



International Centre for Diarrhoeal Disease Research, Bangladesh
CENTRE FOR HEALTH AND POPULATION RESEARCH
Mail : ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh
Phone: 880-2-8811751-60, Telex : 642486 ICDD BJ
Fax : 880-2-8823116, 8812530, 8811568, 8826050, 9885657, 8811686, 8812529
Cable : Cholera Dhaka

Memorandum

16 May 2005

PHSD
2000

To : Dr. Shams El Arifeen
Public Health Sciences Division (PHSD)

From: Professor AKM Nurul Anwar
Chairman
Ethical Review Committee (ERC)

(Signature)

Sub : New Principal Investigator (PI) of
Thymus component of research protocol # 2000-025

The ERC in its meeting held on May 11, 2005 approved Dr. Yukiko Wagatsuma's proposal for designating you the PI of the Thymus component (funded by the London School of Hygiene and Tropical Medicine, UK) of research protocol # 2000-025 titled "Combined interventions to promote Maternal and Infant Health". Congratulations!

Please submit the modified version of the ERC Face Sheet and relevant pages of the research protocol including the consent forms for the above mentioned component of the research protocols reflecting the change in the PI of the research protocol.

TERMS AND CONDITIONS

As the Principal Investigator, the ultimate responsibility for scientific, and ethical conduct including the protection of the rights and welfare of study participants vest upon you. You shall also be responsible for ensuring the competence, integrity and ethical conduct of the investigators and other staff directly involved in this research protocol.

You shall conduct the study in accordance with the ERC-approved protocol and shall fully comply with any subsequent determinations by the ERC.

You shall obtain prior approval from the Research Review Committee and the ERC for any modification in the approved research protocol and/or approved consent form(s), except in case of emergency to safeguard/eliminate apparent immediate hazards to study participants. Such changes must be immediately reported to the ERC Chairman.

You shall recruit/enroll participants for this study strictly adhering to the criteria mentioned in the research protocol.

You shall obtain legally effective informed consent (i.e. consent should be free from coercion or undue influence) from the selected study participants or their legally responsible representatives, as approved in the protocol, using the approved consent form prior to their enrollment in this study. Before obtaining consent, all prospective study

participants must be adequately informed about the purpose(s) of the study, its methods and procedures, and also what would be done if they agree and also if they do not agree to participate in the study. They must be informed that their participation in the study is voluntary and that they can withdraw their participation any time without any prejudice. Signed consent forms should be preserved for a period of at least five years following official termination of the study.

You shall promptly report the occurrence of any Adverse Event or Serious Adverse Event or unanticipated problems of potential risk to study participants or others to the ERC in writing within 24 hours of such occurrences.

Any significant new findings, developing during the course of this study that might affect the risks and benefits and thus influence either participation in the study or continuation of participation should be reported in writing to the participants and the ERC.

Data/samples should be collected and interviews should be conducted, as specified in the ERC-approved protocol, and confidentiality must be maintained. Data/samples must be protected by reasonable security, safeguarding against risks such as their loss or unauthorized access, destructions, used by others, and modification or disclosure of data. Data/samples should not be disclosed, made available or use for purposes other than those specified in the protocol, and shall be preserved for a period, as specified under Centre's policies/practices.

You shall promptly and fully comply with the decision of the ERC to suspend or withdraw its approval for the research protocol.

You shall report progress of research to the ERC for continuing review of the implementation of the research protocol as stipulated in the ERC Guidelines. Relevant excerpt of ERC Guidelines and '*Annual/Completion Report for Research Protocol involving Human Subjects*' are attached for your information and guidance.

I wish you all success in running the above-mentioned study.

cc: - Director, PHSD
- Director, HSID



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Memorandum

Date : 29th March 2005

To : ERC Chairman

Thru. : Director, HSID 

From : Dr. Yukiko Wagatsuma, Scientist, IDU/HSID 

Subject : Change of PI

CC : Finance
: Executive Director
: Dr. Rashidul Haque, LSD
: Dr. Shams Areefin
: A.S.G. Faruque, CRSC

I would like to transfer my PI responsibility to the following scientist,

- | | |
|------------|---|
| 2001-021 | 1. Kila-azar community study (Funded by CDC) to Dr. Rashidul Haque, LSD |
| 2000-025 | 2. Mini-mat study thymus components (funded by London School of Tropical Medicine and Hygiene) to Dr. Shams Areefin |
| 2004-047-- | 3. Relationship between rainfall and diarrhea to A.S.G. Faruque, CRSC |
| 2000-023 | 4. Dengue response study which I am responsible for entomology components has already assigned to Dr. Abdullah Brooks, IDU/HSID as a PI |



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Memorandum

5 January 2004

To : Dr. Shams El Arifeen
PI of research protocol # 2000-025 & 2002-031
Public Health Sciences Division (PHSD)

From: Professor AKM Nurul Anwar
Chairman, Ethical Review Committee (ERC)

A handwritten signature in black ink, likely belonging to Professor AKM Nurul Anwar.

Sub : Approval of the proposal for amendment of the addendum
to research protocol # 2000-025 & 2002-031

Thank you for your proposal for an amendment of the addendum to research protocol # ~~2000-025~~ titled "Combined interventions to promote maternal and infant health", and 2002-031 (titled "Combined interventions to promote maternal and infant health: effects over a pregnancy a cycle and on children of 0-24 months". I have the pleasure to inform you that the ERC in its meeting held on 31st December 2003 approved the proposal.

As had been mentioned while according approval of the protocols, you shall conduct the study in accordance with the ERC-approved protocol; and shall be responsible for protecting the rights and welfare of the subjects and compliance with the applicable provisions of ERC Guidelines. You shall also submit report(s) as required under ERC Guidelines.

Thank you once again.

Cc: Acting Head, PHSD



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Memorandum

29 June 2003

To : **Dr. Shams El Arifeen**
PI of the research protocol # 2000-025
Public Health Sciences Division

From: Professor AKM Nurul Anwar
Chairman, Ethical Review Committee (ERC)

Sub : Approval of the proposal for an addendum to research protocol # 2000-025

Thank you for your memo dated 18th June 2003 proposing an addendum to research protocol # 2000-025 entitled "Combined interventions to Promote Maternal and Infant Health" (sub-title: Measurement of breastmilk intake using stable isotope methods in infants born in MINIMat study), which the ERC approved in its meeting held on 25th June 2003.

As had been informed while according approval of original protocol, you shall conduct the study in accordance with the ERC-approved protocol; and shall be responsible for protecting the rights and welfare of the subjects and compliance with the applicable provisions of ERC Guidelines. You shall also submit report(s) as required under ERC Guidelines.

Thank you and I wish you all success in running the above-mentioned study.

cc: Acting Head, Public Health Sciences Division
Dr. AKM Iqbal Kabir, Clinical Sciences Division



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Memorandum

Date: June 18, 2003

To: Prof. AKM Nurul Anwar
Chairperson, ERC

Through: Dr. Abbas Bhuyian
Acting Associate Director, PHSD

From: Dr Iqbal Kabir 
Clinical Sciences Division

Dr Shams El Arifeen 
Public Health Sciences Division

Subject: Addendum to 'Combined Interventions to Promote Maternal and Infant Health' Study.

We are requesting approval of an addendum to the ongoing approved project titled 'Combined Interventions to Promote Maternal and Infant Health', (the 'MINIMat study').

The MINIMat Study

Poor maternal nutritional status is an important determinant of long-term maternal health as well as of fetal growth and subsequent infant health and survival. Infections during pregnancy may contribute to impaired fetal development and short gestational period. We are evaluating 4 combined interventions among a single group of pregnant women who live in Matlab upazilla, Bangladesh:

1. We are randomly assigning women to receive advice to begin the food supplementation programme (a) immediately after diagnosis of pregnancy (early care) or (b) at the time of their choosing (usual care).
2. Within each of these groups, we are randomly assigning women to receive a pill that contains (a) 30mg Fe and 400µg folic acid or (b) 60mg Fe and 400µg folic acid (usual care) or (c) 30mg Fe, 400µg folic acid and 13 additional micronutrients.
3. All women are offered screening for Bacterial Vaginosis (BV). Within each of the 6 groups mentioned above, asymptomatic BV-positive women are randomly assigned to (a) 250mg metronidazole orally thrice daily for 7 days or (b) lactose tablets given with the same dose frequency.

4. We are randomly assigning all of the subjects to receive either (a) counselling for exclusive breast feeding or (b) a different health education message of equal intensity.

An ongoing surveillance programme identifies pregnant women within 6-8 weeks of conception. Follow-up of the enrolled pregnant women continues throughout pregnancy. Birth weights are measured by home visits within 72 hours of delivery. Babies born in health facilities (approximately 20-30% of births) are weighed in the facility. Infants are then followed up until they are 24 months of age. Infant nutritional status and morbidity is measured by monthly home visits by trained data collectors.

Date: 18/6/2003

(FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator: DR. IQBAL KABIR

Trainee Investigator (if any):

Application No. DR. SHAM ELARIFEEN

Supporting Agency (if Non-ICDDR,B)

Title of Study: *Addendum measurement of breast milk intake using isotope methods of infants born in the MINIMat Study. (Addendum to combined intervention to promote Maternal and Infant Health Study (MINIMat))*

Project Status: *New Study (Addendum)*

Continuation with change

No change (do not fill out rest of the form)

Circle the appropriate answer to each of the following (If Not Applicable write NA)

1. Source of Population:
 - (a) Ill subjects Yes ☐ No ☒
 - (b) Non-ill subjects ☒ Yes No ☐
 - (c) Minor or persons under guardianship ☒ Yes No ☐
2. Does the Study Involve:
 - (a) Physical risk to the subjects Yes ☐ No ☒
 - (b) Social risk Yes ☐ No ☒
 - (c) Psychological risks to subjects Yes ☐ No ☒
 - (d) Discomfort to subjects ☒ Yes No ☐
 - (e) Invasion of privacy Yes ☐ No ☒
 - (f) Disclosure of information damaging to subject or others Yes ☐ No ☒
3. Does the Study Involve:
 - (a) Use of records (hospital, medical, death or other) ☒ Yes No ☐
 - (b) Use of fetal tissue or abortus Yes ☐ No ☒
 - (c) Use of organs or body fluids ☒ Yes No ☐
4. Are Subjects Clearly Informed About:
 - (a) Nature and purposes of the study ☒ Yes No ☐
 - (b) Procedures to be followed including alternatives used ☒ Yes No ☐
 - (c) Physical risk ☒ Yes No ☐
 - (d) Sensitive questions ☒ Yes No ☐
 - (e) Benefits to be derived ☒ Yes No ☐
 - (f) Right to refuse to participate or to withdraw from study ☒ Yes No ☐
 - (g) Confidential handling of data ☒ Yes No ☐
 - (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure ☒ Yes No ☐
5. Will Signed Consent Form be Required:
 - (a) From subjects ☒ Yes No ☐
 - (b) From parents or guardian ☒ Yes No ☐
(if subjects are minor)
6. Will precautions be taken to protect anonymity of subjects ☒ Yes No ☐
7. Check documents being submitted herewith to Committee:
 - _____ Umbrella proposal - Initially submit an with overview (all other requirements will be submitted with individual studies)
 - _____ Protocol (Required)
 - _____ Abstract Summary (Required)
 - _____ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
 - _____ Informed consent form for subjects
 - _____ Informed consent form for parent or guardian
 - _____ Procedure for maintaining confidentiality
 - _____ Questionnaire or interview schedule
 - * If the final instrument is not completed prior to review, the following information should be included in the abstract summary
 1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy
 2. Example of the type of specific questions to be asked in the sensitive areas
 3. An indication as to when the questionnaire will be presented to the Committee for review

We agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.



Principal Investigator

Trainee

Proposed Addendum: Measurement of breast milk intake using isotopic methods in infants born in the MINIMat study

Principal Investigators: Dr. Iqbal Kabir, Dr. Sophie Moore, Dr. Shams El Arifeen

Co-Investigators: Dr. Andy Coward, Antony Wright, Prof. Andrew Prentice, Prof. Lars Ake Persson

Background

The aim of the proposed addendum is to measure actual breast milk intake in infants born during the MINIMat study, using the dose to the mother deuterium dilution technique (Butte *et al.*, 1988; Coward *et al.*, 1982; Orr-Ewing *et al.*, 1986). In addition to quantifying the actual breast milk intake, this technique significantly allows for the estimation of non-breast milk water intake, and hence can be used to assess breast-feeding and complementary feeding practices. This study will be co-ordinated by Dr Iqbal Kabir and conducted by the MINIMat team in collaboration with Dr Andy Coward and Mr Antony Wright, MRC Human Nutrition Research, Cambridge, UK and Dr Sophie Moore and Professor Andrew Prentice, MRC International Nutrition Group, London School of Hygiene and Tropical Medicine, London, UK.

The promotion of exclusive breast-feeding (EBF), i.e. intake of nothing but breast milk, during the first six months of an infants life is now recommended (WHO, 2001a; WHO 2001b) but in many societies infants are predominantly breast-fed (PBF), that is to say they receive other liquids (such as water, tea and juices). Although breast feeding is almost universal in Bangladesh, appropriate breast feeding practices including EBF for the first months are low, largely due to the lack of appropriate knowledge in the mothers (Ahmed *et al.*, 1999). Furthermore, the common practise of the early initiation of complementary feeding in rural Bangladesh is known to be associated with high rates of infant malnutrition (Haider *et al.*, 1996).

Standard survey questionnaires often overestimate breastfeeding and its exclusivity (Albernaz 2003). It may be possible that mothers who received BF counselling may be reluctant to admit to giving their infants other liquids than breast milk. As such the proportion of mothers thought to be exclusively breast-fed will be higher than reality. Since EBF is an major outcome in the MINIMat study, it is important to we measure this well and include a measurement of bias using standard interviews.

The deuterium dilution technique has recently been used to demonstrate that complementary liquids (water, tea of juice) do not replace breast milk in PBF infants as compared to EBF Brazilian infants (Haisma *et al.*, 2003). In partially breast fed infants however, breast milk intake was reduced to 74% compared to EBF infants (Haisma *et al.*, 2003). In a further study in Brazil, the deuterium dilution technique was additionally used to demonstrate that breast feeding counselling increases breast feeding duration (although not intake) during the first four months of infancy (Albernaz *et al.*, 2003). Currently, there are no published studies looking at breast milk intakes or breast feeding practices using isotopic tracer techniques in rural Bangladesh.

In the MINIMat study, all mothers are randomly assigned to receive either (a) counselling for EBF or (b) a different health education message of equal intensity. Questionnaires administered to the mothers during the infant follow up will help ascertain both actual breast-feeding practices, and practices according to participation in the two intervention groups. The use of the dose to the mother deuterium dilution technique, will help describe *actual* patterns.

The specific objectives of this addendum will be to use the deuterium dilution technique for measuring breast milk intake in order to:

1. Quantify breast milk intakes in infants born in the MINIMat study;
2. Assess the impact of counselling for exclusive breast feeding on breast milk intake compared to health education counselling only; &
3. Validate mother's reports on breast-feeding patterns.

In addition, the results obtained will be used for the appropriate analysis of the data on infant growth and morbidity, thymic size and immune function and cognitive function in the infants (all are measured in the MINMat study), in relation to their breast milk intake and feeding patterns.

Methods

100 mother-infant pairs will be enrolled into the proposed addendum. These pairs will be enrolled from Block B of the Matlab study area when the infants are 13 to 15 weeks of age. Eligibility criteria will be:

- I. Singleton births, with gestational age between 37 and 42 weeks.
- II. Only mothers reporting to be either exclusively or predominantly breast feeding their infants, and do not report the use of formula (alternative) milk.
- III. Mothers and their infants who predict that they will remain in Matlab for the 15 day duration of the study.
- IV. No reported illness in the mother or infant during the previous 7 days.

Classification of Breast-feeding category: (according to WHO)

Exclusive breast-feeding: breast milk only

Predominant breast-feeding: breast milk plus other liquids such as water, tea or juices.

Partially breast-feeding: other foods and milk in addition to breast-milk

Breast milk intake will be measured by the deuterium dilution technique. A baseline sample of saliva (2ml) from the mother and a urine sample (2ml) from the child will be collected at baseline (day 0) following which the mother will receive an oral dose of deuterium (10g). A further 3 samples of saliva from the mother, and 5 urine samples from the baby will then be collected over a 14 day period, as outlined below.

	Days														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Mother	X	X			X										X
Child	X	X		X	X									X	X

Sample collection will be performed having been assured that the mother has not consumed any food or drink during the previous 30 min to 1 hour. Small pieces of cotton wool will be used to collect saliva samples, after which saliva will be expressed using a syringe. Urine samples will be obtained by leaving cotton wool balls in clean disposable nappies, according to the method described by Roberts & Lucas (1985). During the sampling, nappies will be regularly checked and once the

infant has passed urine, the cotton wool balls will be squeezed in a syringe to collect the urine. All samples (saliva and urine) will then be stored at -20°C prior to transportation to Dhaka, and onto the UK. All mothers and infants will be weighed, and their height measured, at the beginning and the end of the deuterium study.

Deuterium enrichment in the saliva and urine samples will be analysed using isotope ratio mass spectrometry (IRMS) at MRC Human Nutrition Research in Cambridge, UK. The protocol used is described elsewhere (Hoffman *et al.*, 2000). Intake of breast milk and water from non-milk sources will be calculated by fitting the isotopic (tracer) data to a model for water (tracee) turnover in the mothers and infants and the transfer of milk from mother to the baby (Coward *et al.*, 1982; Orr-Ewing *et al.*, 1986).

Based on recent work in Brazil (Haisma *et al.*, 2003), it has been estimated that, to detect a 100 ml difference in breast milk intake between groups, a sample size of 41 infants per group is required. This sample size calculation is based only on testing the difference between the main effect of counselling for exclusive breast feeding on breast milk intake versus health education counselling only. This estimation assumes $\alpha=0.05$, $\beta=0.8$, and is based on the standard deviation of 160 obtained from the studies in Brazil. Given the expected low-rates for drop-out from the study, it will be appropriate that about 100 mother-infant pairs should be recruited into the study.

Ethical considerations

Deuterium is a naturally occurring non-radioactive isotope of hydrogen, which has a natural abundance of about 150 ppm. For the measurements in the current study, mothers will be asked to drink a small amount of deuterium (10 g) enriched water that will enrich body water by about an additional 150 ppm. As a consequence of the infant receiving tracer from mother's milk, enrichment in the infant will increase to about 75 ppm above baseline values. After these maximums, the isotope will disappear from the mothers and infants with biological half-lives of about 5 to 6 and 3 to 4 days, respectively. Similar amounts have been used worldwide in many studies and there are no known risks. Isotopic methods have the advantage over the traditional method of test-weighing since they do not interfere with the normal patterns of behaviour, and are not time-consuming for the mothers involved.

Any mother will be free to withdraw her infant from the sub-study at any stage, without affecting any aspects of the routine health care provided in the area, or to her involvement with other aspects of the MINIMat infant follow up study.

References

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- Hoffman DJ, Sawaya AL, Coward WA, Wright A, Martins PA, de Nascimento C, Tucker KL, Roberts SB. Energy Expenditure of stunted and non-stunted boys and girls living in the shantytowns of Sao Paulo, Brazil. *Am J Clin Nutr* 2000 **72**, 1025-31.
- Orr-Ewing AK, Heywood PF, Coward WA. Longitudinal measurements of breast milk output by a $^2\text{H}_2\text{O}$ tracer technique in rural Papua New Guinean women. *Hum Nutr Clin Nutr* 1986 **40**, 451-67.
- Roberts SB, Lucas A. Measurement of urinary constituents and output using disposable napkins. *Arch Dis Child* 1985 **60**, 1021-24.
- WHO. Global strategy for infant and young child feeding: The optimal duration of exclusive breast-feeding. In *Fifty-fourth World Health Assembly*. 2001a. Document A54/INF.DOC./4, Geneva.
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APPENDIX

International Centre for Diarrhoeal Disease Research, Bangladesh: Centre for
Health and Population Research

Voluntary Consent Form

MINIMat Breast Milk Intake Study

Principal Investigators: Dr. Iqbal Kabir, Dr. Shams El Arifeen, Dr. Sophie Moore

Information for the study participants and consent: Please ask our study staff to explain any words or information you don't understand. If you agree to take part in the study, we will give you a copy of this consent form.

Introduction:

As you will be aware, breast milk is the best form of nutrition for your baby during early life. Some mothers perceive breast milk is not enough for baby's growth. However, little is known about the exact amount of breast milk women from Matlab produce each day for their babies. In this study, we would like to measure the amount of breast milk your baby receives from you. To do this, we do not need to take any of your breast milk, we can just measure the amount of water that you transfer to your baby via your milk. We do this using a special form of labeled water.

Purpose of the Study:

The aim of this study is to measure the amount of breast milk your baby is receiving from you.

Study Procedure:

If you agree to participate in this study, the following simple procedures will be done;

1. We will give you a piece of cotton wool and ask you to chew on it for about 5 minutes. We will then collect some of your saliva from the cotton wool using a syringe.
2. We will also collect a urine sample from your baby. To do this we will place some cotton wool balls in a diaper, and then wait for your baby to pass urine. We will then extract a small amount of urine from the cotton wool using a syringe.
3. We will then ask you to drink a small amount of water. This water is specially made so that we can trace its passage from you to your baby through your breast milk. This water looks and tastes just like normal water and is perfectly harmless.
4. We will then come back to visit you again to collect urine from your baby 1, 3, 4, 13, and 14 days later. In days 1, 4 and 14 we will also collect another sample of saliva from you.

Benefits:

There is no direct benefit to you or your child. However, this study will allow us to estimate the amount of breast milk your baby is getting.

Risks:

There are no risks associated with this study methodology. This type of study has been conducted in lots of other mothers and their infants all over the world.

Rights:

Your participation to this study is completely voluntary. You may refuse to participate in the study, and may also withdraw yourself from the study at any time without any penalty.

Confidentiality:

All information obtained from you, your baby and results of all laboratory tests will be stored in a safe place, under lock and key, and only the study staff, the Ethical Review Committee of ICDDRDB would have access to this information. The name and identity of you and your baby would not be disclosed at the time of publication of these results of this study.

Answer to questions:

You are free to ask any question about the study now, and would also be able to contact the investigators of this study afterwards at the address given below. We would be happy to provide answers and results of the study when they become available.

Declarations:

The investigators have informed me on the details of the study, and its purpose and methods, benefits and risk involved, my rights and confidential handling of my/baby's personal information. I understand that I would be able to freely communicate with them to get answer to my questions about the study.

