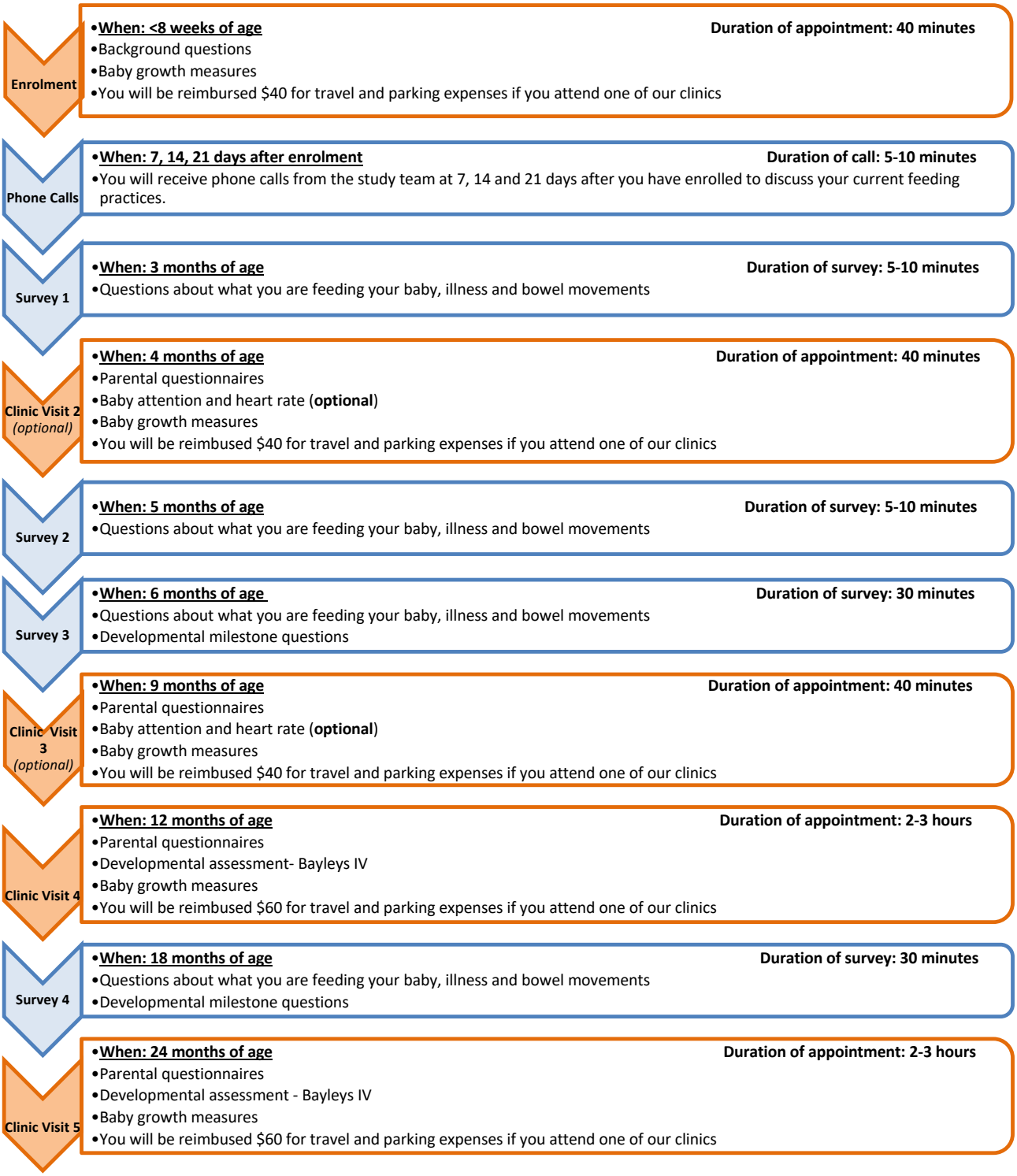
we may contact you with some additional information that you might find helpful (e.g., safe sleeping practices), depending on how you respond to your surveys. Two of the surveys will also include questions about your child's general development (like whether they have started crawling or walking or speaking yet, or whether they are able to hold a crayon).

Outline of study contact



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4. What does my baby have to do?

We ask that you provide us with information about any use of any antibiotics or other medications prescribed or given to your baby during the study.

Whilst your baby is participating in this study, it is important to tell the study team about any treatments or medications you may give your baby, including over-the-counter medications, vitamins or herbal remedies. You should also tell the study team about any changes to these during your baby's participation in the research study.

We will be collecting information about the general health and development of your baby throughout the study and may access your medical records if your baby has been admitted to hospital.

5. Other relevant information about the study

We will collect you and your partners contact details to enable us to communicate with you throughout the study. We will also collect details of up to four alternate contacts. We may contact one or more of the alternate contacts that you have provided to us in the event we have difficulties in reaching you throughout the study.

6. Does my baby have to take part in this study?

Participation in any study is voluntary. If you decide that your baby can take part and later change your mind, you are free to withdraw your baby from the project at any stage.