Name of the investigators:

Name	Telephone number
Dr. Iqbal Kabir	8811751-60 ext: 2312
Dr. Shams El Arifeen	8811751-60 ext: 2233

If you agree to participate please give your signature or thumb impression:

Signature/thumb impression of the subject

Date

Signature of the investigators/representative

Date

Signature of the witness

Date

আন্তর্জাতিক উদারাময় গবেষণা কেন্দ্র, বাংলাদেশ

হেলথ এন্ড পপুলেশন রিসার্চ

সম্মতি পত্র

প্রধান গবেষক : ডাঃ ইকবাল কবির, ডাঃ সামস্ আরেফীন, ডাঃ নোফিয়া মুর

গবেষণায় অর্ন্তভুক্ত করার আগে গবেষণার অর্ন্তভুক্তকারীকে গবেষণার উদ্দেশ্য, পদ্ধতি এবং গবেষণার সুবিধা এবং তাতে কোন ঝুঁকি আছে কিনা এই সব ব্যাপারে জানাতে হবে। যদি গবেষণাকারী এই গবেষণায় আসতে রাজী হন তখন আমরা গবেষণাকারীকে এই সম্মতি পত্র প্রদান করবো।

ভূমিকা :

আমরা জানি যে, একটা ছোট বাচ্চার জন্য যতটুকু পুষ্টির প্রয়োজন তার সবটুকুই বুকের দুধেই আছে। কিন্তু কিছু মা মনে করেন, বুকের দুধ তার বাচ্চার বৃদ্ধির জন্য যথেষ্ট নয়। এই গবেষণা থেকে আমরা জানতে পারবো বাচ্চা কতটুকু বুকের দুধ তার মার কাছ থেকে পায় এবং এটা করতে আলাদা করে আপনার বুকের দুধের প্রয়োজন নেই। মতলবের মায়েরা তাদের বাচ্চাদের প্রতিদিন কতটুকু পরিমাণ বুকের দুধ খাওয়াতে পারে তা কিছুটা জানা গেছে। আমরা আপনাকে কিছু বিশেষ ধরনের পানি খাওয়াবো, তা বাচ্চা বুকের দুধের মাধ্যমে পাবে।

উদ্দেশ্য :

এই গবেষণার লক্ষ্য হচ্ছে বাচ্চা কতটুকু পরিমাণ বুকের দুধ পাচ্ছে তা পরিমাপ করা।

গবেষণার প্রণালী :

যদি আপনি এই গবেষণায় আসতে রাজী হন তাহলে আপনাকে কিছু সহজ নিয়মাবলী পালন করতে হবে।

- ১। আমরা আপনাকে ১টা তুলার টুকরা দিবো এবং এটা প্রায় ৫ মিনিট পর্যন্ত চিবাতে বলবো। এরপর আমরা সিরিঞ্জের মাধ্যমে তুলার টুকরা হতে কিছু লালো সংগ্রহ করবো।
- ২। আমরা আপনার বাচ্চার প্রস্রাবের নমুনা ও সংগ্রহ করবো। এটা করতে বাচ্চার ডায়পারের ঠিক মাঝখানে কয়েক টুকরা তুলার বল রাখবো এবং বাচ্চার প্রস্রাব না হওয়া পর্যন্ত অপেক্ষা করবো এরপর সিরিঞ্জের মাধ্যমে তুলার বল হতে কিছু পরিমাণ প্রস্রাব সংগ্রহ করবো।
- ৩। এরপর আমরা আপনাকে অল্প পরিমাণ পানি পান করতে বলবো। এই পানিটা বিশেষভাবে তৈরী। আপনি যে বাচ্চাকে বুকের দুধ খাওয়াচ্ছেন তার মাধ্যমে আমরা এটাকে সনাক্ত করতে পারবো। এই পানির স্বাদ সাধারণ পানির মতই এবং এটি সম্পূর্ণ নিরাপদ।
- ৪। আমরা আবার আপনার বাসায় আসবো বাচ্চার প্রস্রাব সংগ্রহ করার জন্য ১,৩,৪,১৩ এবং ১৪ দিনের দিন এবং ১,৪,১৪ দিনের দিন আমরা আপনার লালার নমুনাও সংগ্রহ করবো।

উপকারিতা :

এখানে আপনার ও আপনার বাচ্চার সরাসরি কোন উপকারিতা নাই। কিন্তু এই গবেষণার মাধ্যমে বাচ্চা কতটুকু বুকের দুধ পাচ্ছে তার পরিমাণ জানতে পারবো।

ঝুঁকি :

এই গবেষণায় কোন রকম ঝুঁকি নেই। বিশ্বের অন্যান্য স্থানেও মা ও বাচ্চাদের নিয়ে নিরাপদে এই ধরনের গবেষণা হয়েছে।

অধিকার :

এই গবেষণায় আপনার অংশগ্রহন সম্পূর্ণ ইচ্ছামূলক। আপনি ইচ্ছা করলে এই গবেষণা থেকে বিরত থাকতে পারেন এবং আপনি যে কোন সময় এই গবেষণা ছেড়ে দিতে পারেন।

গোপনীয়তা :

আপনার ও আপনার বাচ্চার থেকে নেয়া সবরকম তথ্য, গবেষণার পরীক্ষার ফল নিরাপদ জায়গায় রাখা হবে যেখানে তালী চাবির ব্যবস্থা আছে। এই সব তথ্য কেবল মাত্র গবেষকগণ এবং আইসিডিডিআর,বি-র এগিক্যাল কমিটির সদস্যগণ অবগত হতে পারবেন।

গবেষণা প্রকাশনীর সময় আপনার ও আপনার বাচ্চার নাম এবং পরিচয় প্রকাশ করা হবে না।

প্রশ্নোত্তর :

গবেষণা সম্পর্কে যে কোন প্রশ্ন আপনি খোলাখোলী আলোচনা করতে পারেন। এবং এই গবেষণার গবেষকদের সাথে যে কোন সময় আপনি যোগাযোগ করতে পারেন তাদের নাম ঠিকানা লিখা থাকবে। আপনাদের উত্তর তারা আনন্দের সাথে গ্রহন করবে।

প্রত্যয়ন :

গবেষকগণ গবেষণার সমস্ত তথ্য বিস্তারিত ভাবে আপনাকে জানাবেন এবং এর উদ্দেশ্য পদ্ধতি উপকারিতা এবং ঝুঁকি আছে কি না, আমার অধিকার এবং আমার ও আপনার বাচ্চার ব্যক্তিগত সকল তথ্য গোপনীয়তা সম্পর্কে। আমি তাদের সাথে আমরা যোগাযোগ করতে পারবো এবং গবেষণা সম্পর্কে যে কোন প্রশ্ন সম্পর্কে খোলাখোলী আলোচনা করবো।

গবেষকদের নাম :

নাম : টেলিফোন নং

ডাঃ ইকবাল কবির ৮৮১৭৫১-৬০ # ২৩১২

ডাঃ সামস্ এল আরেফিন ৮৮১৭৫১-৬০ # ২২৩৩

আপনি যদি এই গবেষণায় অংশ নিতে সম্মতি হন তাহলে আপনার নাম সই করুন বা বাম হাতের বৃদ্ধাঙ্গুলির ছাপ দিন।

স্বাক্ষর/বৃদ্ধাঙ্গুলির ছাপ

তারিখ

Date 30 Oct 2000

ETHICAL REVIEW COMMITTEE, ICDR, B.

Principal Investigator Prof. Lars Ake Persson

Trainee Investigator (if any) -

Application No. 2000-025

Supporting Agency (if Non-ICDR, B)

Title of Study Combined interventions

Project status:

to promote maternal and infant health

- (✓) New Study
 () Continuation with change
 () No change (do not fill out rest of form)

Circle the appropriate answer to each of the following (If Not Applicable write NA).

1. Source of Population:
 - (a) Ill subjects ☒ Yes ☐ No
 - (b) Non-ill subjects ☒ Yes ☐ No
 - (c) Minors or persons under guardianship ☒ Yes ☐ No
2. Does the study involve:
 - (a) Physical risks to the subjects ☐ Yes ☒ No
 - (b) Social Risks ☐ Yes ☒ No
 - (c) Psychological risks to subjects ☐ Yes ☒ No
 - (d) Discomfort to subjects ☒ Yes ☐ No
 - (e) Invasion of privacy ☐ Yes ☒ No
 - (f) Disclosure of information damaging to subject or others ☐ Yes ☒ No
3. Does the study involve:
 - (a) Use of records, (hospital, medical, death, birth or other) ☒ Yes ☐ No
 - (b) Use of fetal tissue or abortion ☐ Yes ☒ No
 - (c) Use of organs or body fluids ☐ Yes ☒ No
4. Are subjects clearly informed about:
 - (a) Nature and purposes of study ☒ Yes ☐ No
 - (b) Procedures to be followed including alternatives used ☒ Yes ☐ No
 - (c) Physical risks ☐ Yes ☒ No
 - (d) Sensitive questions ☐ Yes ☒ No
 - (e) Benefits to be derived ☒ Yes ☐ No
 - (f) Right to refuse to participate or to withdraw from study ☒ Yes ☐ No
 - (g) Confidential handling of data ☒ Yes ☐ No
 - (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure ☒ Yes ☐ No
5. Will signed consent form be required:
 - (a) From subjects ☒ Yes ☐ No
 - (b) From parent or guardian (if subjects are minors) ☒ Yes ☐ No
6. Will precautions be taken to protect anonymity of subjects ☒ Yes ☐ No
7. Check documents being submitted herewith to Committee:
 - ☐ Umbrella proposal - Initially submit an overview (all other requirements will be submitted with individual studies).
 - ☒ Protocol (Required)
 - ☒ Abstract Summary (Required)
 - ☒ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw (Required)
 - ☒ Informed consent form for subjects
 - ☒ Informed consent form for parent or guardian
 - ☐ Procedure for maintaining confidentiality
 - ☐ Questionnaire or interview schedule
8. If the final instrument is not completed prior to review, the following information should be included in the abstract summary:
 1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy.
 2. Examples of the type of specific questions to be asked in the sensitive areas.
 3. An indication as to when the questionnaire will be presented to the Cttee. for review.

I agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

Principal Investigator

Trainee

Lars
 Budget
 Just birth

RESEARCH PROTOCOL

Protocol No.:2000-025

FOR OFFICE USE ONLY

RRC Approval: Yes/ No 2000-025 Date: 19/09/2000

ERC Approval: Yes/No Date:

AEEC Approval: Yes/No Date:

Project Title: Combined Interventions to Promote Maternal and Infant Health

Theme: (Check all that apply)

- | | |
|---|--|
| ☒ Nutrition | ☐ Environmental Health |
| ☐ Emerging and Re-emerging Infectious Diseases | ☐ Health Services |
| ☐ Population Dynamics | ☐ Child Health |
| ☒ Reproductive Health | ☐ Clinical Case Management |
| ☐ Vaccine evaluation | ☐ Social and Behavioural Sciences |

Key words: Food supplementation, micronutrient supplementation, intra-uterine growth retardation, birth weight

Principal Investigator: Lars Ake Persson

Division: PHSD

Phone: 2200 1111 5133

Address: ICDDR,B

Email: persson@icddr.org

Co-Principal Investigator(s): George Fuchs, CSD

Co-Investigator(s): See list in protocol

Student Investigator/Intern:

Collaborating Institute(s):

Population: Inclusion of special groups (Check all that apply):

- | | |
|---|--|
| Gender | ☒ Pregnant Women |
| ☒ Male | ☐ Fetuses |
| ☒ Females | ☐ Prisoners |
| Age | ☐ Destitutes |
| ☒ 0 - 5 years | ☐ Service providers |
| ☐ 5 - 9 years | ☐ Cognitively Impaired |
| ☒ 10 - 19 years | ☐ CSW |
| ☒ 20 + | ☐ Others (specify _____) |
| ☐ > 65 | ☐ Animal |

Project / study Site (Check all the apply):

- | | |
|---|--|
| ☐ Dhaka Hospital | ☐ Mirsarai |
| ☐ Matlab Hospital | ☐ Patya |
| ☒ Matlab DSS area | ☐ Other areas in Bangladesh _____ |
| ☐ Matlab non-DSS area | ☐ Outside Bangladesh _____ |
| ☐ Mirzapur | name of country: _____ |
| ☐ Dhaka Community | ☐ Multi centre trial |
| ☐ Chakaria | (Name other countries involved) |
| ☐ Abnayanagar | |

Revised on: 30 May 2000

Type of Study (Check all that apply):

- | | |
|--|---|
| ☐ Case Control study | ☐ Cross sectional survey |
| ☒ Community based trial / intervention | ☐ Longitudinal Study (cohort or follow-up) |
| ☐ Program Project (Umbrella) | ☐ Record Review |
| ☐ Secondary Data Analysis | ☐ Prophylactic trial |
| ☐ Clinical Trial (Hospital/Clinic) | ☐ Surveillance / monitoring |
| ☐ Family follow-up study | ☐ Others |

Targeted Population (Check all that apply):

- | | |
|---|--------------------------------------|
| ☒ No ethnic selection (Bangladeshi) | ☐ Expatriates |
| ☐ Bangalee | ☐ Immigrants |
| ☐ Tribal groups | ☐ Refugee |

Consent Process (Check all that apply):

- | | |
|---|--|
| ☒ Written | ☒ Bengali language |
| ☒ Oral | ☐ English language |
| ☐ None | |

Proposed Sample size: _____

Total sample size: ~ 5000 ☐

Sub-group _____ ☐

_____ ☐

Determination of Risk: Does the Research Involve (Check all that apply):

- | | |
|---|--|
| ☐ Human exposure to radioactive agents? | ☐ Human exposure to infectious agents? |
| ☐ Fetal tissue or abortion? | ☐ Investigational new drug |
| ☐ Investigational new device? | ☐ Existing data available via public archives/source |
| (specify _____) | ☐ Pathological or diagnostic clinical specimen only |
| ☐ Existing data available from Co-investigator | ☐ Observation of public behaviour |
| | ☒ New treatment regime Food and micronutrients |

Yes/No

- ☐ ☒ Is the information recorded in such a manner that subjects can be identified from information provided directly or through identifiers linked to the subjects?
- ☐ ☒ Does the research deal with sensitive aspects of the subject's behaviour: sexual behaviour, alcohol use or illegal conduct such as drug use?

Could the information recorded about the individual if it became known outside of the research:

- ☐ ☒ a. place the subject at risk of criminal or civil liability?
- ☐ ☒ b. damage the subject's financial standing, reputation or employability: social rejection, lead to stigma, divorce etc

Do you consider this research (Check one):

- | | |
|--|---|
| ☐ greater than minimal risk | ☒ no more than minimal risk |
| ☐ no risk | ☐ only part of the diagnostic test |

Minimal Risk is "a risk where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests. For example, the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than the risk of doing so as a part of routine physical examination".

Yes/No

☒ ☐ Is the proposal funded? Yes

If yes, sponsor Name: UNICEF (partly committed)

Yes/No

☒ ☐ Is the proposal being submitted for funding?

If yes, name of funding agency: (1) UNICEF

(2) NIH

Do any of the participating investigators and/or their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test-product or device to be studied or serve as a consultant to any of the above? N.A.

IF YES, submit a written statement of disclosure to the Director.

Dates of Proposed Period of Support

Cost Required for the Budget Period (\$)

(Day, Month, Year - DD/MM/YY)

a. 1st Year 2nd Year 3rd Year Other years

Beginning date: November 2000

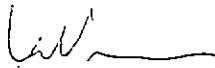
End date: October 2005

b. Direct Cost: _____ Total Cost: US\$2,582,339

Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

LA PERSSON



30/10/2000

Name of the Division Director

Signature

Date of Approval

Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

Signature of PI



Date:

20/10/2000

Name of Contact Person (if applicable)

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Principal Investigator: Lars Åke Persson

PROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application. (Type text within the space provided)

Principal Investigator (s) Lars Åke Persson

Project Name Combined Interventions to Promote Maternal and Infant Health

Total Budget \$2,865,757 Beginning Date Nov 2000 Ending Date Oct 2005

Poor maternal nutritional status remains an important determinant of long-term maternal health as well as of foetal growth and subsequent infant health and survival, especially in South Asia. Infections during pregnancy may contribute to impaired foetal development and short gestational period. We will evaluate 4 combined interventions among a single group of approx. 5,000 undernourished women who live in Matlab upazilla, Bangladesh, the well-established field site of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). An on-going surveillance program identifies pregnant women within 5-8 wk of conception. An on-going government program provides a food supplement to pregnant and lactating women that contains 600 kcal/d (6 d/wk) and a pill that contains 60 mg iron (Fe) and 400 µg folic acid daily. Intervention 1: We will randomly assign women to receive advice to begin the food supplementation program (a) immediately after diagnosis of pregnancy (early care) or (b) at the time of their choosing (usual care). *We postulate that those in the early care group will have higher-birthweight infants than those in the usual care group.* Intervention 2: Within each of these groups, we will randomly assign women to receive a pill that contains (a) 30 mg Fe and 400 µg folic acid or (b) 60 mg Fe and 400 µg folic acid (usual care) or (c) 30 mg Fe, 400 µg folic acid and 13 additional micronutrients. *We postulate that the multiple micronutrient supplement will increase maternal haemoglobin concentration and birth weight compared to usual care. We postulate that the lower-iron treatment will have the same effect on maternal haemoglobin (Hb) and birth weight as usual care and thus that the additional micronutrient effect may be attributed to the increased effect of multiple micronutrient supplementation.* Intervention 3: All women will be offered screening for Bacterial Vaginosis (BV). Within each of the 6 groups mentioned above asymptomatic BV-positive women will be randomly assigned to (a) 250 mg metronidazole orally thrice daily for 7 days or (b) lactose tablets given with the same dose frequency. *We postulate that women in the metronidazole group will have longer gestation than women receiving placebo.* Intervention 4: We will randomly assign all of the subjects to receive either (a) counselling for exclusive breastfeeding (EBF) or (b) a different health education message of equivalent intensity. *We postulate that those who receive counselling for EBF will have a longer duration of EBF than those who did not receive this counselling. We postulate that this treatment will increase infant growth and reduce infant morbidity.* Each of these trials is designed to address an important scientific issue and also uses an intervention that could be readily incorporated into public health programs. The combinations of the interventions will allow for analysis of combined effects and interactions between the interventions.

KEY PERSONNEL (List names of all investigators including PI and their respective specialties)

Name	Professional Discipline / Specialty	Role in the Project
1. Lars Åke Persson	Division Director, PHSD, ICDDR,B	Princ. Investigator
2. George Fuchs	Division Director, CSD, ICDDR,B	Co-Princ. Investigator
3. Shams El Arifeen	Epidemiologist (Child Health Programme, PHSD)	Co-Investigator
4. Eva-Charlotte Ekström	Scientific Consultant, CSD	Co-Investigator
5. Dewan S Alam	Sr. Medical Officer, MHRP, PHSD	Co-Investigator
6. K Rasmussen	Professor, International Nutrition, Cornell University	Co-Investigator
7. JP Habicht	Professor, International Nutrition, Cornell University	Co-Investigator
8. EA Frongillo, Jr.	Professor, International Nutrition, Cornell University	Co-Investigator
9. Robert Black	Professor, Johns Hopkins University, USA	Co-Investigator
10. Ruchira T Naved	Gender & Reproductive Health Specialist, SBSP, PHSD	Co-Investigator
11. Rubina Shaheen	Sr. Medical Off., Reproductive Health Prog., PHSD	Co-Investigator
12. AKM Iqbal Kabir	Scientist, Clinical Sciences Division	Co-Investigator
13. Saskia Osendarp	Nutrition Scientist, CSD	Co-Investigator
14. Lauren Blum	Medical/Nutritional Anthropologist, SBSP, PHSD	Co-Investigator
15. Yukiko Wagatsuma	Scientist, PHSD	Co-Investigator
16. MA Wahed	Head, Nutritional Biochemistry Laboratory	Co-Investigator
17. M Yunus	Senior Scientist and Head, MHRP, PHSD	Co-Investigator
18. Nigar Shahid	Scientist, CHP, PHSD	Co-Investigator
19. Motiur Rahman	Assistant Scientist, LSD	Co-Investigator

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be Tested:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods. Concisely list in order, in the space provided, the hypothesis to be tested in the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

The main hypotheses to be tested relate directly to the randomised study design:

1. Women in the early assignment food supplementation group will have infants with higher birth weight (BW) than women in the usual assignment group.
2. The intervention with 30 mg Fe, 400 µg folic acid and 13 additional micronutrients (MuMS) will increase maternal Hb concentration and birth weight compared to the 60 mg iron - folate (Fe60F) and 30 mg iron - folate (Fe30F) micronutrient interventions. The Fe30F intervention and the Fe60F intervention will not have any significantly different effects on maternal Hb concentrations or birth weight.
3. There will be an interaction between food supplementation and multiple micronutrient supplementation such that those women who began food supplementation early and received MuMS will have infants with the highest BW.
4. Asymptomatic pregnant women with bacterial vaginosis (BV) who receive appropriate treatment will have less pre-term newborns than those who do not receive treatment.
5. Those who receive counselling for exclusive breast feeding (EBF) will increase their duration of EBF compared to those who did not receive this counselling and, consequently, infant growth will increase and infant morbidity will decrease during the period of extended EBF.

This study also provides the opportunity to test a number of other hypotheses that will address important scientific issues and provide information to understand how these interventions can best be used in public health programs. In particular, we will learn about which women most benefit from the interventions and how much exposure to the interventions is needed to obtain a maximal effect. The other hypotheses to be tested are:

6. Total supplemental energy over the course of the pregnancy is the determinant of an increase in BW, so that there will be no differences on BW between early and late supplementation when controlling for total supplemental energy.
7. Only mothers who weigh less than approximately 50 kg before the beginning of pregnancy are likely to have infants who have benefited from food supplementation.
8. There is an asymptote at about 3200 g of BW, and an asymptote for supplementation itself, above which more supplementation will not improve BW much.
9. The differential effect on Hb concentration between the MuMS and Fe30F in comparison with Fe60F will be shown for women with initially low Hb concentrations where the Fe60F group may respond faster and reach the 110 g/L threshold more quickly.
10. There will be an interaction between the various amounts and types of supplementation and women's ability to extend the period of EBF. In particular, women receiving food supplementation earlier or who have high total supplemental energy will be able to extend the period of EBF longer and with less decrease in maternal weight.

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods.

In the developing world, and particularly in South Asia, poor maternal nutritional status remains an important determinant of long-term maternal health as well as of foetal growth and subsequent infant health and survival. We propose to conduct a set of randomised trials that will improve maternal nutritional status through supplementation and treat bacterial vaginosis so as to reduce maternal anaemia, increase birthweight (BW) and

reduce pre-term deliveries; and, in addition, will increase the duration of exclusive breastfeeding (EBF) so as to improve infant health and growth.

The proposed research consists of 4 combined intervention trials, all to be conducted in a single group of about 5,000 poor and undernourished women who live in the well-established ICDDR,B field site in Matlab upazilla, Bangladesh. An on-going, government-supported program in this area provides a food supplement to pregnant and lactating women that contains 600 kcal/d (6 d/wk) and a pill that contains 60 mg iron and 400 µg folic acid (Fe60F) daily. An on-going surveillance program for reproductive events identifies women who have become pregnant within 6-8 wk of conception. The 4 interventions and the primary hypotheses that we will test are:

1. Bangladesh has exceptionally high proportion of foetal growth retardation that has not been eliminated with the current government program of food supplementation that begins in the latter half of pregnancy. As part of the surveillance-for reproductive events, we will identify women within 6-8 wk of conception. After obtaining informed consent, we will randomly assign women (stratified by maternal weight and parity) to receive advice to begin the government's supplementation program immediately after diagnosis of pregnancy (early assignment) or at the time of their choosing (later assignment; usual care in this community). *We postulate that women in the early assignment group will have higher-BW infants than those in the usual assignment group.*
2. It is thought that an inadequate diet makes a considerable contribution to maternal anaemia. Anaemia in pregnancy is associated with low birth weight. The government program addresses two of the primary causes of nutritional anaemia, deficiencies of Fe and folic acid, but it is possible that deficiencies of other micronutrients limit the effect on haematologic status and birth weight of the current iron and folic acid supplementation. Within each of the 2 groups described above, we will randomly assign women to receive a pill that contains (a) 60 mg Fe and 400 µg folic acid (current practice) or (b) 30 mg Fe, 400 µg folic acid and additional micronutrients (MuMS) (equivalent in composition to UNICEF's multiple micronutrient formulation that is currently being evaluated in a number of developing countries) or (c) 20 mg Fe and 400 µg folic acid (Fe30F). *We postulate that MuMS will increase Hb concentration and birth weight compared to Fe60F. In addition, we postulate that there will be an interaction between food and MuMS supplement such that those women who begin earlier with food and receive MuMS have infants with the highest BW. We postulate that the Fe30F will have an effect not significantly different from the effect on birth weight by Fe60F supplementation.*
3. Bacterial vaginosis (BV) produces chorio-amnionitis, decreases foeto-placental circulation and produces scarring of the endometrial lining. This inflammation prevents expansion of the endometrial cavity and adds to the rising pressure within the cavity near mid-pregnancy making it incompatible with full duration of gestation. Within each of the 6 groups described above, we will randomly assign asymptomatic women who are diagnosed with BV to receive a course of: (a) 250 mg metronidazole given orally thrice daily for 7 days or (b) lactose tablets given orally thrice daily for 7 days. *We postulate that women in the metronidazole group will have longer gestation than women receiving the lactose tablets.*
4. Breastfeeding (BF) is nearly universal in the population group that we will study, but the duration of EBF is much shorter than is optimal for infant health. After delivery, we will randomly assign all of the subjects to receive either (a) counselling for EBF or (b) a different health education message of equivalent intensity. *We postulate that those who receive counselling for EBF will have a longer duration of EBF than those who did not receive this counselling. As a result, we postulate that this treatment will increase infant growth and reduce infant morbidity during the period of extended EBF.* Inasmuch as this trial will be conducted among women who also received the 2 sets of treatments described above, we will be able to examine the interactions among these various amounts and types of supplementation and women's ability to extend the period of EBF.

The proposed research will validate the causal impact of these interventions and also evaluate their synergistic effects and cost-effectiveness. This research also will permit us to assess who benefits from the interventions, estimate efficacy and cost-efficacy of the interventions, provide insight into ways to improve participation in the future, and permit extrapolation of these findings to future interventions in other settings.

Background of the Project Including Preliminary Observations

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is no sufficient information on the subject, indicate the need to develop new knowledge. Also include the significance and rationale of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. (Do not exceed 5 pages, use continuation sheets).

Despite overall improvements in living conditions in recent decades, the proportion of malnourished women and young children remains alarmingly high in many areas of the world. Nowhere is this more evident than in South Asia. For example, the rate of low birthweight (LBW) in and in the countries around Bangladesh is 28% in the most recent figures available (ACC/SCN, 2000). In studies in different localities in Bangladesh it has been shown to be as high as 50% (Arifeen *et al.* in press; Goodburn and Chowdhury, 1994; Osendarp *et al.* 2000). Inasmuch as the rate of LBW reflects both the mother's historic and current nutritional status and affects the prognosis for infant health, growth and development, this is an alarmingly high statistic. Despite the success in Bangladesh in recent years of preventing unnecessary deaths of infants and young children from diseases such as diarrhoea, many underlying causes of illness remain--particularly inadequate breast feeding and poor maternal and infant nutrition.

Bangladesh, like many developing countries, has a variety of programs, some provided by the government and others by various non-governmental organisations, that are designed to improve the health of mothers and their infants. The multidisciplinary research proposed here will improve evidence-based medicine related to public-health interventions in several ways. First, we will examine whether an on-going intervention, food supplementation, can be improved. We will do this by making it available to pregnant women earlier than has been the usual practice. Second, we will examine whether the on-going iron and folic acid intervention may be made more effective by reducing the amount of iron and thus reducing side-effects thereby improving compliance. Third, we will examine whether the MuMS intervention that UNICEF proposes should be implemented world-wide provides any additional benefits to women who are already receiving food and Fe60F supplements. Fourth, we will assess whether treatment of asymptomatic BV impacts on duration of pregnancy. Finally, we will examine whether synergistic effects between interventions are possible. We will do this by examining interactions between the 2 nutritional interventions offered during pregnancy (food and micronutrient supplementation) and a counseling intervention (promotion of EBF) offered postpartum. We focus on 3 primary outcomes: BW, maternal Hb concentration and the duration of EBF, each of which has important implications for either maternal or infant health. The population that we will study is similar to many others in the developing world, so that one can extrapolate our findings to other locations.

1. Rationale for intervention to change the timing of food supplementation

In typical research and program settings, food supplementation programs have started in mid-pregnancy. This reflects both the reality of many program settings in which women do not begin prenatal care until this time (or later) as well as the biological concept that food supplementation is likely to have the greatest effect at the time the foetus is growing most rapidly, namely the last trimester of pregnancy. However, there are reasons to think that this biological concept is at least inadequate. The setting of the proposed research provides a unique opportunity to examine this proposition because the active surveillance program for reproductive events will enable us to identify not only women who intend to become pregnant but also new pregnancies just a few weeks after conception. Thus, we will be able to offer one group of women a much longer period of supplementation than has been possible in the past.

Growth retardation in foetuses is evident as early as 8 wk of gestation (Smith *et al.*, 1998). In the United Kingdom, foetuses with lower than expected crown-rump length were at 1.8 times the risk of weighing < 2500 g at birth than those with normal length (Smith *et al.*, 1998), which indicates that intrauterine stunting begins in the first trimester. In Guatemala, where the rate of LBW and intrauterine stunting remains high, foetuses who were described as growth-retarded at birth were growth retarded (as assessed by foetal femur length by ultrasound) as early as 17-18 wk of gestation (Neufeld *et al.*, 1999). Epidemiological data indicate that chronically malnourished mothers with low pre-pregnancy weight and height, and malnourished mothers during early pregnancy (as indicated by low second trimester weight gain), have newborns who are shorter, lighter, and have smaller head circumferences

(Thame *et al.*, 1997; Arbuckle and Sherman, 1989). Maternal skinfold change in the first half of the second trimester is associated positively with BW. In addition, 1 kg of maternal weight gain in the second trimester results in more than twice the effect on BW than 1 kg of maternal weight gain in the third trimester (Li *et al.*, 1998). Thinner women also have lower placental/birth weight ratio. This indicates some insult in the placenta (Thame *et al.*, 1997), which is most susceptible to insult in the first half of gestation (Owens *et al.*, 1995). Similarly, in animal studies, chronic undernutrition reduces placental weight and foetal growth (Harding and Johnson, 1995) and is thought to be due to limited substrate availability.

Evidence from food supplementation trials conducted in developing countries is quite consistent across a variety of different kinds of food supplements: the more supplement a woman consumes, the bigger her baby. For a woman to consume more total food, she has to begin to accept supplementation earlier in pregnancy or consistently consume more food daily. In Guatemala, those who consumed $\geq 20,000$ kcal had babies who were 131 g heavier than those who consumed $< 20,000$ kcal (Lechtig *et al.*, 1975). In Indonesia, those who participated for > 90 d had infants who were 96 and 71 g larger (females and males, respectively) than those who participated for ≤ 45 d (Kardjati *et al.*, 1988). In Colombia, the effect was very small, but mothers of male who received the supplement for > 13 wk had babies who were 105 g bigger than controls, but the overall effect by participation for any duration was 95 g (Mora *et al.*, 1979). The effect on BW appears to be biggest when women receive the supplement throughout the reproductive period and consume it in large amounts. Compared to those who consistently consumed the least supplement in Guatemala, those who consistently consumed the most had second study infants who were 238 g larger than first study siblings (Villar and Rivera, 1988).

The fact that foetal growth retardation is evident by the middle of the first trimester, the observation that better maternal nutritional status in the second trimester is associated with better foetal growth, and the observation that those who consume more supplement daily or consume it for a longer period of time have larger infants all suggest that beginning a food supplementation program as early as possible during pregnancy is likely to result in larger infants than the women's usual care, namely starting supplementation later in pregnancy. It is this proposition that we will test in the proposed research.

2. Rationale for intervention to include micronutrients in pill supplementation

As a result of the ongoing program of Fe supplementation during pregnancy, the mean Hb concentration among non-pregnant women is higher (129 g/L) in women in Matlab than it was in 1975 (111 g/L) or than it now is in the rest of Bangladesh (120 g/L) (HKI & IPHN, 1999; Stoltzfus *et al.*, 1999). Nevertheless 23% of the women are anaemic (Hb < 120 g/L). This cannot be explained by haemoglobinopathies, because those are of low prevalence.

The expected size of the effect of iron/folate supplementation on intrauterine growth among anaemic or iron deficient women is not well known (Cook *et al.*, 1994), only few studies have addressed the issue. However, two randomised trials have reported an effect on birth weight (30 and 56 g) (Menendez *et al.* 1994; Preziosi *et al.* 1997). Furthermore, an observational study demonstrated as much as 172 grams increase in birth weight (Achadi *et al.* 1995). Still the efficacy of iron/folate supplementation in improving pregnancy outcome is currently debated. It is increasingly proposed that iron/folate supplementation should be replaced by a multiple micronutrient supplement. The justification for use of a multiple micronutrient supplement is that deficiencies do not happen in isolation and all the deficient micronutrients should be supplied. Deficiency of one nutrient may prevent the optimal use of others and thus a combination of nutrients may be necessary to produce the maximum effect on pregnancy outcome. For example, experimental studies have shown that vitamin A supplementation may have a positive effect on haemoglobin concentration and iron status and may improve haematological response to iron supplementation (Bloem, *et al.* 1989; Mejia *et al.* 1988; Muhilal *et al.* 1988; Suharno *et al.* 1996). There is a lack of knowledge on the effects of a number of other micronutrients, or combinations of micronutrients and energy/protein supplementation (de Onis *et al.* 1998).

There are three reasons for including a Fe30F pill in the study. (a) The efficacy and effectiveness of MuMS will be compared with that of Fe30F to evaluate the proportion of response attributable to the 13 additional micronutrients in the MuMS. To reduce the risk of side-effects of high total metal content the MuMS supplement contains only 30 mg iron and not 60 mg, that is the current recommendation. UNICEF is now promoting MuMS world-wide, but information on the efficacy and effectiveness of a lower iron content is still missing. (b) A lower dose of iron will reduce the side-effects and thus increase compliance (Ekström *et al.*, 1996). It may well be that a Fe30F tablet provides the same total amount of iron as the Fe60F supplement will. (c) There are reasons to believe that 60 mg of iron is higher than necessary and that 30 mg would be sufficient. In a similar region of Bangladesh, pregnant women (initial Hb 111 g/L) reached a plateau of response to Fe supplementation after ingesting a total of 2.5 g of

elemental Fe taken in doses of 60 mg/d (Ekström 1999, unpubl. data); 2.5 g of Fe corresponds to 15 mg/d over 2 trimesters.

The sample size of the proposed study is large enough to identify very small changes in Hb concentrations. If no significant difference is found comparing 30 and 60 mg iron/d, one can accept 30 mg/d of Fe as adequate. Furthermore, if no statistically significant difference in maternal Hb is found comparing MuMS and 60 mg iron/d, one can be confident that micronutrients provide no additional benefit.

Although the sample size is also adequate to detect a small (70 g) difference between the micronutrient supplement in birth weight it may be that the effect on birth weight will only be manifested in presence of food supplements. The interaction between food and micronutrients and the effect on birth weight will be examined.

3. Rationale for intervention to treat asymptomatic bacterial vaginosis

BV is a syndrome marked by an increase in the vaginal pH, milky creamy discharge, and amine or fishy odour. Microscopically, bacterial vaginosis is characterised by a shift in the vaginal flora from the dominant flora of *Lactobacillus* sp to a mixed vaginal flora that includes *Gardnerella vaginalis*, *Bacteroides* sp., *Mobiluncus* sp. and *Mycoplasma hominis* (Romero *et al.*, 1988; Gravett *et al.*, 1986; Hiller *et al.*, 1995).

Bacterial vaginosis is responsible for 40–50% vaginal infections in sexually active women and occurs in 12–20% of women of reproductive age including those who are pregnant (Hiller *et al.*, 1988). The prevalence of BV among pregnant women varies widely between different studies. BV was found in 31.8% of pregnant women at the University Hospital in Seattle (Gravett *et al.*, 1983). Prevalence was higher in black as compared to white clinic patients (29% vs 19.5%) and higher in the low socio-economic group and women with IUD devices. There are limited data on the prevalence of BV in pregnant women in developing countries. Longitudinal studies on BV among pregnant women have shown that, women whose vaginal flora was normal initially, rarely had developed BV at end of pregnancy (Hay *et al.*, 1994; Kurki *et al.*, 1992). However, spontaneous recovery has been observed in 50% of women who had BV at early pregnancy (Hay *et al.*, 1994; Christensen *et al.*, 1993). The pathogenesis of BV in pregnancy is not well understood. It is postulated that in the mid-ovarian cycle there are active uterine contractions associated with "in suck" of vaginal/cervical fluid and prompt transport to the fallopian tubes. This results in rapid ascent of pathogenic microbes during pregnancy leading to chorioamnionitis in case of pregnancy. The membranes of the expanding pregnancy seal the endometrial cavity near mid pregnancy, resulting in increased pressure, premature rupture of the membranes, and premature labour (Parsons & McGregor 1997).

There is a school of thought that BV is a self-limiting condition during pregnancy. Thus, controversy remains as to the rational of treating BV during pregnancy because women with a positive laboratory diagnosis in early pregnancy may come up with a negative laboratory diagnosis in late pregnancy. Since asymptomatic BV infections are as common as symptomatic infections, there is again the controversy of exposing pregnant women to unnecessary drugs keeping in mind the self-limiting nature of BV infections. The absence of the organisms in the lower genital tract does not necessarily mean that the infection is cleared from the upper reproductive tract. There is evidence that the organisms in the vagina ascend and cause chorio-amnionitis and endometritis resulting in premature termination of pregnancy, thus there is justification for treatment during pregnancy. In addition, treatment of BV at varying gestational ages may have differences in pregnancy outcomes.

Several studies have shown an association between BV and pre-term delivery. BV has now been considered as a major independent risk factor for pre-term birth and low birth weight (LBW). Results of prospective studies have shown that women who have BV at the beginning of second trimester have a 5-fold increase in risk of having pre-term labour or late miscarriage and pre-term low birth weight infants independent of other recognised risk factors (Hay *et al.*, 94). However, BV associated pathogenesis occurs in early gestation (late first trimester) although, the outcome of the pathogenesis is often late (third trimester). It has been reported, that BV after 26 weeks gestation is associated with a relative risk (RR) of pre-term delivery of 1.4–1.9, whereas before 16 weeks gestation, the RR is 5–7.5. A hospital based study in Indonesia examined the association between BV and pre-term delivery in a low-income group of women (N= 490). The prevalence of BV in this population was 17% at 16–20 weeks and 15% at 28–32 weeks (Riduan *et al.*, 1993). The rates of pre-term delivery were double in women who had BV in early pregnancy (20.5%) as compared to women who had BV in late pregnancy (10.7%) (Riduan *et al.*, 1993).

Results of studies conducted to assess the impact of diagnosis and treatment of BV for the reduction of pre-term deliveries are mixed. Application of treatment (week of gestation), treatment regimen, dose, duration of treatment seems to play a vital role in the reduction of pre-term birth. In a recent review paper it was concluded that the identification and treatment of BV in pregnancy lead to substantial reduction in pre-term birth and LBW (Paige *et*

(1998). Although some studies have documented a significant reduction of pre-term labour by treating BV, others have not found such an effect (Morales *et al.*, 1994; Hauth *et al.*, 1995; Mc Donalds *et al.*, 1997; Carey *et al.*, 2000). Studies adopting diagnosis of BV in first trimester and early treatment with metronidazole have had better success. It has been shown that treatment of BV instituted either in late first trimester or in early second trimester results in significant reduction of pre-term labour compared to treatment in late second trimester (Morales *et al.*, 1994; Hauth *et al.*, 1995). However, in a recent multi-centre study in which the intervention regime (two 2-gm doses of metronidazole vs placebo) was given after 20 weeks of gestation observed no effect of treatment (Carey *et al.*, 2000). These results suggest that though efficacy seems highly likely, the evidence to support such a conclusion is not yet adequate.

The prevalence of BV among pregnant women in Bangladesh is not well documented. Data from etiological studies among women attending an urban delivery unit showed that the prevalence of BV was 40-44% in women reporting with RTIs (Bogaerts *et al.*, 1999). Population based studies among females from the general population in rural Bangladesh showed a prevalence of 9%. In the same population the prevalence of BV was found to be 20% among patients with symptoms RTIs (Hawkes *et al.*, 1999).

Metronidazole (tablet and vaginal suppository) and clindamycin (tablet and vaginal suppository) have long been used for treatment of BV. In 1998 the recommended treatment guideline for sexually transmitted infections (STI) of the Centres for Disease Control (CDC) recommended treatment for BV in pregnancy to be metronidazole 250 mg three times daily for 7 days. A 2g single dose treatment of metronidazole was recommended as an alternate regimen but discouraged due to high failure rates (CDC 1998, Larson *et al.*, 1992). Metronidazole vaginal suppository and clindamycin cream was not recommended in pregnancy (CDC 1998).

Results of studies conducted to assess the impact of diagnosis and treatment of BV for the reduction of pre-term delivery are mixed. Timing of treatment, treatment regimen, dose, duration of treatment seem to play a vital role in the reduction of pre-term birth. Although some studies have documented a significant reduction of pre-term labour by treating BV, others have failed (Morales *et al.*, 1994; Hauth *et al.*, 1995; Mc Donalds *et al.*, 1997; Carey *et al.*, 2000). Studies adopting diagnosis of BV in first trimester and early treatment with metronidazole have had better success. It has been shown that treatment of BV instituted either in late first trimester or in early second trimester results in significant reduction of pre-term labour compared to treatment in late second trimester (Morales *et al.*, 1994; Hauth *et al.*, 1995). However, a recent multi-centre study in which the intervention regime (two 2-gm doses of metronidazole vs. placebo) was given after 20 weeks of gestation observed no effect of treatment (Carey *et al.*, 2000).

BV is an important cause of RTIs in both developing and developed countries and has been incriminated to cause premature rupture of membranes (PROMS) and preterm (PT) deliveries. In Bangladesh, as part of the WHO-recommended syndromic management strategy, metronidazole is the recommended treatment for abnormal vaginal discharge, (Bangladesh MHPW, NIPHP, Technical standard 1999). The recommendations are to give 400 mg of metronidazole twice daily for 7 days. This empirical regime is prescribed for women coming to a health facility with the complaints of vaginal discharge and is not contraindicated after the first trimester of pregnancy. This recommendation only applies to symptomatic women. We are only proposing to enroll asymptomatic women who would not otherwise receive the current standard of care in the country. Since the efficacy of treating BV still remains debated, we feel that it is ethical to conduct a placebo-controlled clinical trial to establish this efficacy. As stated above, the study procedures will not result in depriving any women from receiving the standard care that she is eligible for.

This study will provide data on prevalence of BV (symptomatic and asymptomatic) in pregnant women. It will also examine cure rates for BV during pregnancy of both the symptomatic and asymptomatic groups with and without treatment. Randomisation of treatment in BV positive cases will provide the necessary data on whether treatment during pregnancy is required at all since there are indications that the infection may be self limiting in later pregnancy. Randomising treatment in asymptomatic BV cases in early pregnancy will provide evidence in preventing colonization and subsequent inflammatory process which may lead to preterm labour (Lamont RF 2000).

Bangladesh has one of the highest reported frequencies of pre-term delivery. Community-based studies in Dhaka and Matlab have found rates of pre-term deliveries ranging from 13-17% (Arifeen 1997; Alam, unpublished results). Such high rates contribute to the high level of LBW in Bangladesh. To the best of our understanding treatment trials of BV have never been conducted in developing country with high rates of both BV and pre-term delivery. We will examine the possible effect of treatment of BV on the length of the gestational period.

4. Rationale for intervention to increase the duration of EBF

BF confers significant health, nutritional, immunologic, developmental, psychological, social, economic, and environmental benefits to infants, mothers, families, and society. "Human milk is uniquely superior for infant feeding and is species-specific: all substitute feeding options differ markedly from it. The breast-fed infant is the reference or normative model against which all alternative feeding methods must be measured with regard to growth, health, development, and all other short- and long-term outcomes" (American Academy of Pediatrics, 1997). Feeding recommendations endorsed by international agencies and academies of paediatrics specify that infants should be exclusively breast-fed for 4-to-6 months, with BF continuing thereafter while the infant is also fed appropriate, adequate, and safe complementary foods.

A large percentage of infants in the world, however, are not fed according to these recommendations. In Bangladesh, only 53.5% of infants <4 mo of age are exclusively breast-fed, while the median duration of partial BF is about 3 y (ACC/SCN, 2000). Therefore, there is a need to improve the duration of EBF in Bangladesh, especially because it is this BF behaviour that is most associated with infant health and survival (ACC/SCN, 2000).

Several studies have demonstrated that BF promotion is effective in increasing the duration of EBF, with interpersonal counselling being the crucial intervention (ACC/SCN, 2000; Alberman et al., 1998). BF promotion is understood to be one of the most cost-effective interventions for child health, comparable to immunisations, with important effects on reducing morbidity and mortality (WHO Collaborative Study Team, 2000). Furthermore, concerted efforts to increase EBF in countries are related to shifts in population-level changes in behaviours (ACC/SCN, 2000).

The timing of an intervention to promote EBF is important so as to be able to positively affect mother's decision making, motivation and problem-solving, and persistence in the face of negative influences and obstacles. Inasmuch as EBF declines sharply in the first few weeks after delivery, it is important to especially counsel mothers right after delivery, and during the first weeks (ACC/SCN, 2000). Work already completed in Bangladesh has shown that community-based peer counselling increases the duration of EBF (Hinder et al., 2000).

Although mothers in Bangladesh are poorly nourished on average, their lactational capacity is not severely impaired. Nevertheless, mothers' milk production is somewhat limited by their nutritional status and may be able to be increased through improvement in nutritional status (Brown et al., 1986). Consequently, interventions to improve nutritional status of mothers during gestation and lactation may have a synergistic effect with promotion of EBF on both the duration of EBF and the well-being of infants. This potential benefit for the well-being of infants needs to be studied along with the potential benefits and costs for mothers (Frongillo and Habicht, 1997). The research proposed here will provide a unique opportunity to examine these issues in a comprehensive manner, with attention to multiple maternal and infant outcomes as well as to cultural, social, behavioural, and biological factors. This research is needed to understand how best to promote the well-being of mothers and infants in Bangladesh, but also to understand the trade-offs implied by alternate interventions and public-health actions (Frongillo and Habicht, 1997).