Your decision that your baby can or cannot take part, or that they can take part and then be withdrawn, will not affect their routine treatment, relationship with those treating them, their hospital or relationship with SAHMRI.

7. What are the possible benefits of taking part?

We cannot promise that your baby will receive any benefits from this research. We can offer you access to a lactation consultant until your baby is 6 months of age and the developmental assessments may assist in detecting a problem or delay.

This study will lead to an improved understanding of whether supplementing infant formula with additional MFGM will benefit infant health and development.

8. What are the possible risks and disadvantages of taking part?

There a minimal risks for taking part in this study. Attending the clinic visits and participating in this study will involve a time commitment for you and your baby.

9. What if I withdraw my baby from this study?

If you decide to withdraw your baby from the project, please notify a member of the research team before you withdraw them. This notice will allow that person or the research supervisor to further discuss any special requirements linked to withdrawing.

If you do withdraw your baby during the study, study staff will not collect additional personal information, although personal information already collected will be retained to ensure that the results of the study can be measured properly and to comply with law.

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10. What happens when the study ends?

Following completion of this study, all records identifying you and your baby will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

The results of this study may be published in medical journals or presented at professional meetings, but you or your baby will not be identified in any way. We will send you a newsletter with the results of the study.

We may contact you following the completion of this study to see if you are interested in participating in any potential follow up studies, pending approval by the Human Research Ethics Committee.

11. What will happen to information about you and your baby?

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12. Complaints and Compensation

If your baby requires medical attention as a result of effects attributable to the consumption of any of the study formulas, you will be reimbursed for such medical costs by Fonterra Cooperative Group Limited. Such compensation shall be in accordance with the Australian Pharmaceutical Manufacturers Association (APMA) Clinical Trials Compensation Guidelines.

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14. Who has reviewed the study?

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If you would like to contact us, the person you may need to contact will depend on the nature of your query. If you want any further information concerning this project or if the baby has any medical problems which may be related to their involvement in the project (for example, any side effects), you can contact a member of the study team on 8128 4436 or any of the following people:

Study related matters

Name	Dr Jacqueline Gould
Position	Chief Investigator
Telephone	
Email	

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

Reviewing HREC approving this research and HREC Executive Officer details

Reviewing HREC name	
HREC Executive Officer	
Telephone	
Email	

Local HREC Office contact (Single Site - Research Governance Officer)

Name	
Position	
Telephone	
Email	

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Infant Feeding Study CONSENT FORM

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I _____

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4. I understand that I can withdraw my child from the study at any stage and that this will not affect medical care or any other aspects of my / my child's relationship with this healthcare service.
5. I understand that there will be no direct payment to me or my child for taking part in this study. I understand I will be reimbursed $40 for the first three study visits, and $60 for the final two study visits should they require travel to the hospital clinics. All visits completed in the home will not be reimbursed.
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11. I understand that I may be contacted following the completion of this study to see if I am interested in participating in a follow-up of this study.

Infant Feeding Study CONSENT FORM

14. I consent to my baby's de-identified data being used in other studies, provided the project has the approval of the Women's & Children's Hospital Research Ethics Committee.

Full name of Parent/Guardian: .....

Signature of Parent/Guardian: .....

Relationship to baby: .....

Full name of baby: .....

Dated: .....

I certify that I have explained the study to the parent/guardian and consider that he/she understands what is involved.

Researcher Name: .....

Researcher Signature: .....

Title: .....

Dated: .....

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Supplementary Table. Timeline of key study milestones and information collected*

	Visit 1 Birth to 60 days virtual	**Survey 1 91 days (3 mo's)**	**Visit 2 120 days (4 mo's) clinic or** virtual	**Survey 2 152 days (5 mo's)**	**Survey 3 182 days (6 mo's)**	**Visit 3 273 Days (9 mo's) clinic or** vritual	**Visit 4 365 days (12 mo's) clinic or home**	**Survey 4 547 days (18 mo's)**	**Visit 5 730 days (24 mo's) clinic or home**
Background/ demographic /clinical information	x								
Feeding recall	x	x	x	x	x	x	x	x	x
Indicators of dietary tolerance	x	x	x	x	x	x	x		
Illness and SAE's		x	x	x	x	x	x	x	x
Childcare attendance	x		x			x	x		x
Attention Assessment			x			x			
Bayley-IV Assessment							x		x
MCDI							x		x
ASQ-3					x			x	
Home environment							x		x
Infant height, weight, head circumferenc e	x		x			x	x		x

* Additional phone calls at 4, 7, 14 and 21 days after enrolment collected information on receipt of study formula (where applicable) and feeding recall to monitor early compliance.

ASQ-3: Ages and Stages Questionnaire, Third Edition; Bayley-IV: Bayley Scales of Infant Development, Fourth Edition; MCDI-3: MacArthur-Bates Communicative Development Inventory, Third Edition; SAE: Serious Adverse Event