5. Role of behavioural sciences in understanding the determinants of utilisation of the interventions

In the proposed research we will obtain social and cultural data to identify the determinants of utilisation of the intervention by subjects in the study villages. This information can be used to: (a) improve the effectiveness of programs, so as to improve participation and response in the future; (b) elucidate the impact of the intervention by permitting an investigation of the interplay between behaviours that affect energy intake (participation and home diet) and energy expenditure (reduction in energy intensive activities), and (c) increase the power of the data analysis by reducing error terms.

The extent to which individual women utilise the two of the supplementation components, namely the food and micronutrient supplements, can be regarded as a function of the socio-cultural appeal and power of "complying" with program requests in relation to the competing and complementary power of other demands to comply with socio-cultural expectations about behaviour in pregnancy. These expectations derive from several sources, including, but not limited to, long-standing cultural beliefs about how to act during pregnancy to ensure a positive outcome as well as family power structure, which determines whose beliefs and desires influence the behaviour and choices of the pregnant women. Although cultural beliefs and expectations provide a general framework for individual behaviour, intra-cultural diversity (e.g., in the strength of specific beliefs, psychological motivations and

household social context) results in behavioural differences, including different responses to health and nutrition interventions. In this component of the study, we will identify and measure a set of socio-demographic and cultural factors that we postulate will affect the extent to which subjects will utilise the food and micronutrient supplements.

We postulate that socio-cultural factors that potentially affect participation in the proposed research by women in Matlab include: (a) type of family structure, (b) age and parity, which may affect whether a woman returns to her own family during pregnancy and how much deviation from routine activities she is permitted, (c) education of the subject and family members, which may affect their understanding of the purpose of the supplements and willingness to engage in new activities, (d) prior pregnancy experience; (e) work roles and responsibilities of the subject as well as household socio-economic organisation, which may also influence willingness to participate and affect her potential to reduce energy expenditure; (f) the structure for decision-making in the household, and the exposure of the woman to chronic stress, e.g. domestic violence; and (g) cultural beliefs concerning what to avoid and what to do to ensure a safe pregnancy and delivery and a positive outcome for the child. The strength of such beliefs is likely to affect participation. As cultural beliefs and social expectations interact to produce behavioural responses, we expect that interactions among these factors, rather than the strength of individual characteristics, will account for differences in utilisation of the interventions.

Violence may also lead to LBW through stress and anxiety (Wadhwa 1996). Stress, acting through the neuroendocrine axis, causes the release of catecholamines, beta-endorphin, and cortisol, which have the effect of vasoconstriction, fetal hypoxia, growth restriction and LBW, as well as provoking the release of prostaglandin, thereby contributing to preterm labor (Istivan, 1986). We will measure stress through standardised questionnaires, which will enable us to study interactions with the intervention effects, and associations to compliance.

6. Efficacy, effectiveness, compliance and cost-effectiveness

The proposed randomised design with high quality measures of compliance will enable estimations of both efficacy and effectiveness of the interventions. In "intention-to-treat" analysis, disregarding the information on compliance, estimates of effectiveness will be calculated. These estimates may be used for cost-effectiveness evaluation.

As compliance is estimated it is also possible to evaluate the effect in relation to dose of intervention. This means that measures of efficacy will be calculated. Effectiveness is the product of efficacy, quality of program implementation and coverage. For the food and micronutrient supplements, dose will be measured by amount ingested, and is directly related to compliance. Compliance is expected to be high but with enough variability to ascertain a range of doses. Response also depends on the nutritional deficiency of the individual (e.g., there is no dose response if there is no deficiency). Thus, overall efficacy depends on the distribution of deficiency in the population. This variation in efficacy is not usually taken into account in cost-effectiveness analyses. Our proposed study will ascertain this variation (Winkvist et al., 1998; Ekstrom et al., 1996, 1999) and include it in our cost-effectiveness calculations.

The quality of the program implementation will be high and constant in this study. Coverage of the target population is high. Limitations in effectiveness are mostly due to variation in compliance and in efficacy and no true estimation of program effectiveness will be achieved. However, using estimates of efficacy and compliance from this study, one can calculate the expected program effectiveness in other settings where coverage and the degrees of nutritional deficiency are known. In these simulations to plan interventions, one must also consider equity, which may be impaired by higher cost-effective interventions.

7. Conclusion

We propose here a unique set of linked investigations that will not only contribute to the knowledge base on which common public-health interventions are based, but also will provide practical guidance for implementation of similar programs elsewhere. The primary health variables addressed, namely maternal Hb concentration, BW, pre-term delivery and duration of EBF, are among the most important determinants of maternal and infant health and survival and infant performance. Finally, we have designed this multidisciplinary investigation to measure, and to account for statistically, factors that predict participation in these investigations. The proposed research will validate the causal effects of these interventions and also evaluate their synergistic effects and cost-effectiveness. This research also will permit us to assess who really benefits from the interventions, estimate their efficacy and

cost-efficacy, provide insight into ways to improve participation in the future, and permit extrapolation of these findings to future interventions in other settings.

PRELIMINARY OBSERVATIONS

In this section we first describe the Matlab field station of ICDDR,B in which the proposed research will occur. Next, to provide evidence of the suitability of this area for the proposed studies, we provide information on the health and nutritional status of women and their infants in Matlab and similar areas of Bangladesh. Finally, to provide evidence of our ability to execute intervention trials and use methods that might be suitable for the research that we proposed here, we describe the results of recent relevant interventions.

1. The Matlab Field Station

Matlab is an *upazilla* (sub-district), which is the administrative unit with the lowest-level management unit in the health system. The population of Matlab is about 500,000 and is divided into 22 unions, the smallest local government body. The population of each union is about 22,000.

ICDDR,B's activities in Matlab involve extensive service delivery and data collection. A Health and Demographic Surveillance System (HDSS) is in place, which covers a population of about 220,000 people in over 140 villages. Started in 1966, the surveillance has been in operation continuously, and is the largest longitudinal demographic data collection system in a developing country. Demographic data are routinely collected on vital events--including births, deaths, cause of deaths, marriages and migration. Use of a unique identifying system allows every individual to be tracked over time and across studies and databases. ICDDR,B provides community-based reproductive and child health services in half of the area. In the other half, only government services are available. HDSS also collects data on morbidity and service utilisation. Community health workers (CHW) are now collecting all data during their monthly household visits.

The area where ICDDR,B provides services had a population of 109,573 in 1998, which included 28,352 currently married women. The total fertility rate is 3.0, with 2,287 live births annually. The neonatal and postneonatal mortality rates are 36.8 and 13.8/1,000 live births, respectively. This area is divided into 4 blocks, each with about 25,000-30,000 population and a health sub-centre. A sub-centre is staffed with 2 medical assistants, one family welfare visitor and one nurse-midwife. The sub-centres provide a mix of sick child management and reproductive health services. Complicated illnesses are referred to the main primary care facility, operated by ICDDR,B in the same premises as its administrative offices at Matlab Bazar.

In the ICDDR,B-served area, female CHW have been recruited from the villages and trained to provide reproductive and child health services. Most of what they do takes place when they visit each household monthly. However, some services, like immunisation, are provided by the CHW from cluster points. A CHW is responsible for about 400 households in the HDSS intervention area. They provide immunisation, family planning services, and treatment for cases of diarrhoea and respiratory infection. More complicated cases and reproductive health cases (e.g., severe pneumonia, deliveries or reproductive tract infections) are referred to the sub-centre or primary care facility. Education and counselling on reproductive and child health are also an important component of the CHW activities.

2. Maternal and childhood nutritional status and birth weight in Matlab and Bangladesh

2.1 Maternal nutritional status and pregnancy weight gain

Low body weight is quite common among rural Bangladeshi women. The data are consistent among studies and provide no evidence that maternal nutritional status has improved over time. Women in Matlab appear no better nourished than do women elsewhere in Bangladesh. Typically, Bangladeshi women are slender (weight of 40-45 kg) and short (148-151 cm tall) (Fauveau and Chakraborty, 1994; Bhuiya, 1993; Stoltzfus et al., 1998; DS Alam, unpubl. data; L Kiess, unpubl. data). We estimate their average body mass index (BMI) as 18.3-20.4 kg/m² from data on weight and height in recent surveys.

In a study conducted in Matlab more than a decade ago, it was observed that over a period of about 15 wk beginning at 20 wk of gestation, women gained an average of only 3 kg. More recently, women gained an average 200 g/wk or an estimated total of 4 kg weight during the last 20 wk of pregnancy (DS Alam, unpubl. data). These

are about half what is expected among well-nourished women (IOM, 1990). Notably, women lost about 1 kg weight during the first 6 mo of lactation. As evident from low pregnancy weight gain and the small weight change postpartum, women do not compensate for the energy stress during pregnancy and lactation by gaining or losing much body weight.

2.2 Birth Weight

An early study in Matlab had found that 50% of new-borns were LBW (Khan et al., 1979). More recently, DS Alam (unpubl. data) reported LBW rate of 48% from an intervention study in Matlab. In a study to assess the effect of the BINP supplementation program in Shaharasthi, an *upazilla* adjoining Matlab, the average BW of the participating women was 2,513 g and 47% were LBW (Shaheen et al., 2000). Infants who were born in 1994-95 in selected slum areas of Dhaka had a mean BW of 2,516 g and 46% were LBW ($n = 1,654$) (Arifeen, 1997). Thus, this high rate of LBW is widespread and, unfortunately, seems to be unchanged after 2 decades.

The Dhaka data provide information about foetal growth retardation. In that study, babies with BW $< 10^{th}$ percentile of a reference that was specific for gestational age were classified as small-for-gestational-age (SGA). SGA infants with low ponderal index (PI) (i.e., less than 10^{th} percentile of a reference chart) were classified as asymmetric-SGA and those with normal PI were defined as symmetric-SGA. Seventy per cent were SGA: 44% of the sample were symmetric SGA and 26% were asymmetric SGA. About 17% of the babies were premature; a similar pre-term rate was reported from a study in Matlab (DS Alam, unpubl. data). Inasmuch as maternal undernutrition is associated with symmetric foetal growth retardation, the high proportion of these characteristics in Bangladesh suggests that LBW is primarily the consequence of chronic restrictions during gestation.

2.3 Risk Factors for LBW

In the study in the Dhaka slums (Arifeen, 1997), the mothers were 149 (± 5) cm tall and weighed 43 (± 6) kg after delivery. In an analysis of a sub-sample ($n=1,060$), Antelman (1997) found that maternal height and postpartum (7 d after birth) weight and arm circumference were associated with foetal growth retardation. Low intake of animal protein was also associated with foetal growth restriction. We (Alam, 1998) found that maternal height and weight in pregnancy also were positively associated with BW in an intervention trial in Matlab.

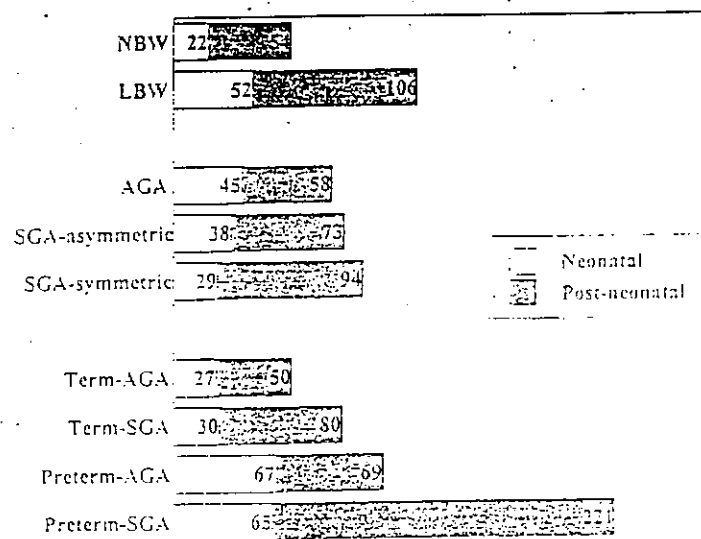
2.4 Consequences of LBW

LBW infants in slums of Dhaka remained persistently lighter throughout infancy (Arifeen, 1997). The growth of the infants in our sample closely tracked the -2 SD curve of a pooled sample of breast-fed infants from affluent countries (WHO, 1994). Their SD-score at birth was -2.38 compared to a reference population; it improved slightly in the next 3 mo, and was again similar to birth at 12 mo of age (-2.34 SD-scores). Catch-up growth was not seen and differences between infants grouped according to BW, foetal growth retardation and/or pre-term status were retained throughout infancy, even after adjusting for other variables.

Compared to normal-BW infants, LBW infants were at greater risk of death from all causes ($RR=2.08$), acute respiratory infection ($RR=2.52$) and diarrhoea ($RR=2.79$). Pre-term, growth-retarded infants were at the greatest risk ($RR=4.1$ compared to normal-BW infants, $RR=3.2$ compared to other pre-term infants), especially for deaths due to acute respiratory infection ($RR=6.03$). Although both pre-term delivery and foetal growth retardation were associated with increased risk of death, the timing of the effect varied: foetal growth retardation contributed to greater post-neonatal mortality (Figure 1).

In Matlab, only limited data on childhood nutrition are available. However, data collected as part of nutritional surveillance show that the mean height-for-age SD-score was just below -3.5 in 1970-71. By 1974, this had increased to about -2.7, where it remained until about 1988. Since 1991, the height-for-age SD-score has remained fairly constant at just over -2.5. Thus, child nutritional status in Matlab is still alarmingly poor and has not been improving recently.

Figure 1: Estimated mortality risk per 1000 live births by status at birth: Dhaka Slums, 1994-95



3. Bacterial vaginosis and rates of pre-term delivery

Data from etiological studies among women attending an urban health care delivery unit in Bangladesh showed that the prevalence of BV is 40-44 % in women reporting with RTIs (Bogaerts *et al.*, 1999). Population based studies among females from general population in rural Bangladesh show that the prevalence of BV is 9%. In the same population the prevalence of BV was found to be 20% among patients with symptoms RTIs (Hawkes *et al.*, 1999). Community-based studies in Dhaka and Matlab have found rates of pre-term deliveries ranging from 13-17% (Arifeen 1997; Alam, unpubl. results).

4. Data on relevant interventions

4.1 Data on effectiveness of interventions in preventing LBW

There are very limited data on effectiveness of interventions in preventing LBW in Matlab or Bangladesh. In a randomised clinical trial in a slum population in Bangladesh, daily supplementation of pregnant women with 30 mg of zinc had no effect on BW or gestational age at birth (Osendarp, 2000).

Shaheen (2000 and unpubl. data) recently evaluated the relationship between duration of food supplementation and BW within the BINP supplementation program in Shaharasthi. Women with a BMI <18.5 kg/m² early in pregnancy were invited to participate in the program. The study sample comprised of 716 births. The duration of supplementation duration was normally distributed, with an average supplementation period of 118 d, and a range from 4 to 229 d. In this study, BW improved with longer duration of supplementation. Women who weighed <50 kg benefited from increasing duration of supplementation, especially those below 42 kg. There were very few who weighed 50 kg or more, and there was no conclusive pattern in the response to supplementation among these women.

Interesting patterns were also observed in terms of variations BW by season at birth. BW was lowest among children born in the winter.

There has been no trial in Bangladesh evaluating the effect of treating BV on pre-term delivery.

4.2 Iron and micronutrient supplementation, and anaemia

Anaemia is prevalent among women in Bangladesh [60 and 85% among rural pregnant and non-pregnant women, respectively in the latest national nutrition survey (Jahan and Hossain, 1998)]. However, this is much less so in Matlab, where a long-term Fe (60 mg/d) supplementation program in pregnancy has raised mean Hb concentrations (Stoltzfus *et al.*, 1998) to the values (124-127 g/L) observed worldwide after adequate Fe supplementation. (Ekstrom *et al.*, 1996, IOM, 1990). A similar concentration of Hb (120 g/L) was achieved from a baseline value of 110 g/L in a recent supplementation trial of Fe supplementation during pregnancy in Bangladesh. A maximum

target was attained at a total intake of Fe of 2.4 g (Ekstrom et al., 1999). We think that a total Fe intake of 2.4 g could be attained with 30 mg of Fe daily in Matlab given current compliance rates ($70\% \pm 25\%$) and initiation time (second trimester). Although 30 mg/d is half the international recommended daily dose during pregnancy (INACG, 1998), this dose should prevent anaemia in Matlab because it was high enough to cure anaemia in another, similar setting in Bangladesh (Ekstrom et al., 1999). Inclusion of an intervention with a 30-mg iron dose will enable an evaluation of the effect of additional micronutrients.

In a recent iron supplementation trial of 162 pregnant women in a rural area of Bangladesh, serum zinc levels were very low (8.37, 7.56 and 8.02 $\mu\text{mol/L}$ in second trimester, third trimester and 6 wk postpartum, respectively). Almost two thirds had serum zinc concentrations $<10.7 \mu\text{mol/L}$ in the third trimester (LA Persson, unpubl data). There was no association between serum zinc concentrations in the third trimester and the dose of iron supplements.

4.3 Exclusive breast-feeding (EBF) and counselling

In a hospital-based intervention study on mothers of partially breast-fed infants admitted to hospital for diarrhoea, lactation counsellors advised a randomly selected group of these mothers on EBF (Haider, 1996). When followed up at home 2 wk later, 75% of mothers in the intervention group were EBF compared with only 8% in the control group. Case studies of the mothers in the intervention groups who were not practicing EBF revealed factors relating to family support and workload as responsible (Haider, 1997). The investigators repeated the study in the community, randomising 40 clusters to either intervention or control. A local woman was trained as peer counsellor in each intervention cluster. Of the 573 women who completed follow-up until the infant was 5 mo old, 70% of those in intervention clusters breast-fed exclusively for 5 mo compared to only 6% in the control clusters ($p < 0.0001$). The mean weight-for-length SD-score was 0.1 in the intervention group and -0.9 in the control group ($p < 0.0001$) (Haider, 1998).

As is the case elsewhere in Bangladesh, rates of EBF in the first 4-6 mo remain low in Matlab. In his 1995-7 study, DS Alam (unpubl. data) found that 60% of the infants were exclusively breast-fed during the first 3 mo, which dropped to 31% at 6 mo. Unpublished 1999 data from the Matlab HDHS show that 64% of infants aged 0-1 mo were exclusively breast-fed, declining to 27% in 2-3 mo old infants and 15% at 4-5 mo of age.

5. Summary and conclusions

An exceptionally strong infrastructure for research of the type proposed here is present in Matlab *upazilla*. We will be using the existing surveillance of reproductive events to identify women who are likely to become pregnant or who have just recently conceived. The type of maternal undernutrition that is present (very low BMI values) and its association with high rates of foetal growth retardation as expressed in the rates of LBW and symmetric foetal growth retardation suggest that: (a) undernutrition is a chronic condition for mothers and their fetuses and (b) improvements in maternal nutrition have the potential to improve BW. Although Matlab *upazilla* is maybe no longer characterised by the exceptionally high rates of maternal anaemia that have been reported previously in South Asia, the rates remain too high and interventions to lower them are appropriate. Rates of BV and pre-term delivery is high. Finally, although BF is nearly universal, extended EBF is not the norm. We have experience with a counselling intervention, similar to that proposed here, which was effective in improving the duration of EBF.

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the project. Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scientific validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the specific aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project. (Do not exceed ten pages, use continuation sheets).

The proposed study will be conducted in collaboration with the Bangladesh Integrated Nutrition Project (BINP) and its follow-on National Nutrition Project (NNP). This will ensure that the study findings are incorporated into on-going national programmes. Similarly, a close collaboration exists with BRAC, a non-governmental organisation, which is assisting the government in implementing the BINP program in the Matlab *upazilla*.

1. STUDY SITE

The Matlab study site has been described in detail in Preliminary Results. The Matlab Health Research Programme of ICDDR,B has instituted a program of routine collection of weights of all non-pregnant women 13-44 y old. It is estimated that there are 33,000 women aged 13-44 years, who reside in the Matlab intervention area. In mid-2000, special field workers were recruited to weigh these women. They are using the UNISCALE, a scale produced by UNICEF, which is both portable and easy to use. The scales will be standardised every morning. An independent quality control team will do repeat assessments of weights in a 5% sub-sample. In a small sub-sample we will obtain repeated measurements over 12 mo to assess seasonal variation, because data from other sources suggest that maternal weight may vary by season.

2. STUDY DESIGN AND INTERVENTIONS

The study is an experiment to evaluate the efficacy and effectiveness of 4 public health nutrition interventions designed to improve the health of pregnant women and their new-born infants. The outcomes will be assessed during pregnancy, at delivery, and in the infant. Three of these interventions will be delivered when the woman is still pregnant, and the fourth intervention will be delivered to mothers from the same cohort who have just delivered a live born infant. Women will be individually randomised to the intervention groups, and comprehensive mechanisms will be set up to obtain data on the outcomes as well as factors likely to be confounders or effect-modifiers.

2.1 Interventions to be delivered during pregnancy

2.1.1 Daily food supplementation (early/usual): An on-going, government-supported program provides a food (energy-protein) supplement to pregnant and lactating women that contains 600 kcal/d (6 d/wk), of which a little less than 25% will come from vegetable protein. It is made up of fried rice powder (80 g), fried pulse powder (40 g), molasses (20 g), and soybean oil (12 mL). These supplements are made available through community nutrition centres (CNC) run by the program. Pregnant women with BMI $<18.5 \text{ kg/m}^2$ are required to consume the supplements under supervision 6 d a week at the CNC. As part of the study, 2 changes will be made in this supplementation program. All pregnant women, irrespective of BMI, will be enrolled in the supplementation program. Pregnant women will be individually randomised to enrolment into the feeding program either: (a) immediately after diagnosis of pregnancy (early assignment), or (b) at the time of their choosing (usual care in this community).

2.1.2 Daily micronutrient supplementation: The government recommendation for Bangladesh is that all pregnant and lactating women receive a tablet that contains 60 mg Fe and 400 μg folic acid daily. Currently, this supplement is made available through the CNC, to be taken home by the woman for daily ingestion. In the proposed study, all pregnant women will be individually randomised to receive one of the following 3 types of micronutrient supplements daily in lieu of the current iron-folate supplements: (a) 30 mg iron-folate (Fe30F), which contains 30 mg Fe and 400 μg of folic acid; (b) 60 mg iron-folate (Fe60F), which contains 60 mg of iron and 400 μg of folic acid; and (c) multiple micronutrient supplement (MuMS) has recently been developed by UNICEF for evaluation in a number of countries. One tablet (daily dose) contains 800 μg RE vitamin A, 200 IU vitamin D,

10 mg vitamin E, 70 mg vitamin C, 1.4 mg vitamin B₁, 1.4 mg vitamin B₂, 18 mg niacin, 1.9 mg vitamin B₆, 2.6 µg vitamin B₁₂, 400 µg folic acid, 30 mg iron, 15 mg zinc, 2 mg copper, 65 µg selenium and 150 µg iodine.

2.1.3 Treatment of bacterial vaginosis: The Centers for Disease Control (CDC) has recommended that BV in pregnancy be treated by metronidazole 250 mg three times daily for 7 days. Eligible pregnant women will be randomised to receive either (a) oral metronidazole 250 mg, or (b) oral lactose, both three times daily for 7 days.

2.2 Intervention to be delivered after the birth of the child

2.2.1 Counselling to promote EBF: Mothers will be counselled right after delivery and during the first weeks to be able to optimally bolster mothers' decision-making, motivation and problem-solving, and persistence in the face of negative influences and obstacles. The lactation counselling intervention will be modelled on community-based interventions already shown to be effective in Bangladesh (Haider et al., 2000) and Brazil (Albernaz et al., 1998). The alternative intervention will consist of standard health education on maternal and new-born/child care. It will be delivered with the same frequency and intensity as the BF intervention. Four *Counsellors*, each an expert in only 1 of the 2 interventions (e.g. lactation counselling or health education), will be based in each of the sub-centres. The BF *Counsellors* will be trained by a pair of expert lactation trainers in the employ of ICDDR,B. The *Counsellors* will first visit each infant within 3 d of birth. The visits will be repeated at 15 d of age of the infant and again at 1, 2, 3 and 4 mo. They will provide counselling on the intervention assigned to the mother-child pair.

3. STUDY PROCEDURES

Seven types of field workers will be used to implement specific study activities as outlined below. These will include: paramedics, follow-up interviewers, dietary interviewers, CNC monitors, ultrasonographers, interviewers trained in qualitative research methods, and counsellors.

3.1 Measurement of determinants of utilisation of the pregnancy interventions

We propose to obtain social and cultural data to identify the determinants of utilisation of the intervention by subjects in the study villages. In preparation for this research, informal ethnographic interviewing revealed a number of common pregnancy-related beliefs and practices that potentially affect BW and that may affect utilisation of the intervention (see Preliminary Results). As a result, the proposed research will include an ethnographic study that will focus on the following sectors of information. (a) Attitudes toward consumption of micronutrient "tablets" during pregnancy and familiarity with prenatal supplements. It should be noted that from a community perspective, 2 distinctly different interventions are being undertaken—food, provided at a community center, and "tablets", taken at home. This information is necessary to determine the best way to present the micronutrient intervention to the community. (b) Specific beliefs concerning eating and foods, pregnancy events, and individual attributes that may affect consumption of micronutrient and food supplements. (c) Household characteristics related to authority over women's actions, household workload and flexibility to participate in the intervention. It is expected that other insights into potential constraints and facilitators of participation will also emerge from this study. This research step will be conducted when the project is formally initiated and will take 6 wk. Data will be collected by means of key informant interviewing, verification with respondents, and the use of formal ethnographic techniques, as appropriate to the type of information being collected. We will use the data obtained in this focused ethnographic study to design specific questions for the baseline sociocultural data on determinants from all study subjects.

3.2 Identification, enrollment and randomization of pregnant women

CHW of ICDDR,B visit households monthly to collect information on selected health and demographic indicators. They routinely ask currently married women whether they have had their menses since the last visit. This system will be reinforced by offering them a pregnancy test, whenever a woman reports that her last menstrual period (LMP) is overdue by 2 wk or more or that she is pregnant after a continuous period of amenorrhea. Informed consent will be obtained for the pregnancy test and the woman's privacy will be maintained. The woman will be informed of the result of the test. After giving verbal informed consent, a woman who tests positive will be enrolled in the study. Date of her LMP also will be recorded and used to estimate gestational age.

The name and identification of this pregnant woman will be communicated to the project office, where she will be randomised to one of the following 6 groups by entering her name into a computerised tracking system that will then assign her to a group. Randomisation will be done in blocks of 12 (Table 1).

Table 1: Schematic description of the interventions during pregnancy. The metronidazole/lactose treatment courses are only randomised among BV-positive asymptomatic women.

		Daily multiple micro-nutrient supplementation (MuMS)		Daily supplementation with 30 mg Fe and 400 µg of folate (Fe30F)		Daily supplementation with 60 mg Fe and 400 µg of folate (Fe60F)	
		A1	A2	B1	B2	C1	C2
Routine food supplementation	Early start						
	Usual start	D1	D2	E1	E2	F1	F2
		7-day course of metronidazole	7-day course of lactose	7-day course of metronidazole	7-day course of lactose	7-day course of metronidazole	7-day course of lactose

A *Follow-up Interviewer* will visit every pregnant woman within a few days of randomization (at 8-10 weeks of gestation). They will collect socio-economic, demographic and anthropometric information from the women. Baseline anthropometric information will include height and mid-upper arm circumference. Pre-pregnant weights will be available as part of the routine collection of weights of all women 15-44 y old.

Pregnant women in groups A, B and C will be strongly urged to contact the local community nutrition promoter (CNP) of BINP for enrolment in the routine food supplementation program. The names of these women will also be communicated to the local CNP, who will know about the study activities and be expected to enrol pregnant women in the feeding program. We anticipate that almost all the women in the 3 groups will have started on the daily food supplement by the end of the first trimester. During the first visit to the CNC, the woman will receive a feeding card (green) from the CNP. All visits to the CNC will be recorded in this card, which will later be used to assess compliance with food supplementation.

No encouragement to join the feeding program will be given to the pregnant women in groups D, E and F. As was found in neighbouring Sharasthi *upazilla*, the time of entry of these women ("usual start") will vary considerably, with a mean of about 17 (± 7) weeks of gestation (Shaheen et al., 2000).

During this first post-randomisation contact with the *Follow-up Interviewer*, all pregnant women will be informed of the need and reasons for visiting the nearby ICDDR,B sub-centre within a few days of enrolment. The exact dates for her visit will be discussed and confirmed with the woman. The woman will be given a referral slip showing her name, identification number, and the date and place of the sub-centre visit. One counterfoil of the referral slip will be given to the local CHW who will then ensure that the woman does not forget to go to the sub-centre as scheduled. A second counterfoil will be sent to the relevant sub-centre.

3.3 First visit to sub-centre: testing of vaginal swabs for BV and enrolment in the BV treatment trial

When the pregnant woman comes for the first time to the sub-centre at 8-10 wk of gestation, her referral slip will be matched with the counterfoil present in the clinic. Pregnant women missing their scheduled clinic visits by more than 7 d will be contacted at home by a *Follow-up Interviewer*. This will ensure a high compliance on the part of the women scheduled to visit sub-centres.

Female *Paramedics* will be located at the sub-centres and will take informed written consent for obtaining blood and high vaginal samples and micronutrient supplementation. All women will be offered screening and treatment for bacterial vaginosis. They will also do a full antenatal check-up. The paramedics will be available to provide medical back up when required for the study-participants.

Women will be asked how they are doing and whether they have any problem in their reproductive area/tract. Irrespective of the response to this screening question, a high vaginal swab will be collected from every woman giving consent. Women not reporting an abnormal vaginal discharge in response to the screening question and whose vaginal swab tests positive to BV will be enrolled in the BV treatment component of the study. However, women matching any of the following criteria will be excluded from BV study component:

- History of chronic heart disease, diabetes mellitus, pregnancy induced hypertension, hypertension requiring treatment.
- Antibiotic treatment in previous two weeks and an allergy to metronidazole.

- Foetal death or known life threatening foetal anomaly and multifoetal gestation.
- Previous history of congenital abnormality in off-springs.

High vaginal swab will be collected from the posterior vaginal fornix by using a sterile non-lubricated speculum swabs. Smears will be made on glass slides in duplicates by the *Female Paramedic* at the sub-centres. The slides will be transported to Matlab at normal temperature where they will be gram stained and read daily (same day) by a trained research officer according to the Neugent criteria which characterises bacterial morphotypes and has a scoring system (Neugent *et al.*, 1991):

- Grade I -normal, comprising predominantly lactobacillus morphotypes.
- Grade II -intermediate, lactobacillus are reduced and mixed with other bacterial morphotypes.
- Grade III -few or no lactobacillus morphotypes with predominant gram positive and negative coccus bacilli for

Grades II and III will be classified as BV. The results from the slides will be entered into the computerized study tracking system the following day. Using a pre-defined algorithm, the computerized tracking system will individually randomize asymptomatic women classified with BV to either treatment with metronidazole for 7 days or placebo. Women who report an abnormal vaginal discharge will be treated based on the result of the vaginal swabs and will NOT be enrolled in the BV treatment component of the study, but will also be followed as a third study group in this trial.

The full course of 21 tablets will be delivered to the women enrolled in the BV trial in a special pill bottle (eDEM®, described below) at 13 wk of gestation through her local Community Health Worker. The metronidazole and placebo tablets will be identical in appearance. The CHW will explain the course to the woman and will follow-up after 7 more days to assess compliance. Symptomatic women classified with BV will be treated with metronidazole for 7 days, also through the CHW. At the follow-up visit, the CHW will remind the woman to go to the sub-centre within a week (14-15 wk).

3.4 Second visit to sub-centre: Initiation of micronutrient supplementation.

At the second sub-centre visit at 14-15 wk of gestation, a full antenatal check-up will be repeated again. The *Paramedics* will collect venous blood samples (4 mL). At present, pregnant women in BINP *upazillas* receive iron-folate supplements through the CNP. Arrangements will be made with the program to discontinue this practice, which will be replaced by a system of delivery by study personnel. Micronutrient supplementation will be initiated during this sub-centre visit. At the first sub-centre visit, women will receive identical looking-tablets that contain either multiple micronutrients, 30 mg iron and 400 µg of folate, or 60 mg iron and 400 µg of folate. These tablets will be made available in bottles labelled with the letter indicating the randomisation group. Each bottle will contain 200 tablets, and will therefore last until delivery, with some to spare. Assignment of the bottles at the sub-centre will be based on computer printouts listing all enrolled women and their group code.

3.5 Monitoring and follow-up during pregnancy

3.5.1 Dietary Assessment: The women will be followed through pregnancy and delivery. *Dietary Interviewers* will be responsible for administering dietary questionnaires. Using 24-h recalls, detailed dietary information will be collected from each woman on 3 occasions in the second and third trimesters of pregnancy. These interviews will be carried out in the participants' homes. The schedule in Table 2 outlines the time of these visits and the tasks to be performed. The 24-h dietary-recall assessment will be based on procedures well-established and tested in ICDDR,B and will involve the use of standardised household measures (serving units). The *Dietary Interviewers* will be trained to conduct these tasks and will be accompanied by a logistical assistant (porter). Energy and nutrient intake data will be calculated based on food composition tables for Indian and Bangladeshi foods (NTN, 1989; HKI, 1988). As part of a previous project carried out in the study area, representative recipes for multi-ingredient foods consumed in the community were obtained and assessed. All locally used household measures were also standardised for respective food items. The information thus obtained was used to update the food composition database, which has been computerised.

3.5.2 Compliance with supplementation

Compliance with micronutrient supplementation. A special equipment called eDEM® will be used for monitoring of compliance. Similar equipment has previously been used successfully in micronutrient supplementation studies (Ekstrom *et al.*, 1996, 1999). It consists of an ordinary pill/bottle equipped with a counting

device and a small microprocessor embedded in the cap. Each time the pill-bottle is opened and closed, the time and date is recorded. Use of this equipment permits continuous recording of information on compliance, which will be collected until delivery. The information in the caps will be downloaded into a computer from bottles collected from the enrolled women. This method will be compared with other measures of compliance such as pill-counts and recalls of the pregnant women.

Compliance with food supplementation: At selected home/sub-centre visits, the feeding cards will be examined by the *Follow-up Interviewers* (Table 2). They will transfer the information on compliance with food supplementation, which has been recorded on the cards by the CNPs, to a structured form. The feeding card will be pre-tested at the beginning of the study. The women will also be asked about visits to the CNC on each of the previous 7 d and how many packets she consumed on the last visit. *CNC Monitors* will visit CNC in the study area once every 2 wk and collect information from CNP records on days study women came to the CNC to receive the supplementation. It is our understanding that in many BINP *upazillas* it is the unofficial practice to allow women at 7 mo or more of pregnancy to be able to collect the BINP food packets from the CNC and take them home. In the proposed study, we intend to aggressively discourage this practice and ensure that all feedings are at the CNC. However, we anticipate that this practice is likely to continue among some women, especially in advanced stages of pregnancy. During our home visits, we will collect data on the extent of this practice and how much of the supplement is being shared with other family members.

Compliance with metronidazole/placebo course will be measured with the same eDEM equipment used for the micronutrient supplements.

Home-based measurement of morbidity and side-effects: Morbidity will be assessed at home 3 times in pregnancy (i.e., every 8-14 wk) through 1 wk/1 mo recalls (Table 2). Trained *Dietary Interviewers* will perform these home-based interviews using a structured questionnaire that will include probing. It will contain questions related to duration of illness, and attributes of the severity of disease (such as pain, self-care, mobility, cognition, emotion and fertility). Prevalence and severity of side-effects to micronutrients will be assessed using a list of pre-tested symptoms. Women will also be probed for their severity. To reduce bias, the list will also contain symptoms that are not side-effects. Other morbidity that may have a possible association with compliance and/or outcomes will be assessed clinically using specific case definitions. They include urinary tract infections in the last month; backache in the last week, well-being at the time of the visit, axillary temperature at the time of the visit, and any sickness that interfered with normal work and confined the subject to her bed in the last month.

3.5.3 Sub-centre follow-up visits and ultrasonographic assessment: The schedule of visits during pregnancy and at delivery is shown in Table 2. After the first visit at 8-10 wk of pregnancy, 3 more visits will be made to the sub-centre. A full antenatal control will be conducted at these visits. There will also be questions and physical examinations for morbidity, and measurement of weights. We will monitor for pre-eclampsia by checking for tibial oedema, conducting dipstick test for albumin in urine, and measuring blood pressure.

From a sub-sample of enrolled women, a second vaginal sample will be collected 7 weeks after the collection of the initial sample to study the efficacy of the treatment. Specimens will be collected from 50 women from each of the following 5 categories:

- i. Asymptomatic women classified with BV and receiving treatment with metronidazole for 7 days.
- ii. Asymptomatic women classified with BV and receiving placebo for 7 days.
- iii. Symptomatic women classified with BV and receiving treatment with metronidazole for 7 days.
- iv. Asymptomatic women NOT classified with BV and NOT receiving any treatment.
- v. Symptomatic women NOT classified with BV and NOT receiving any treatment for abnormal vaginal discharge.

We also propose to use ultrasound for a direct assessment of foetal growth. All enrolled women will be examined by ultrasound at the sub-centre visits, first at 8-10 wk of gestational age. This will give the most accurate ultrasound gestational age estimate. On the basis of available data on the validity of one-point measurement of ultrasound foetal growth indicators, the best combination of measurements to assess "ultrasound estimated foetal weight" and available curve modelling techniques requires 3 more examinations of the pregnant woman during the 2nd and 3rd trimesters. Each examination will take approximately 10 min and will be done at the sub-centre visits.

We will use 4 small portable ultrasound machines, each placed one in one of the sub-centres. Four *Sonographers* (trained female nurses/technicians) will be trained for the standard measurements on foetal growth parameters. They will be blinded to the woman's group assignment. A quality controller will re-examine 5% of the total examinations without knowing the previous results.

Principal Investigator: Lars Ake Persson

Table 2: Schedule of contacts during pregnancy and delivery

Gestational week	8-10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	at birth
Household visit																														
Enrollment	C/F																													
24-h dietary recall	D													D								D								
Compliance to food supplement													D									D								
Collection of pillbottles													D									D								P
Morbidity, side-effects	D												D									D								P
newborn anthropometry																														
delivery history																														P
metronidazole for BV+				C																										
Sub-centers visit																														
antenatal check-up, maternal morbidity	P				P					P												P								
Weight measurement	P				P					P												P								
Initiation of micronutrient suppl					P																									
Venous blood					P																	P								
capillary blood										P																				
Vaginal swab	P									P*																				
Compliance to food supplement					P																									
Fetal ultra sound	S				S					S												S								

*subsample

P=paramedic

S=sonographer

F=follow-up

D=dietary

C=CHW

Various major sonographic indicators to be measured include: crown-rump length, biparietal diameter, head circumference, femur length and abdominal circumference. Estimated foetal weight will be calculated by combining 2-3 appropriate parameters for specific gestation periods. Based on the international reference values on foetal sonographic biometry for either single or combined parameters, fetuses will be classified in terms of adequacy of growth for their gestational age.

3.5.4 Sampling of blood during pregnancy: Blood samples will be collected 3 times during pregnancy. At the second sub-centre visit at 14-15 wk, the baseline sample of 4 mL venous blood will be collected. A capillary blood sample for assessment of Hb will be taken after about 4-5 wk of micronutrient supplementation (19-20 wk) at a sub-centre visit. During the sub-centre visit at around 30 wk of gestation (after 3-4 mo of supplementation), the *Paramedic* will again collect 4 mL of venous blood.

If the hemoglobin concentration fall below 80 g/L in the first venous blood sample, the women will be excluded from the trial and referred to the Matlab Hospital for appropriate investigation and therapy. After about 4 weeks of supplementation the women's haemoglobin concentration will be assessed from a capillary blood sample. As capillary blood is 5g/L lower than that of venous women who have a haemoglobin concentration below 75g/L will be excluded from further participation. They will be referred for medical investigation as outlined above.

3.5.5 Notification of births and measurements: A birth notification system will be established to ensure that study staff become aware of births as soon as they occur. When a pregnant woman is first enrolled in the study, she will be given a yellow notification card with her name and address (village and *bari*). A family member will be identified to notify the sub-centre immediately following the delivery. The location of the nearest sub-centre will be explained to the family member and the family will be promised US \$2 as reimbursement if the delivery is reported as soon as possible. Half of the amount will cover transportation costs and the rest will be reimbursement for time-spent. As a back up to this system, CHW and CNP will be encouraged to check on enrolled pregnant women on an almost-daily basis close to the expected time of delivery.

Because this study will be done in a community where most births occur at home, it will not be possible to measure BW immediately after birth. We propose to measure weight within 72 h of birth. Previous studies have shown that in poor Bangladesh populations, there is very little change in weights in the first 3 d (Arifeen, 1997; Goodburn et al., 1994). However, we also propose to obtain weight measurements repeated twice-daily in a sub-sample of 100 new-borns, who will first be weighed within 12 h of birth. Weighing will be continued until 96 h following birth. This data will be used to test the hypothesis of negligible weight change in new-borns in the first 72 h and to develop correction factors if deviation from this hypothesis is significant.

A female *Paramedic* based in the sub-centre will visit the newly delivered woman within 72 h of birth to measure weight, length, head circumference, and knee-heel length of the new-born. Data on the delivery will also be collected at this time. This will include type of delivery, duration of labour, and complications, if any. The axillary temperature of the mother will be taken and her lower abdomen palpated for tenderness. If both fever and lower abdominal tenderness are present postpartum infection will be suspected and she will be referred to the sub-centre, where she will be assessed. A high vaginal swab will be taken for culture and blood sampled for a blood count. The sick woman will be managed appropriately. The female *Paramedic* and the local CHW will be trained on care and referral for a LBW baby. This will enable them to provide the appropriate advice or referral when a LBW baby is found.

3.5 Assignment to postpartum interventions

Immediately after delivery of a live born baby, mothers will be randomised to 1 of 2 interventions. One group will receive counselling on BF while other mothers will receive standard health education on general health of mother and infant. After the study has actually commenced and more accurate information on the study population, clustering and study logistics will be available and will be reviewed. A decision on either individual or *bari*-level randomization will be taken. Randomization will be in blocks of 4 to either of these 2 interventions. A randomization log for new-borns will be kept in each sub-centre. All births, once reported, will be recorded in the log in the order the information is received at the sub-centre. The log will indicate the intervention group to which the mother is assigned.

During the first contact (see Table 3) immediately after birth, discussion will centre on the advantages of BF and doing so exclusively for at least 4 mo. Previous experience BF by the woman and others she knows will be discussed. Women will be invited to talk about their feelings about previous and current BF experiences and any

difficulties they encountered. About 20% of women in the study area give birth at a sub-centre. For these women, during the first 24 h after birth, counselling on the advantages of BF and correct technique will be first provided by clinic staff, to be followed-up later in their homes by the *Counsellors*. The objective of the follow-up visits is to talk with the mothers about their experiences BF so far, their concerns and difficulties, and to provide positive feedback. For all women, counselling will be reinforced during the monthly visits of the CHW to the families after the birth. The importance of EBF to at least 4 mo for the health of the infant is reinforced, as is the availability of assistance from the CHW if needed.

Table 3: Schedule of postpartum contacts (Home Visits)

Activities	Worker Type	Infant age (mo)										
		0	1	2	3	4	5	6	8	10	12	
Counseling of mothers	Counselor	3d	15d	+	+	+	+					
Postpartum maternal morbidity	Paramedic		15d									
Reinforcement of BF	CHW	+		+	+	+	+					
Infant anthropometrics	Follow-up			+	+	+	+	+	+	+	+	
Infant feeding information	Follow-up			+	+	+	+	+	+	+	+	
Follow-up of diarrhoea and respiratory infections in infants	Follow-up			+	+	+	+	+	+	+	+	
Maternal weight	Follow-up			+					+	+	+	

3.6.1 Postpartum monitoring

3.6.1 Follow-up of infant growth, morbidity and survival: Infants will be weighed and have their lengths measured every month until they reach 6 mo of age and then every 2 mo until they are 12 mo old. These measurements will be taken within ± 7 d of the scheduled dates by Follow-up Interviewers. At each of these visits, data will also be collected on infant feeding and morbidity. The infant feeding information collected will include food frequencies in the previous 24 h and feeding practices in the previous 7 d. This information will be used to obtain a good categorisation of BF status and assess the adequacy of complementary feeding. Important changes in feeding practices since the last visit will also be noted. The child's caretaker will also be asked about diarrhoea and respiratory infections in the previous 7 d and additional information will be obtained to assess severity of disease episodes. The data collection instruments will be adapted from those being currently used by the World Health Organization Multicentre Growth Reference Study in several countries including India. Data on occurrence and cause of deaths of any infant in the study cohort will be available from the Matlab Demographic Surveillance System.

3.6.2 Maternal follow-up postpartum: A Paramedic will visit every woman again at 2 wk (± 3 d) after delivery if the mother does not come to the sub-centre, as is now recommended by the ICDDR,B program. The Paramedic will use a structured questionnaire to assess postpartum maternal morbidity, and also recovery from any postpartum infection detected at the "at birth" visit. Axillary temperatures will be taken and the lower abdomen and breast palpated (for mastitis). Maternal weight will be taken by the Follow-up Interviewers at 1 mo postpartum and every 2 mo from 6 mo onwards (a total of 5 postpartum measurements).

3.7 Techniques of anthropometric measurements

Up to 6 mo of age, all infant weights (including BW) will be measured by beam scales, which are accurate to 10 g. All maternal weights and infant weights beyond 6 mo of age will be measured with electronic scales (UNISCALE), which are accurate to 100 g. Locally manufactured, collapsible length boards, which are precise to 1 mm, will be used to measure the recumbent length of the infant. The recumbent length will be measured according to standard procedures (WHO, 1983).

3.8 Laboratory tests

Hb concentration will be assessed from a drop of venous blood at approximately 12 and 27 wk of gestation, and from a capillary sample at 16 wk by use of HemoCue® system, which has a good validity for field use (Mills and Meadows, 1989). The accuracy of the HemoCue® machines will be checked daily using control cuvettes.

To determine what may be causing maternal anaemia, 4 mL of blood will be collected from venipuncture in trace-element-free tubes at approximately 12 and 27 wk of gestation. The tubes will be protected from light and heat and will not be allowed to stand or be shaken. Serum will be separated and frozen at either -20 or -70 °C depending on period of storage. Serum ferritin will be assessed using immuno-radiometric assay (R & D Systems, Minneapolis, MN, USA). Soluble s transferrin receptors will be assessed by an enzyme immunoassay (double-sandwich method) (Ramco Laboratories, Houston) (Flowers et al., 1989). Vitamins will be assayed in HPLC, while folate will be tested with a bioassay. CRP will be analysed by immunoturbidimetry using a Roche kit in a Hitachi autoanalyzer. All trace elements will be measured using atomic absorption spectrophotometry.

The examination of vaginal swabs on slides has been described under 3.3. All slides will later be transported to Dhaka and re-read by another technician.

3.10 Quality of data

Most of the data will be collected with structured questionnaires, which will include both pre-coded and open-ended questions. Questionnaires on morbidity and infant feeding will be adapted from similar questionnaires used in previous community-based studies conducted by ICDDR,B. These questionnaires will be pretested in a small sample of women with new-borns and then revised. The verbal autopsy instrument will be based on verbal autopsy questionnaires previously used by ICDDR,B and in national surveys in Bangladesh. These will be pre-tested in a small sample of deaths identified through the HDSS system. All questionnaires will be first prepared in English and then translated into Bengali. Interviews will be conducted in Bengali and responses to open-ended questions will be recorded in Bengali. The interviewers will be requested to write down, as well as possible, the actual words used by the respondents. Well-designed guidelines will be used for qualitative data collection. Collected data will be reviewed once every 2 wk by the responsible investigators and discussed with those collecting the data.

Refresher training of the *Interviewers* on methods to collect data and anthropometric measurements will be repeated every 3 mo. Reliability data will be collected on these occasions as per WHO guidelines (WHO, 1983). Weighing equipment will be standardised daily with standard weights. On a continuing basis, supervisors will repeat a 5% random sample of the interviews/measurements collected by the *Interviewers*. In addition to implementing standard quality control systems in the laboratory, 5% of the specimens will be sent out for re-assessment in an internationally recognised reference laboratory.

4. OUTCOME MEASURES

In the proposed research, our primary outcome variables are BW, maternal Hb concentration, pre-term delivery (<37 wk of gestation at birth) and duration of EBF. In addition, we will measure: weight gain during pregnancy; ultrasound-estimated foetal growth; growth in weight and length among infants 0-12 mo old; infant morbidity; and change in maternal weight postpartum. We also will assess: SGA at birth, and the concentrations of transferrin, ferritin and various micronutrients in blood. The study will also provide a measure of cost-effectiveness of the interventions and effects on equity.

5. ESTIMATION OF SAMPLE SIZE

The sample size needed was estimated primarily considering the key outcome of BW. We departed from the notion, that the size of the effect we would be able to demonstrate should have an importance or significance from a public health program point of view. Calculations confirm that this sample size is adequate for other outcomes as well. The study uses a design with 12 cells in a 2x3x2 factorial. For BW, the design is 2x3 with 6 cells. We assume that the power should be 0.90 (Type II error rate of 0.10) with testing at 0.05 (Type I error rate), and estimate the sample size needed for the minimum important difference (i.e., the smallest difference that is substantively important) given the expected variation.

For BW, the standard deviation is 400 g from previous data in Bangladesh, and the minimum important difference was deemed to be 70 g. The sample size required for comparison of any 2 of the 6 cells is then 686 or a total of 4116 across the 6 cells. A total of 5300 women will be recruited to ensure that this sample size is reached

(accounting for 5% refusal, 11% loss during pregnancy and 9% loss during infancy due to death and out-migration). This means enrolment over 23 months at current rates of pregnancy occurrence in this population. This calculation assumes that interactions among the interventions are likely to be important, and so there needs to be sufficient power to compare individual cells (i.e., simple effects) as well as main effects and interactions. With this sample size, the minimum difference detectable with adequate power for comparing the main effects for BW of the food interventions is 40 g and of the micronutrient interventions is 50 g. Furthermore, with this sample size, we can differentiate a 44 kcal (about 2.6%) substitution of diet by the supplementation between those who start early and those who start at the usual time from the first trimester dietary interviews, assuming the SD is 440 kcal; it is important to know how much of the energy from the supplement is substituting for energy that would otherwise come from the home diet.

With this sample size, the minimum difference for comparing the main effects of the 3 micronutrient interventions on the Hb status of women that can be detected with adequate power is very small, 1.7 g/L, assuming a standard deviation of 14 g/L (Ekstrom et al., 1996, 1999). The minimum difference for comparison of any 2 cells is 2.4 g/L. These differences are not biologically important, so there will be sufficient power to determine equivalence of the 3 interventions to about 2 g/L. Also, with other outcomes as examples, the minimum detectable difference in the percentage being exclusively breast-fed comparing any 2 of the 12 cells for the BF intervention is 12%. That is, there will be sufficient power to detect differences of 50% vs. 62% in the percentage of infants being exclusively breast-fed at, say, 4 mo. The sample size of about 350 per cell is comparable to that used in other studies that have found important effects of a counselling intervention on the duration of EBF (Lutier et al., 1997). The minimum detectable difference for infant weight and length gain for a 2-mo interval from 4 to 6 mo will be very small, 80 g and 0.27 cm, respectively, for comparisons across any 2 of the 12 cells (Frongillo and Habicht, 1997).

Current estimates of pre-term births in Matlab is 17%. We also expect that 80% of the women will be asymptomatic and we assume that the proportion with BV+ among these women is 15%. Of the 5300 pregnant women to be enrolled in the full study, 3200 will be enrolled in the BV component. We expect 2900 live births in this sub-sample. If 20% refuse this component of the study then we expect that there will 280 asymptomatic BV- women available to be enrolled. We assume also that BV increases the risk of pre-term delivery by 2-fold. We should therefore expect rates of pre-term among BV+ and BV- women to be 30% and 15%. Pre-term rate among BV+ women who receive treatment with metronidazole should also be 15%. The power of this study to detect the difference between 30% and 15% in the untreated and treated groups with 140 women per group is 86%.

7. Timetable

Activities	Year 1				Year 2				Year 3				Year 4				Year 5			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Preparatory activities	X	X																		
Preliminary focused ethnographic study	X																			
Enrollment & randomization of pregnant women		X	X	X	X	X	X	X												
Follow-up of pregnant women		X	X	X	X	X	X	X	X	X	X									
Enrolment of new-borns				X	X	X	X	X	X	X	X									
Follow-up of infants				X	X	X	X	X	X	X	X	X	X	X	X					
Data entry and cleaning		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Laboratory analyses								X	X	X	X	X	X	X	X	X	X	X	X	
Data analysis							X	X	X	X	X	X	X	X	X	X	X	X	X	X
Reporting						X						X								X

Facilities Available

Describe the availability of physical facilities at the place where the study will be carried out. For clinical and laboratory-based studies, indicate the provision of hospital and other types of patient's care facilities and adequate laboratory support. Point out the laboratory facilities and major equipments that will be required for the study. For field studies, describe the field area including its size, population, and means of communications. (Type within the provided space).

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) has been established by the Government of Bangladesh as an international and independent health research institute. The Centre has an impressive record on international health and population research, specially for intervention and analytical studies in the areas of family planning, reproductive health, nutrition and child survival, including diarrhoea and acute respiratory illnesses. ICDDR,B maintains demographic, health and disease surveillance systems in both rural and urban populations, as well as community based health services and health facilities. The Centre's large multi-disciplinary scientific research staff include both international and national members. ICDDR,B has a well established and sophisticated research infrastructure, including inpatient hospital facilities, microbiological laboratories and computerised data handling capacity.

The Matlab field study area offers excellent facilities for this study. ICDDR,B has established means of communication within Matlab based on road and river transport. The twice a day shuttle between Matlab and Dhaka will ensure that the data forms reach Dhaka on time. The Health and Demographic Surveillance System, is a regularly updated demographic information system on 210,000 population and can provide much of the data required for this study. The existing ICDDR,B field data collection staff in Matlab offers a pool of highly experienced and trained individuals to choose from. Both the Matlab and Dhaka-based data processing units of the Public Health Sciences Division have long experience in handling both longitudinal and special project data.

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical software packages will be used, and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to monitor further progress of the study. (Type within the provided space).

This study is designed as a randomised, controlled experiment with longitudinal follow-up. Women will be randomly assigned to 1 of 12 cells of a 2x3x2 complete factorial design. Compliance to the interventions will be carefully assessed permitting evaluation of both efficacy and effectiveness of single interventions as well as for combinations of them.

Analysis of effectiveness:

Effectiveness will be assessed by "intention-to-treat" analysis of the outcomes. This analysis includes all subjects randomised into each group, even if some of those subjects did not comply with the conditions of the group (Pocock, 1983). The resulting statistical probabilities are statements about causality and not just about statistical association (Habicht et al., 1999). General linear models (i.e., analysis of variance and regression) with interactions will be used with continuous outcomes such as birth weight and haemoglobin values. Logistic regression will be used with binary outcomes such as rates of LBW deliveries and anaemia. Proportional hazards regression will be used with outcomes that capture time to an event such as duration of EBF. Negative binomial or TOBIT regression may be needed to model morbidity outcomes (Ramakrishnan et al., 1995). In all analyses, standard regression diagnostics will be done to evaluate model assumptions, including examining distributions, performing any needed transformations, and examining overall fit, residuals, and leverage. The proportionality assumption of the proportional hazards regression will be evaluated using plots of the product-limit estimator, residuals, and modelling using time-varying covariates. Inasmuch as some data will be collected at multiple times, static, developmental curve, and dynamic approaches for longitudinal analysis will be used (Frongillo and Rowe, 1999).

Handling drop-outs: One of the difficulties in a longitudinal study is that cases are subject to attrition over time, resulting in a partial series of measurements for some cases. A typical pattern is that some cases are followed up until a certain time, after which those cases drop out of the study. If the drop-out process in a study is non-ignorable (i.e., not missing at random), then estimates of difference among groups or regression effects may be

biased (Frongillo and Rowe, 1999). When this occurs, estimates of group differences or regression effects can still be obtained by developing an instrumental variable representing each case's propensity to drop-out using logistic regression, and then including this variable in the regression model. This requires that information is available that is important for determining the propensity to drop-out for each case, but that is not important for determining the responses of interest. An alternative approach is to obtain at least one late follow-up measurement on each individual, even if they otherwise drop out, and use that measurement to adjust for any potential selection bias. Both of these strategies will be employed in this study.

Compliance as a determinant of effectiveness: Not all study participants will fully comply with the experimental interventions, even among those who remain in the study throughout the follow-up period. Information on compliance for food supplementation, EBF and pill ingestion is expected to be of high quality given the monitoring done by health workers and counsellors and the electronic monitoring of the pill bottles (Urquhart and De Klerk, 1998). Predictors of compliance will be estimated from regression analyses of the socio-demographic, cultural and behavioural data. The qualitative information from behavioural studies will both guide the analyses and be used to interpret the results. Simulations with these predictors can estimate improvements in compliance that may ensue if future interventions change the predictors' distributions.

The measure of effectiveness is not affected by limitations in coverage of the target populations as often is the case. Thus, to predict effectiveness of future interventions in different settings, simulations must include effect-modified estimates of efficacy, determinants of compliance as well as coverage of the intended target population.

Analysis of efficacy: Compliance information can also be used to estimate the dose-response to the biological intervention (e.g., more supplemental food). Dose-response estimates based on compliance information are subject to potential bias because compliance can be confounded by other biological or psychological factors that are related to the outcomes. Many of these will be known from our analyses of compliance and taken into account. We will test for lack of bias by showing that estimates of effectiveness derived from the dose-response curves is similar to that using the "intention-to-treat" analyses.

Estimating efficacy from the dose-response curves requires not only that the curves are unbiased, but also that they must properly model for changes in efficacy. Thus the relationships between compliance and outcomes may not exhibit straight lines, so a non-linear or threshold model may be most appropriate for some outcomes (Albert, 1999). This is certainly the case for the nutritional supplements that have less impact at higher doses as nutrition improves (Ekstrom et al., 1996, 1999). For the same reason individuals with different levels of initial deficiency will respond differently to a same dose and thus modify the effects of the intervention.

Nutritional status and other effect-modifiers can be used as predictors of benefit to select women for the interventions (Habicht and Pelletier, 1990). Predictors of benefit will be identified in regression models by evaluating interactions between potential predictors of benefit and the interventions. An indicator of benefit consists of the predictor of benefit, and a cutoff point (IOM, 1996). For predictors of benefit found to be important, cutoff points will then be examined in relation to the tradeoff between sensitivity and specificity for separating those who will or will not likely benefit.

Analysis of cost-effectiveness: The project will include health economics evaluation of the interventions. This will include costing of the interventions, cost-effectiveness analyses, and evaluation of incremental costs and benefits for combinations of interventions (Pettiti, 1994; Gold et al., 1996). Data on costs will mainly be collected by prospective micro-costing by the project personnel administering the interventions. That is, the details of all input costs will be collected and recorded. Estimates of possible savings in health care will be collected. Value of the gains will be assessed by a "willingness-to-pay" survey. The indirect costs will be equal to the value of production lost due to participation in the program. The resources needed for implementation of the intervention and the effects caused by the intervention will be identified, measured and valued. Resources will be valued in monetary units, effects will be measured in natural and appropriate physical units, and indication on value will be given by a ratio between costs and effects (C/E) measured in non-monetary unit (e.g., cost per 100-g gain in birth weight).

We will estimate the incremental costs and the incremental effects of the interventions compared to the alternative of no intervention. The cost-effectiveness analyses will focus on three aspects: (a) how the choice of intervention components influences cost-effectiveness, that is, does one component dominate another in terms being more effective at less cost, (b) the incremental cost-effectiveness of increasing compliance, and (c) how the choice of target groups influences cost-effectiveness. We will examine the extent to which program costs of the

biased (Frongillo and Rowe, 1999). When this occurs, estimates of group differences or regression effects can still be obtained by developing an instrumental variable representing each case's propensity to drop-out using logistic regression, and then including this variable in the regression model. This requires that information is available that is important for determining the propensity to drop-out for each case, but that is not important for determining the responses of interest. An alternative approach is to obtain at least one late follow-up measurement on each individual, even if they otherwise drop out, and use that measurement to adjust for any potential selection bias. Both of these strategies will be employed in this study.

Compliance as a determinant of effectiveness: Not all study participants will fully comply with the experimental interventions, even among those who remain in the study throughout the follow-up period. Information on compliance for food supplementation, EBF and pill ingestion is expected to be of high quality given the monitoring done by health workers and counsellors and the electronic monitoring of the pill bottles (Urquhart and De Klerk, 1998). Predictors of compliance will be estimated from regression analyses of the socio-demographic, cultural and behavioural data. The qualitative information from behavioural studies will both guide the analyses and be used to interpret the results. Simulations with these predictors can estimate improvements in compliance that may ensue if future interventions change the predictors' distributions.

The measure of effectiveness is not affected by limitations in coverage of the target populations as often is the case. Thus, to predict effectiveness of future interventions in different settings, simulations must include effect-modified estimates of efficacy, determinants of compliance as well as coverage of the intended target population.

Analysis of efficacy: Compliance information can also be used to estimate the dose-response to the biological intervention (e.g., more supplemental food). Dose-response estimates based on compliance information are subject to potential bias because compliance can be confounded by other biological or psychological factors that are related to the outcomes. Many of these will be known from our analyses of compliance and taken into account. We will test for lack of bias by showing that estimates of effectiveness derived from the dose-response curves is similar to that using the "intention-to-treat" analyses.

Estimating efficacy from the dose-response curves requires not only that the curves are unbiased, but also that they must properly model for changes in efficacy. Thus the relationships between compliance and outcomes may not exhibit straight lines, so a non-linear or threshold model may be most appropriate for some outcomes (Albert, 1999). This is certainly the case for the nutritional supplements that have less impact at higher doses as nutrition improves (Ekstrom et al., 1996, 1999). For the same reason individuals with different levels of initial deficiency will respond differently to a same dose and thus modify the effects of the intervention.

Nutritional status and other effect-modifiers can be used as predictors of benefit to select women for the interventions (Habicht and Pelletier, 1990). Predictors of benefit will be identified in regression models by evaluating interactions between potential predictors of benefit and the interventions. An indicator of benefit consists of the predictor of benefit, and a cutoff point (IOM, 1996). For predictors of benefit found to be important, cutoff points will then be examined in relation to the tradeoff between sensitivity and specificity for separating those who will or will not likely benefit.

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Interventions are offset by health care savings, yielding an increment of effectiveness and reduced costs. The choices of intervention components will create a series of mutually exclusive measures, and these can be directed towards target groups defined in pre-pregnant weight (in kg). Donald Kenkel, Professor of Policy Analysis and Management at Cornell, will provide assistance with the analysis.

Data from the trial will enable us to measure cost-effectiveness in several ways. For example, cost/100-g increase in mean BW, cost/aversion of LBW, cost/kg net maternal weight gain, cost/life-years gained, cost/reductions in infant death, etc. The data will also permit estimates of cost-efficacy analyses that are useful for future policy making and program planning

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or risk to each subject of the study.

Women will be enrolled in the study after giving their informed consent. The regular ICDDR,B Community Health Worker (CHW), who has years of experience of working in this community, will initially obtain a verbal consent to conduct the pregnancy test while maintaining absolute privacy. If the pregnancy test is positive, the CHW will explain the entire study and study procedures to the woman (see verbal consent form, Appendix 1) and will obtain verbal informed consent from the woman to participate in the study. At the first visit by the enrolled pregnant woman to the sub-centre, female *Paramedics* will obtain written informed consent (see written consent form, Appendix 1) for collecting blood samples, undergoing ultrasonographic examination and providing micronutrient supplementation.

Any enrolled woman may withdraw from the study at any point without affecting her access to and use of routine ICDDR,B and government services. She may also withdraw from any particular component of the study without affecting her participation in other components. For example, she may refuse to give blood or allow ultrasonographic examination. Confidentiality of information will be strictly followed, and access to data forms will be strictly limited.

None of the women will be deprived of currently available or planned routine services or standard of care. At present only women with BMI <18.5 are eligible for the food supplements in the national program. That remains the minimum standard in the proposed project. All pregnant women--irrespective of BMI--will be eligible and some women will be encouraged to join the program earlier than they would have otherwise. All women will receive iron-folate supplements, although two-thirds will receive only half of the standard iron supplement. There is evidence of the adequacy of the half-strength iron [see Preliminary Observations; this is the amount recommended in the United States (IOM, 1990)]. Women who have a haemoglobin concentration below 80 g/L at enrolment will be excluded and referred for clinical investigations. Metronidazole has been used in the past in pregnant women in developed countries and found to be safe and without any evidence of potential teratogenicity (Burtin, 1995). In Bangladesh, the recommended treatment for BV in syndromic management is metronidazole 400 mg twice daily for 7 days. This empirical regime is prescribed for symptomatic women coming to a health facility with the complaints of vaginal discharge. In the proposed study all symptomatic women who test positive for BV will receive treatment, only asymptomatic women will be included in the treatment randomisation. Health education on breast-feeding is the current standard, and all mothers will receive this. However, half of the mothers will receive intensive "counselling" on exclusive breast-feeding; the remainder will receive health education about infant care of equivalent intensity and duration. The follow-up during and after pregnancy will also ensure that all women have better-than-usual access to antenatal and postnatal care, detection and management of illness, and new-born and infant care.

Ethical approval for this study will be sought from institutional review board at ICDDR,B. In addition, ethical approval for the entire investigation will be sought from the University Committee on Human Subjects at Cornell University.

Use of Animals

Describe in the space provided the type and species of animal that will be used in the study. Justify with reasons the use of particular animal species in the experiment and the compliance of the animal ethical guidelines for conducting the proposed procedures.

No animal will be used in this study.

Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication is anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesh through a training programme.

Early findings will be disseminated through short reports and working papers. Important results and conclusions will be disseminated through working papers, journal articles, policy reports and presentations at national, regional and international conferences and meetings. A comprehensive report will be produced in collaboration with UNICEF and be published by UNICEF.

Collaborative Arrangements

Describe briefly if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization. (Do not exceed one page)

ICDDR,B will collaborate with the Division of Nutritional Sciences, Cornell University, Ithaca, New York, USA, Department of International Health, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland, USA, and UNICEF on study design and implementation, data analysis and report preparation. We have also established contacts with Department of Nutritional Epidemiology, Wageningen, for capacity building activities in conjunction with the project. We will also collaborate with the Bangladesh Integrated Nutrition Project with respect to selection of study site, implementation of the intervention, and use of study findings, and with BRAC.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

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Budget

Summary

	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Total Study Budget	693,155	589,841	604,433	495,288	483,040	2,865,757
Requested from UNICEF ¹	216,486	142,454	87,754	0	0	446,694

¹Funding requested from UNICEF is adequate for the implementation of the first three interventions/experiments, as described under specific aims. However, the ultrasonographic monitoring, and behavioral and economic components are not included in this request. The period for the UNICEF support is 3 years, up until enrollment of births as the primary outcome, for a total amount of \$446,694.

Funding requested from other sources covers additional costs for the implementation of the first three interventions/experiments, including the ultrasonographic monitoring, and behavioral and economic components. Costs of the fourth intervention and infant follow up as an outcome of all four interventions is covered under this request.

In preparing ICDDR,B's budget request, specific attention has been given to assuring that there is NO OVERLAP in funding for the portions of that are common to both funding sources.

Budget Details Follows in Next Pages

Combined Interventions to Promote Maternal and Infant Health
DETAILED BUDGET - Total

PERSONNEL		% EFFORT ON PROJ.	ANNUAL SALARY	Year 1			Year 2			Year 3			Year 4			Year 5		
NAME	ROLE ON PROJECT			%	Person-	Totals	%	Person-	Totals	%	Person-	Totals	%	Person-	Totals	%	Person-	Totals
				Salary	Month		Salary	Month		Salary	Month		Salary	Month		Salary	Month	
Lars Ake Persson	Princ-Invest	20%	152,820	10%	12	15,282	20%	12	31,328	20%	12	32,111	20%	12	32,914	20%	12	33,737
George Fuchs	Co-Princ-Inv.	10%	186,192	10%	12	18,619	0%	0	0	0%	0	0	0%	0	0	0%	0	0
Shams El Arifeen	Co-Invest.	40%	14,088	40%	12	5,635	40%	12	5,776	40%	12	5,920	40%	12	6,068	40%	12	6,220
Kathy Rasmussen	Co-Invest.	25%		0%	12	0			0			0			0			0
Jean-Pierre Habicht	Co-Invest.	10%		0%	12	0			0			0			0			0
Edward A Frongillo Jr.	Co-Invest.	10%		0%	12	0			0			0			0			0
Dewan S Alam	Co-Invest.	100%	13,958	100%	12	13,956	100%	12	14,305	100%	12	14,653	100%	12	15,029	100%	12	15,405
Eva-Charlotte Ekstrom	Co-Invest.	20%	48,000	20%	12	9,600	20%	12	9,840	20%	12	10,086	20%	12	10,338	20%	12	10,597
Rubina Shaheen	Co-Invest.	20%	11,520	20%	12	2,304	20%	12	2,362	20%	12	2,421	20%	12	2,481	20%	12	2,543
Ruchira T Naved	Co-Invest.	15%	16,668	15%	12	2,500	15%	12	2,563	15%	12	2,627	15%	12	2,692	15%	12	2,760
Lauren Blum	Co-Invest.	15%	133,140	15%	12	19,971	15%	12	20,470	15%	12	20,982	15%	12	21,507	15%	12	22,044
Yukiko Wagatsuma	Co-Invest.	20%	100,836	20%	12	20,167	20%	12	20,671	20%	12	21,188	20%	12	21,718	20%	12	22,261
Saskia Osendarp	Co-Invest.	15%	36,000	15%	12	5,400	15%	12	5,535	0%	0	0	0%	0	0	0%	0	0
Iqbal Kabir	Co-Invest.	10%	21,600	10%	12	2,160	10%	12	2,214	10%	12	2,269	10%	12	2,326	10%	12	2,384
MA Wahed	Co-Invest.	10%	17,736	10%	12	1,774	10%	12	1,818	10%	12	1,863	10%	12	1,910	10%	12	1,958
M Yunus	Co-Invest.	10%	26,640	10%	12	2,664	10%	12	2,731	10%	12	2,799	10%	12	2,869	10%	12	2,941
Nigar Shahid	Co-Invest.	15%	21,300	15%	12	3,195	15%	12	3,275	15%	12	3,357	0%	0	0	0%	0	0
Motiur Rahman	Co-Invest.	15%	10,860	15%	12	1,629	15%	12	1,670	15%	12	1,711	0%	0	0	0%	0	0
Sr. Manager, Comm. Hlth Research	Field Support	10%	16,800	10%	12	1,680	10%	12	1,722	10%	12	1,765	10%	12	1,805	10%	12	1,854
Field Manager (NOA)	Field Support	100%	7,044	100%	12	7,044	100%	12	7,220	100%	12	7,401	100%	12	7,586	100%	12	7,775
Clerk, GS3 (Mallab)	Field Support	100%	2,520	100%	12	2,520	100%	12	2,583	100%	12	2,648	100%	12	2,714	100%	12	2,782
Admin assistant, GS4 (Dhaka)	Field Support	100%	3,000	100%	12	3,000	100%	12	3,075	100%	12	3,152	100%	12	3,231	100%	12	3,311
Sr Field Research Assistant, GS4	Field Staff	100%	3,000	100%	36	9,000	100%	36	9,225	100%	36	9,456	100%	36	9,696	0%	0	0
Female paramedic/LFPV, GS4	Field Staff	100%	3,000	100%	90	22,500	100%	133	31,081	100%	69	18,123	0%	0	0	0%	0	0
Sonographers, GS4	Field Staff	100%	3,000	100%	48	12,000	100%	48	12,300	100%	20	5,250	0%	0	0	0%	0	0
Interviewers (Follow-up), GS3	Field Staff	100%	2,520	100%	80	16,800	100%	155	39,364	100%	132	29,123	100%	77	17,413	0%	0	0
Interviewers (Dietary), GS3	Field Staff	100%	2,520	100%	104	21,840	100%	104	22,366	100%	43	9,487	0%	0	0	0%	0	0
Interviewers (CNC monitors), GS3	Field Staff	100%	2,520	100%	60	12,600	100%	60	12,915	100%	25	5,516	0%	0	0	0%	0	0
Ethnographers, GS3	Field Staff	100%	2,520	100%	24	5,040	100%	72	15,456	100%	36	7,943	0%	0	0	0%	0	0
Counselors, GS3	Field Staff	100%	2,520	100%	94	10,740	100%	188	40,467	100%	172	37,949	100%	0	0	0%	0	0
Helper/daily wage/messenger	Field Staff	100%	600	100%	145	7,250	100%	265	13,581	100%	229	12,030	100%	101	5,438	0%	0	0
Community health worker, GS2	Field Staff	5%	2,112	5%	684	6,019	5%	684	6,170	5%	312	3,162	0%	0	0	0%	0	0
Research Officer, GS5	Lab Staff	100%	3,900	100%	12	3,900	100%	12	3,998	100%	6	2,645	0%	0	0	0%	0	0
Lab Technician, GS4	Lab Staff	50%	3,000	50%	12	1,500	50%	12	1,536	50%	6	758	0%	0	0	0%	0	0
Data manager, NOA/6	Data Staff	20%	8,556	20%	12	1,711	20%	12	1,754	20%	12	1,798	20%	12	1,843	20%	12	1,889
Programmer/Analyst GS6/3	Data Staff	60%/20%	5,508	60%	12	3,305	20%	12	1,129	20%	12	1,157	100%	12	7,586	100%	12	7,775
Data Analyst NOA	Data Staff	100%	7,044	0%	0	0	0%	0	0	0%	0	0	100%	12	2,714	100%	12	2,782
Data management assistants GS3	Data Staff	100%	2,520	100%	24	5,040	100%	24	5,166	100%	24	5,295	100%	12	2,714	100%	12	2,782
Subtotal----->						287,345			352,730			386,092			176,218			152,234
CONSULTANT COSTS						6,000			6,000			6,000			6,000			6,000
						6,000			6,000			6,000			6,000			6,000

1,254,619

30,000
Shafiqul Haque Moin
Controller, Budget & Costing

ITEM DESCRIPTION	Year 1	Year 2	Year 3	Year 4	Year 5	
	Totals	Totals	Totals	Totals	Totals	
EQUIPMENT						
Hemocues (4x\$400)	1,600	0	0	0	0	
eDEM microchip pill bottles (2000 x \$30)	57,000	3,000	0	0	0	
Microscope (2x \$2000)	4,000	0	0	0	0	
Refrigerator/Freezer	2,000	0	0	0	0	
Sonograph (3)	46,800	0	0	0	0	
Office Furniture (desks+chairs, desks+chairs, \$160x6 sets)	1,000	0	0	0	0	
Wooden length board/height stick (20x\$30)	600	0	0	0	0	
Computers+UPS/accessories (5 x \$1800) and printer (1 x \$1200)	10,200	0	0	0	0	
	123,200	3,000	0	0	0	126,200
SUPPLIES						
Office supplies	2,500	2,500	2,500	2,500	2,500	
Lab supplies (blood collection, \$1.91x5,300 subjects)	10,123	0	0	0	0	
Metronidazole for treating BV (50.035x10,000 tablets)	350	0	0	0	0	
Hemocue cuvettes (15,900x \$0.22)	3,498	0	0	0	0	
Food Supplements	30,769	30,769	30,769	0	0	
Micronutrient supplements (\$1.86x5,300 subjects)	9,854	0	0	0	0	
Miscellaneous supplies	3,000	3,000	3,000	2,000	2,000	
	60,094	36,269	36,269	4,500	4,500	141,632
TRAVEL AND TRANSPORTATION						
Local travel, in/between Dhaka Mallab	4,000	4,000	3,000	2,000	1,000	
International Travel	10,000	10,000	10,000	16,000	16,000	
	14,000	14,000	13,000	18,000	17,000	76,000
PATIENT CARE COSTS						
INPATIENT						
OUTPATIENT						
ALTERATIONS AND RENOVATIONS						
OTHER EXPENSES						
Printing, photocopies	3,000	2,000	2,000	2,000	4,000	
Library and other interdepartmental costs	1,500	1,100	1,000	1,000	1,100	
Dissemination	1,500	1,100	1,000	4,000	6,000	
Communication (email, fax, tel)	1,150	1,200	1,000	1,300	1,500	
Utilities and other costs (Mallab)	500	500	600	800	1,100	
Rent and facility charges (Mallab)	1,000	1,000	1,000	1,000	1,300	
Testing for BV (supplies and test costs; \$2.75x3,200 subjects)	8,800	0	0	0	0	
Analyses of serum for micronutrients (supplies and test costs)	0	11,000	93,000	144,000	155,000	
Software licences	2,000	2,000	1,400	2,000	2,000	
Equipment maintenance	500	1,000	1,200	1,200	1,100	
Unforeseen expenditures	10,000	10,000	10,000	10,000	10,000	
Charges for HDSS data and resources	1,688%	8,615	7,308	6,111	5,956	
Rent, utilities and other charges (Dhaka)	3.00%	15,312	12,582	13,316	10,861	
Security, cleaning, maintenance and other general services	2.00%	10,208	8,656	8,877	7,240	
	63,885	59,874	142,185	191,512	205,698	664,154
TOTAL DIRECT COSTS						
OVERHEAD	25%					
	159,631	117,968	120,887	99,058	96,608	2,292,605
TOTAL COSTS						
	693,155	589,841	603,433	495,288	483,040	2,865,757
TOTAL DIRECT COSTS REQUESTED TO UNICEF						
OVERHEAD (Allowable by UNICEF)	10%					
	19,881	12,950	7,978	0	0	406,065
TOTAL COSTS (Allowable by UNICEF)						
	216,436	142,454	87,754	0	0	446,654

Sharnima Moin
Controller, Budget & Costing

Budget Justification

Personnel

Dr. L.A. Persson, will serve as Principal Investigator (20% time commitment). In his capacity as the Associate Director of the Public Health Sciences Division of ICDDR,B, he will ensure coordination and provide administrative support as necessary, and will provide scientific expertise in the portions of the project related to the nutritional epidemiology and on the development and analysis of the results of the various interventions. Dr. G. Fuchs will provide scientific and administrative support as the Co-Principal Investigator (10% time commitment). Dr. S. E. Arifeen will serve as Co-Investigator and will commit 40% of his time to the proposed research project. He will assist the PI in coordination and administration of the project as well as, coordination with other partners. Dr. Arifeen will also provide scientific expertise particularly in the portions of the project related to dietary supplementation and intrauterine growth. Dr D.S. Alam will serve as co-Investigator at 100% time. He will provide the technical co-ordination of the field work. He will also provide expertise in dietary intake measurements and infant follow-up. Drs. Ekstrom, Shaheen, Wagatsuma (20% time commitments), and Drs. Osendarp, Naved and Blum will serve as co-Investigators (15% time commitments). Dr. Ekstrom will provide scientific expertise on the micronutrients and compliance aspects of the project, while Dr. Osendarp will provide scientific expertise on the dietary assessment and micronutrient interventions. Dr. Shaheen has a special interest in maternal morbidity and will provide scientific expertise on that area. She will be responsible for the health economic evaluation of the intervention. Dr. Wagatsuma will provide scientific expertise on portions of the project related to fetal growth ultrasound measurements; Drs. Naved and Blum will provide scientific expertise on the social and behavioral aspects of the interventions and outcomes. Dr. Kabir is a member of the ICDDR,B team of investigators responsible for the intervention trials on promotion of exclusive breastfeeding. He will provide scientific expertise on the post-partum intervention (10% time commitment). Mr. Wahed is the Head of the Biochemistry Laboratories of ICDDR,B. He will provide scientific and technical expertise on the assessment of biochemical outcomes (10% time commitment). Drs Shahid and Rahman will provide scientific and technical expertise on the bacterial vaginosis component (15% time commitment each). Dr. Yunus (10% time commitments), in his capacity as the Head of the Matlab Health Research Programme will guide and facilitate the implementation of the project activities in Matlab.

There is firm commitment from UNICEF to provide partial funding for a portion of the project, including salary, up until enrollment of births as the primary outcome, but not including the ultrasound and behavioral components. The period for the UNICEF support is 3 years. Consequently, support from UNICEF will not cover costs in years 4-5. Dr. Persson requests only 38% reimbursement for his time in years 2 and 3 and none in year 1. Drs. Ekstrom, Shaheen, and Yunus request only 50% reimbursement for their time from UNICEF. Dr. Osendarp request only 67% reimbursement for her time in year 1 only. Dr. Alam request only 35% reimbursement for his time. Dr. Arifeen request 50% reimbursement for his time in years 1-2 and 100% reimbursement in year 3. We are requesting 100% reimbursement of the time given by Drs Shahid and Rahman. Drs. Fuchs, Wagatsuma, Naved, Blum, Kabir and Wahed do not request any salary support from UNICEF.

Field activities in Matlab. The Senior Manager, Community Health Research of the Matlab Health Research Program (10% time commitment, 50% reimbursement for his time from UNICEF) will coordinate the community health services with the fieldwork of the study. He will be assisted by a Field Manager, and a clerk, both with 100% time commitments and 100% reimbursement. An administrative assistant will provide assistance to the investigators in Dhaka (100% time commitment, 100% reimbursement for his/her time). Three full-time Senior Field Research Assistants will be paid for from UNICEF funds. They will assist the Field Manager in field monitoring, coordination, supervision and support.

Funding needs for the field data collection staff is calculated based on the month-to-month volume of interviews and measurements on recruited mothers, delivering mothers and follow-up of babies. Data is collected at home visits, at the Community Nutrition Centre, as well as at planned visits to clinics. The responsibilities of the full-time field and clinic staff are described in detail in the Methods Section of Appendix 2.

A total of 293 person-months of *Female Paramedics* will be required for activities in the sub-centre and enrolment of newborns over 3 years of which salary support requested is for 80 person-months. This is based on one paramedic in each of the 4 sub-centres and a few more making an average of 3 home visits per day. *Sonographers* will be needed for 116 person-months over 3 years (one in each sub-centre), but no salary support has been requested. *Follow-up Interviewers* will make home visits (6 per day) during and after pregnancy to collect data. The total needed is 446 person-months over 4 years of which support for 56 is being requested. *Dietary Interviewers* will be female graduates or equivalent preferably with previous experience of conducting dietary interviews or interviewing women on health or nutrition related issues. The need is for 251 person-months (3-4

Principal Investigator: Lars Ake Persson

interviews per day), request is for 151 person-months. *CNC Monitors* will be male graduates preferably with some previous experience in field survey. Their main activity will be to visit Community Nutrition Centres within the study area every 2 weeks and collect information on supplement distribution (through BINP) among study women. We will need 145 person-months of *CNC Monitors*, of which we are requesting support for 100 person-months. We are also require support for *Ethnographers* for 132 person-months. They will collect ethnographic and behavioural data. The *Counsellors* will be responsible for the post-partum intervention. The requirement and request is for 454 person-months. We expect that they will be able to make 3-4 home visits per day. However, no salary support is requested from UNICEF for the *Ethnographers* and *Counsellors*. *Helpers/messengers* will be male or female workers who will accompany interviewers during anthropometric measurements and other fieldwork, which might need assistance. A *Research Officer* and a *Lab Technician* will be responsible for the testing of vaginal swabs specimens and supervision of the collection of blood samples for Hb and micronutrient assessments and reassessment of BV slides in Dhaka.

The person-months and percentage of effort for which reimbursement has been requested from UNICEF for the field staff has been detailed in the budget.

UNICEF funds will be used to reimburse 20% effort of a Data Manager, 50% effort of a Programmer in year 1 and 20% effort of the Programmer in years 2 and 3, and 2 full-time Data Management Assistants (DMA). The Data Manager will supervise all data management activities while the Programmer will develop programs for data entry and preliminary analysis. The 2 DMAs will be responsible for data entry. In the last 2 years, we propose the reimbursement of 25% effort of the Data Manager and the Programmer and one full-time DMA from another source. To assist with data analysis in the last 2 years, an expert Data Analyst will be recruited full-time. None of the costs in the last 2 years will be requested from UNICEF.

Consultant Costs

A health economist from Umea University in Sweden and Rukhsana Haider of John Snow Inc., Dhaka will be hired as consultants to provide assistance with the economic and breastfeeding aspects. The health economist will make one 5 d trip to Dhaka every year (airfare \$1,500). Each of the consultants will provide 2 weeks of FTE every year for the project. However, none of these costs will be requested from UNICEF.

Equipment

Funds are requested from UNICEF for procuring 2 computers and a printer. The computers will be used by the two Data Management Assistants who are likely to be new recruits and will require computers. Three more computers will be procured from another source to replace those that are outmoded and to be used by the Field Manager and the Clerk in Matlab and the Administrative Assistant in Dhaka.

Some funds have been requested from UNICEF to procure two microscopes for the BV testing (\$4,000), essential field office furniture and length boards. One refrigerator will also be procured with UNICEF support to store blood/serum specimens in Matlab before transportation to Dhaka.

UNICEF funds will be used to procure another 500 eDEM pill bottles. Other funds are also requested for procuring another 2,000 bottles. Four Hemocue machines are available now and another four will be procured through UNICEF funds. Four portable sonograph machines will be procured from other sources of funds available with ICDDR,B.

Supplies

Of the 15,900 Hemocue cuvettes needed for the entire study, funds from UNICEF will be used to procure 9,600. Other funds are requested for procuring the remaining amount. In addition, funds are requested to cover the cost of basic office supplies, and other miscellaneous supplies. UNICEF funds will be used to cover the costs of procuring metronidazole and placebo tablets for treating BV. \$6,112 has been allocated from the UNICEF budget to cover the cost of supplies necessary for collection and storage of blood specimens. Not included in the budget request is US\$5,949 as costs of micronutrient supplements which will be procured directly by UNICEF.

Food supplements will be required for 5,300 women for an average of 120 d in pregnancy and another 120 d in lactation. Every day, they receive 4 packets costing \$0.048. Forty percent of the costs will need to be covered from project sources (non-UNICEF) as the proportion of women not currently targeted for supplementation by the on-going national program. (It is 40%, but split over 3 years, i.e. about 13% each year. The remaining 60% of the costs will be available from the on-going government supplementation program)

Principal Investigator: Lars Ake Persson

Travel

Local travel: Funds are requested to cover expenses for travel within Dhaka and between Dhaka and Matlab by investigators, using ICDDR,B transportation. The study also involves extensive travelling within Matlab by field staff and supervisors, using both ICDDR,B and local public transportation. The request for support from UNICEF is \$3,500 in year 1, and \$2,500 in years 2 and 3.

International travel: Funds are budgeted annually for the PI/Co-Investigators to travel to Cornell for a coordination meeting, for 10 d each trip, including a stopover in Washington, DC, for a Global Network meeting; and for one of the co-Investigators to make a trip to Ithaca/US, for 10 d. The round-trip airfare Ithaca-Dhaka is \$2,950 and applicable *per diem* is \$184/d. In Years 4 and 5, funds are requested for the two of the ICDDR,B staff members to present results of this work at scientific meetings each year (\$3,000 requested to cover the costs of airfare, *per diem* and meeting registration fees for each trip). All travel uses coach-class airfares and the *per diem* rates of WHO. The travel costs will also include other admissible costs such as visas, in-transit per-diem or lay-over costs, airport-hotel taxis, etc. None of these costs will be requested from UNICEF.

Other Expenses

Funds are requested to cover printing and photocopying, library and other ICDDR,B interdepartmental costs, dissemination, telephone and other communication, Matlab utilities, rent and facility charges in Matlab, testing for BV, micronutrient laboratory analysis, software, and equipment maintenance as detailed in the budget. Some allocation has been reserved for unforeseen needs. Each of the 5,300 women will provide 2 samples of blood for assessment of selected micronutrients. The specimens will be tested for Hb, transferrin, ferritin, CRP, Vitamin B12, folate, vitamin A, zinc and copper. A rate of \$39.2 per specimen has been used to estimate total costs of these tests. These charges include test kits; equipment use charges; Dhaka laboratory personnel and other laboratory supplies. The total amount of support needed and the amount sought from UNICEF have been detailed in the budget.

The Matlab Health and Demographic Surveillance System (HDSS) provides the population basis for this study. Basic demographic data on this population, information on location and current status of the eligible population, data on pre-pregnancy weights, mortality and numerous confounding variables (including spatial GIS information) will be made available by HDSS to the project at charges of \$8,615 in year 1, \$7,308 in year 2, \$7,492 in year 3, \$6,111 in year 4, and \$5,956 in year 5. Only a portion of these charges have been requested from UNICEF. The project plans to use office space and utilities in the Dhaka buildings of ICDDR,B. These will be charged as indicated in the budget. Costs of using the ICDDR,B security, cleaning, dispatcher and transport, maintenance and other general services in Dhaka have been itemized under "Security, cleaning, maintenance and other general services". The total amount of support needed and the amount sought from UNICEF have been detailed in the budget.

Abstract Summary

Combined Interventions to Promote Maternal and Infant Health.

Study summary

Poor maternal nutritional status remains an important determinant of long-term maternal health as well as of foetal growth and subsequent infant health and survival, especially in South Asia. Infections during pregnancy may contribute to impaired foetal development and short gestational period.

We will evaluate combinations of 4 different type of interventions among a single group of approx. 5,000 undernourished women who live in Matlab *upazila*, Bangladesh, the well-established field site of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). An on-going surveillance program identifies pregnant women within 6-8 wk of conception and an on-going government program provides a food supplement to pregnant and lactating women that contains 600 kcal/d (6 d/wk) and a pill that contains 60 mg iron (Fe) and 400 mg folic acid daily.

The 4 study interventions are; *1 Food supplementation* Pregnant women will randomly be assigned to receive advice to enroll in the food supplementation program (a) immediately after diagnosis of pregnancy or (b) at the time of their choosing. *2 Micronutrient supplementation.* All pregnant women will randomly be assigned to receive a pill that contains (a) 30 mg Fe and 400 µg folic acid or (b) 60 mg Fe and 400 µg folic acid or (c) 30 mg Fe, 400 µg folic acid and 13 additional micronutrients. *3 Bacterial vaginosis treatment.* All women will be offered screening for Bacterial Vaginosis (BV). Asymptomatic BV-positive women will be randomly assigned to (a) 250 mg metronidazole orally thrice daily for 7 days or (b) lactose tablets given with the same dose frequency. *4 Exclusive breastfeeding counseling.* The women will be randomly assigned to receive either (a) counseling for exclusive breastfeeding (EBF) or (b) a different health education message of equivalent intensity.

Each of these interventions is designed to address an important scientific issue and also uses an intervention that could be readily incorporated into public health programs. The combinations of the interventions will allow for analysis of combined effects and interactions between the interventions.

The final data collection instruments have not been completed and will need to be pre-tested. These instruments are not expected to contain any question which could be considered either sensitive or an invasion or privacy. The completed draft instruments will be presented to the committee in its next meeting.

Strategies to address specific ethical issues.

1. The study is designed to evaluate the effect of a combination of 4 different interventions for pregnant women aimed at reducing prevalence of low birth weight. Thus, the subjects are pregnant women in a population known to have high prevalence of low birth weight.
2. Participation in the study does not involve any significant risk. The content of iron (30 mg/day) in two of the micronutrient supplements are lower than recommended by WHO. However, it is substantially higher than the 120 mg/wk recently evaluated in WHO multi-center studies that was found to have only a small difference in effect from 60 mg/day currently recommended. Treatment of bacterial vaginosis with metronidazole is recommended by the Centers for Disease Control, USA, and by the Government of Bangladesh as part of the syndromic management of vaginal discharge. These recommendations do not contraindicate metronidazole after the first trimester of pregnancy. Research on the association between bacterial vaginosis and preterm delivery is inconclusive and this study aims to investigate whether such an association exist and whether treatment with metronidazole reduces preterm delivery.

3. Women with a hemoglobin concentration below 75 g/L will be excluded and referred for further examination and treatment. Women complaining of abnormal vaginal discharge will all be treated and not included in "bacterial vaginosis" component of the study. In the interviews, the women will be informed that she may refuse to answer. Privacy will strictly be ensured as well as confidentiality of the subject. Clinical examinations including blood collection will be done by appropriately trained health workers under aseptic conditions.
4. Confidentiality of collected information will be maintained by keeping all data forms private and locked at the Matlab Diarrhoea hospital and the ICDDR,B Dhaka offices with access limited only to staff working with the study. Study subject will be identified by study numbers in the computer databases used for analyses and no names will be used in dissemination of results from the study.
5. Consent will be sought from the subjects in two stages. As some of the subjects are illiterate emphasis has been put on good quality oral communication. A first brief information about the study and what participation may involve will be provided by community health workers at the woman's house and a verbal consent will be obtained. As the woman enrolls in the antenatal care program at the sub-center more detailed information on the clinical procedures will be provided by a paramedic and a written consent will be sought after the woman has an opportunity to ask specific questions to the paramedic who would be better able to answer those questions than the community health workers.
6. The interviews will be done at the woman's house. The interviews will be conducted in Bangla by a trained person. The interviews are expected to take maximum one hour and privacy will be ensured. Some additional questions relating to the clinical aspects of the study will be asked when the women come to the sub-centres.
7. The women will benefit from an intensified antenatal care program that includes a combination of interventions not otherwise readily available. The ICDDR,B will ensure that any woman in need is offered treatment at Matlab Hospital or referred to an appropriate facility and assistance provided with transportation. The results from the study will contribute to the understanding of the efficacy of combinations of different interventions. This is the first study where the potential synergistic effects of combinations of interventions are evaluated.
8. Study women will be selected within Matlab demographic surveillance area. Selected pieces of information routinely collected within the surveillance system will later be linked to the study subjects. Additional information will be extracted from the records of the government nutrition programme. Blood sample will be collected 3 times during pregnancy; at about wk 14, 19 and 30.

Combined Interventions to Promote Maternal and Infant Health

VERBAL CONSENT FORM

Good maternal nutrition and treatment of infection during pregnancy are very important for the health and well being of the mother and her baby. Poor maternal nutrition and infection during pregnancy are very common in Bangladesh, as in Matlab, which results in lack of energy/protein, vitamins and minerals. Because of this, a lot of illnesses and deaths take place among mothers and their babies.

ICDDR,B in collaboration with the Government of Bangladesh, Cornell University of USA and UNICEF, is under-taking a study to improve maternal and infant nutritional status. The study will assist in the ongoing "Pushi" program by helping some of you to start the feeding program earlier, to provide with either iron tablets at standard or half doses, or a mix of 15 vitamins and minerals including half dose of iron. This mix has been specially produced by UNICEF for pregnant women. These tablets will be given to you today in bottles, which you can take home. The decision on who will receive what will be decided at random (by chance). After the birth of your child, we will provide you with either counseling to help you with breast feeding your baby or health education on care for yourself and the baby.

If you agree to participate, we and other members of our team will visit you several times when you are pregnant and also after the birth of your child until the baby is 12 months old. We will also request you to visit the ICDDR,B sub-centre at least 4 times during pregnancy. In the sub-centre, we will give you a full antenatal check up, including physical and pelvic examination and ultrasonographic monitoring of the baby inside you. We will also collect 4 ml of blood (less than one tea spoonful) from your veins two times and once from a finger prick. We will test this blood for anaemia, and status of other vitamins and minerals. On your first visit to the sub-centre, if you agree, we will test whether you have an infection in the vagina, which is known to result in early delivery of babies. If you have the infection, but do not have an abnormal vaginal discharge then you will either receive a seven-day treatment course with a drug called metronidazole or with something that will look similar to it but will not have the drug. No one will know who will receive what which will be decided by chance.

We will set up a system so that we will be able to measure the weight, length and other measurements of your newborn soon after birth. We will request your family to notify us immediately after birth so that we can do this. We will reimburse you for cost incurred. We will check on your physical condition after birth to assess if you have any health problems and provide necessary management. We will visit you to take the weight and length of your child once every month till 6 months of age, and every 2 months thereafter till 12 months of age. We will also ask you about feeding and health of the baby.

The purpose of doing all this is to assess the benefit of the food and micronutrient supplementation, treatment of the infection in the vagina, and of the breastfeeding counseling. The information that will be available from this will help the policy makers to decide on the best way to provide these services for communities like ours.

We assure you that we shall maintain the confidentiality about the information we collect from you. All records from this study at the Matlab Diarrhoea Hospital or the Dhaka offices of ICDDR,B will be kept private and in a locked location. Only people doing the study will be able to look at them. Any study records that are taken from ICDDR,B will not have any of the names of who took part in the study.

You may ask any question regarding the study and shall be happy to answer then for you. Your participation is absolutely voluntary. You are at liberty to withdraw from the study at any time during the study without any penalty or change in the routine care you or your child receives. If you decide not to take part in these parts of the study, it will not change the care you, your child or your family receives from ICDDR,B in any way. Also, once you have agreed to take part in the study, you may remove yourself or your child from the study at any time without any penalty or change in the routine care you or your child receives. You will still receive our routine care and necessary support and treatment.

Once you have agreed to participate in the study, you can contact your home healthcare worker if you have any problems or questions. You may also contact the Matlab Hospital of ICDDR,B or Dr. Lars Ake Persson at the following phone number at any time: 988 5155 (Dhaka).

Do you have any questions? Yes No

Do you understand what this study is doing? Yes No

Do you agree to participate in this study? Yes No

Date: _____

Signature of Community Health Worker

Combined Interventions to Promote Maternal and Infant Health

Verbal Consent Form

মৌখিক সম্মতি প্রত্ন

গর্ভকালীন অবস্থায় মায়ের যথাযথ পুষ্টি বিধান এবং সংক্রামক রোগের চিকিৎসা মা ও তার শিশুর সুস্বাস্থ্য এবং মঙ্গলের জন্য খুবই গুরুত্বপূর্ণ। গর্ভাবস্থায় মায়ের অপুষ্টি এবং সংক্রামক রোগ বাংলাদেশে খুবই সাধারণ একটি ঘটনা যা মতনবের বেলায়ও প্রযোজ্য। এই অপুষ্টির ফলে মায়ের শক্তি, আশিষ, ভিটামিন ও খনিজ পদার্থের অভাব হয়। এই কারণে মা ও শিশুর অনেক অনস্বস্ততা এবং মৃত্যুর মুখোমুখি হতে হয়।

আই সি ডি ডি আর, বি, বাংলাদেশ সরকার, যুক্তরাষ্ট্রের কর্ণেল ইউনিভার্সিটি ও ইউনিসেফ এর সহযোগিতায় একটি গবেষণা প্রকল্প হাতে নিতে যাচ্ছে যা মা ও শিশুর পুষ্টি অবস্থার উন্নতি সাধন করবে। এই প্রকল্পের আওতায় চলতি 'পুষ্টি' কর্মসূচিকে সহযোগিতা করে আপনাদের মধ্যে কাউকে কাউকে শীঘ্র পুষ্টি কর্মসূচিতে অংশগ্রহণ করতে সাহায্য করা হবে, কেউ সাতাষিক বা অর্ধেক মাত্রায় আরও ট্যাবলেট পাবে অথবা ১৫টি ভিটামিন ও খনিজ পদার্থ মিশ্রণ সম্বলিত একটি ট্যাবলেট পাবে (যার মধ্যে অর্ধেক মাত্রায় আরও অত্যন্ত) এর মাধ্যমে এই গবেষণাটি চলতি 'পুষ্টি' কর্মসূচিকে সহযোগিতা করবে। ইউনিসেফ ভিটামিন এবং খনিজের এই সংমিশ্রণটি গর্ভবতী মহিলাদের জন্য বিশেষ ভাবে তৈরী করেছে। এই ট্যাবলেটগুলো আপনাদেরকে বোতলে ভরে দেয়া হবে যা আপনারা বাসায় নিয়ে যেতে পারবেন। কে কোন ধরনের ট্যাবলেট পাবে তা নটরীতে নির্ধারণ করা হবে। শিশুর জন্মের পর আমরা আপনাকে শিশুকে বুকের দুধ খাওয়ানোর ওপর পরামর্শ দেব অথবা শিশুর যত্নের বিষয়ে স্বাস্থ্য পরামর্শ প্রদান করব।

আপনি যদি এই গবেষণায় অংশগ্রহণে সম্মত হন তবে আমরা এবং আমাদের দলের অন্যান্য সদস্যরা আপনার গর্ভকালীন সময়ে এবং আপনার শিশুর জন্মের পর তার ১২ মাস বয়স পর্যন্ত কয়েকবার আপনার সাথে সাক্ষাত করব। গর্ভাবস্থায় মোট ৪ বার আই সি ডি ডি আর, বি-র স্বাস্থ্য কেন্দ্রে যাওয়ার জন্য আমরা আপনাকে অনুরোধ করব। স্বাস্থ্য কেন্দ্রে আমরা আপনাকে শারীরিক পরীক্ষা সহ গর্ভকালীন সকল পরীক্ষা-নিরীক্ষা করব, আলট্রাসোনোগ্রামের মাধ্যমে জরায়ুর ভেতরে শিশুর পর্যবেক্ষণ করব। আমরা দুই বার আপনার শিরা থেকে ৪ মি.লি. (এক চা চামচের কম) এবং একবার আঙ্গুরের মাথা থেকে রক্ত সংগ্রহ করব। এই রক্ত পরীক্ষা করে আমরা আপনার রক্তশূণ্যতা এবং অন্যান্য ভিটামিন ও খনিজ লবণের অবস্থা জানব। আপনি প্রথমবার স্বাস্থ্য কেন্দ্রে এলে, এবং সম্মত হলে, আমরা পরীক্ষা করে দেখব আপনার ঘোনির কোন সংক্রমণ আছে কিনা যার ফলে সময়ের আগেই শিশুর জন্মান ঘটে। আপনার ঘোনির এই সংক্রমণ পাওয়া গেলে এবং কোন অস্বাভাবিক প্রাচ্য না থাকলে আপনাকে হয় মেট্রোনিডাজল ঔষধ দিয়ে সাতদিনের চিকিৎসা দেয়া হবে বা একই রকম দেখতে কিন্তু যার মধ্যে ঐ ঔষধ নাই তা দেয়া হবে। কেউ জানবে না কে কি পাবে - নটরীর মাধ্যমে তা নির্ধারণ করা হবে।

আমরা এমন একটি ব্যবস্থা গ্রহণ করব যার মাধ্যমে জন্মের পর পরই আপনার শিশুর ওজন, দৈর্ঘ্য ও অন্যান্য মাপ করা যাবে। আপনাকে ও আপনার পরিবারকে আমরা অনুরোধ করব যে শিশুর জন্মের পর পরই আপনারা আমাদের জানাবেন যাতে আমরা একাজটি করতে পারি। এর জন্য আপনাদের যা খরচ হবে আমরা তা পরিশোধ করে দেব। আপনার শিশুর জন্মের পর আপনার শারীরিক অবস্থা আমরা পরীক্ষা করে দেখব এবং কোন শারীরিক সমস্যা থাকলে তার জন্য প্রয়োজনীয় ব্যবস্থা প্রদান করব। আপনার শিশুর ৬ মাস বয়স পর্যন্ত প্রতি মাসে একবার এবং এরপর প্রতি দুই মাস পর পর তার ১২ মাস বয়স পর্যন্ত আমরা আপনার শিশুর ওজন এবং দৈর্ঘ্য মাপার জন্য আসব। এছাড়া আপনার শিশু খাওয়া-দাওয়া এবং স্বাস্থ্য সম্পর্কেও আমরা জিজ্ঞাসা করব।

এই সব করার মূল উদ্দেশ্য হলো পুষ্টি খাদ্য ও ভিটামিন/খনিজ পদার্থ, গর্ভাবস্থায় সংক্রমণের চিকিৎসা, এবং বুকের দুধ খাওয়ানোর পরামর্শদানের উপকারিতা যাচাই করা। এ থেকে আমরা যে তথ্য পাব তা আপনাদের জন্য এ সকল সেবা প্রদানের সব চেয়ে ভাল পন্থাটি বাছাই করতে সহায়ক হবে।

আমরা আপনাকে নিশ্চয়তা দিচ্ছি যে আপনার কাছ থেকে যে সকল তথ্য সংগ্রহ করা হবে তা গোপন রাখা হবে। এই গবেষণা কাজ সংক্রান্ত সকল কাগজপত্র আই সি ডি ডি আর, বি-র মতনব ও ঢাকা অফিসে নিরাপদে রাখা হবে। যারা এই গবেষণার সাথে জড়িত শুধুমাত্র তারাই কেবল এ সকল কাগজপত্র দেখতে পারবে। যে সকল কাগজ পত্র আই সি ডি ডি আর, বি-র বাইরে নেয়া হবে সেখানে আপনাদের কারও নাম থাকবে না।

আপনি এই গবেষণা সম্পর্কে যে কোন প্রশ্ন আমাদের করতে পারেন এবং আমরা আপনার সাথে তার জবাব দেব। এই কাজে আপনার অংশগ্রহণ সম্পূর্ণ স্বেচ্ছামূলক। কোন রকম দায়বদ্ধতা ছাড়াই এই গবেষণা থেকে যে কোন সময়ে সরে আসার পুরোপুরি স্বাধীনতা আপনার আছে। আপনি যদি এই গবেষণায় অংশগ্রহণ নাও করেন তবুও আপনি ও আপনার পরিবারের আই সি ডি ডি আর, বি থেকে

কোন চিকিৎসার ক্রটি হবে না। এবং একবার এই গবেষণায় অংশগ্রহণে সম্মত হওয়ার পরও আপনি চাইলে যে কোন সময় এই গবেষণা থেকে আপনি নিজে কিংবা আপনার সন্তানের অংশগ্রহণ প্রত্যাহার করে নিতে পারেন এবং তাতেও নিয়মিত যে সেবা আপনি কিংবা আপনার সন্তান পেয়ে আসছেন তার কোন পরিবর্তন হবে না। আপনি বরাবরের মতই আমাদের নিয়মিত সেবা এবং প্রয়োজনীয় সহযোগিতা ও চিকিৎসা পেয়ে যাবেন।

এই গবেষণায় অংশগ্রহণে সম্মত হওয়ার পর আপনি যে কোন সমস্যা বা প্রশ্নের জন্য আপনার এলাকার স্বাস্থ্যকর্মীর সাথে যোগাযোগ করতে পারবেন। আপনি আই সি ডি ডি আর, বি মতনব হাসপাতালেও যোগাযোগ করতে পারেন অথবা ডাঃ লার্ন অকে পারদর্শনের সাথে নিচের ফোন নম্বরে যে কোন সময় যোগাযোগ করতে পারেনঃ ৯৮৮ ৫১৫৫ (ঢাকা)

আপনার কি কোন প্রশ্ন আছে ?

হ্যাঁ

না

আপনি কি বুঝতে পেরেছেন এই গবেষণা কি নিয়ে কাজ করছে?

হ্যাঁ

না

আপনি কি এই গবেষণায় অংশগ্রহণে সম্মত আছেন ?

হ্যাঁ

না

তারিখ : _____

স্বাস্থ্য কর্মীর স্বাক্ষর

WRITTEN CONSENT FORM

Date: _____

Combined Interventions to Promote Maternal and Infant health

Written Consent Form

লিখিত সন্মতি পত্র

এই গবেষণা কাজে অংশগ্রহণের জন্য এবং আজকে এই সাক্ষ্য কেন্দ্রে আসার জন্য আপনাকে ধন্যবাদ। আপনার নিশ্চয়ই এই গবেষণা কাজ সম্পর্কে সব কিছু মনে আছে, তারপরও আপনার সুবিধার জন্য কিছু তথ্য আমি আবার বলছি।

গর্ভকালীন অবস্থায় মায়ের যথাযথ পুষ্টি বিধান এবং সংক্রমক রোগের চিকিৎসা না ও তার শিশুর দুগ্ধাস্তা এবং মঙ্গলের জন্য খুবই গুরুত্বপূর্ণ। গর্ভাবস্থায় মায়ের অপুষ্টি এবং সংক্রমক রোগ বাংলাদেশে খুবই সাধারণ একটি ঘটনা যা মতলবের বেলায়ও প্রযোজ্য। এই অপুষ্টির কালে মায়ের শক্তি, আঁশ, ভিটামিন ও খনিজ পদার্থের অভাব হয়। এই কারণে না ও শিশুদের অনেক অসুস্থতা এবং মৃত্যুর মুখোমুখি হতে হয়।

আই সি ডি ডি আর, বি, বাংলাদেশ সরকার, যুক্তরাষ্ট্রের কর্ণেল ইউনিভার্সিটি ও ইউনিসেফ এর সহযোগিতায় একটি গবেষণা প্রকল্প হাতে নিতে যাচ্ছে যা মা ও শিশুর পুষ্টি অবস্থার উন্নতি সাধন করবে। এই প্রকল্পের আওতার চুক্তি 'পুষ্টি' কর্মসূচিকে সহযোগিতা করে আপনার মাঝে কাউকে কাউকে শীঘ্র পুষ্টি কর্মসূচিতে অংশগ্রহণ করতে সাহায্য করা হবে, কেউ রাজানিত বা অর্ধেক মাত্রায় আরও ট্যাবলেট পাবে অথবা ১৫টি ভিটামিন ও খনিজ পদার্থ মিশ্রণ সম্বলিত একটি ট্যাবলেট পাবে (যার মাধ্যমে অর্ধেক মাত্রায় আরও অস্তর্ভুক্ত) এর মাধ্যমে এই গবেষণাটি চলতি 'পুষ্টি' কর্মসূচিকে সহযোগিতা করবে। ইউনিসেফ ভিটামিন এবং খনিজের এই সংমিশ্রণটি গর্ভবর্তী মহিলাদের জন্য বিশেষ ভাবে তৈরী করেছে। এই ট্যাবলেটগুলো আপনারদেরকে যেভাবে ভরে দেয়া হবে বা আপনারা বাসার নিচে যেতে পারবেন। কে কোন ধরনের ট্যাবলেট পাবে তা লটারীতে নির্ধারণ করা হবে। শিশুর জন্মের পর আমরা আপনাকে শিশুকে দুগ্ধের দৃশ্য যাওয়ার পর পরামর্শ দেব অথবা শিশুর বাড়ির বিষয়ে স্বাস্থ্য পরামর্শ প্রদান করব।

আপনি যদি সন্মত হন তবে আমরা আজ সাক্ষ্য কেন্দ্রে আসার আপনাকে শারীরিক পরীক্ষা সহ গর্ভকালীন সকল পরীক্ষা-নিরীক্ষা করব, এবং আলট্রাসোনোগ্রামের মাধ্যমে আপনার পেটের ভেতরের শিশুর পর্যবেক্ষণ করব। আমরা আজ আপনার শিরা থেকে ৪ মিলি, (এক চা চামচের কম) রক্ত সংগ্রহ করব। এই রক্ত পরীক্ষা করে আমরা আপনার রক্তসংপূর্ণতা এবং অন্যান্য ভিটামিন ও খনিজ লবণের অবস্থা জানব। আপনি সন্মত হলে, আমরা আজ আপনার যোনির উপরিভাগ থেকে পরীক্ষার জন্য সোজা সংগ্রহ করব এবং পরীক্ষা করে জানব যে যোনির কোন সংক্রমণ আছে কিনা যা রক্তে সময়ের আগেই শিশুর জন্মনাম দাট। আপনার যোনির এই সংক্রমণ পাওয়া গেলে এবং কোন অস্বাভাবিক প্রাণ না থাকলে আপনাকে হয় মেট্রোগিভাজল ঔষধ দিয়ে সন্তানদের চিকিৎসা দেয়া হবে বা একই রকম দেখাতে কিন্তু যার মধ্যে ঔষধ নাই তা দেয়া হবে। কেউ জানবে না যে কি পাবে - লটারীর মাধ্যমে তা নির্ধারণ করা হবে। আমরা গর্ভকালীন সময়ে অন্ততঃ আরো তিনবার এই ক্লিনিকে আসার জন্য আপনাকে অনুরোধ করব। আপনি যখন আসার আসবেন তখন আজকের সবকিছুই আমরা আবার করব। হরমোনের গর্ভাবস্থার আর একবার যোনি থেকে সোজা সংগ্রহ করব - ঔষধ যাওয়ার পর আপনি কতটুকু ভাল হয়েছেন তা দেখার জন্য। এ ছাড়া কয়েক সন্তানের মধ্যেই একজন মাষ্ট কর্নী আপনার বাড়িতে যাবে আপনার আঙ্গুলের মাথা থেকে সুইয়ের রক্ত সংগ্রহের জন্য।

আমরা আপনাকে নিশ্চয়তা দিচ্ছি যে আপনার কাছ থেকে যে সকল তথ্য সংগ্রহ করা হবে তা গোপন রাখা হবে। এই গবেষণা কাজ সংক্রান্ত সকল কাগজপত্র আই সি ডি ডি আর, বি-র মতলব ও ঢাকা অফিসে নিরাপদে রাখা হবে। যারা এই গবেষণার সাথে জড়িত শুধুমাত্র তারাই কেবল এ সকল কাগজপত্র দেখতে পারবে। যে সকল কাগজ পত্র আই সি ডি ডি আর, বি-র বাইরে দেয়া হবে সেখানে আপনার নাম থাকবে না।

এই কাজে আপনার অংশগ্রহণ সম্পূর্ণ স্বেচ্ছানূলক। কোন রকম দায়বদ্ধতা ছাড়াই এই গবেষণা থেকে যে কোন সময়ে সরে আসার পূরোপুরি স্বাধীনতা আপনার আছে। আপনি যদি এই গবেষণায় অংশগ্রহণ নাও করেন তবুও আপনি ও আপনার পরিবারের আই সি ডি ডি আর, বি থেকে কোন চিকিৎসার ভ্রুটি হবে না। এবং একবার এই গবেষণায় অংশগ্রহণে সন্মত হওয়ার পরও আপনি চাইলে যে কোন সময় এই গবেষণা থেকে আপনি নিজে কিংবা আপনার সন্তানের অংশগ্রহণ প্রত্যাহার করে নিতে পারেন এবং তাতেও নিয়মিত যে সেবা আপনি কিংবা আপনার সন্তান পেয়ে আসছেন তার কোন পরিবর্তন হবে না। আপনি বরাবরের মতই আমাদের নিয়মিত সেবা এবং প্রয়োজনীয় সহযোগিতা ও চিকিৎসা পেয়ে যাবেন।

আপনি এই গবেষণা সংক্রান্ত যে কোন প্রশ্ন আমাদের করতে পারেন এবং আমরা আনন্দের সাথে তার জবাব দেব। আপনি যে কোন সমস্যা বা প্রশ্নের জন্য আপনার পারিবারিক স্বাস্থ্যকর্মীর সাথে যোগাযোগ করতে পারবেন।

এই গবেষণা সম্পর্কে আপনার কোন প্রশ্ন থাকলে আমি সানন্দে তার উত্তর দেব। আপনি আই সি ডি ডি আর, বি মতলব হাসপাতালেও যোগাযোগ করতে পারেন অথবা ডাঃ লার্স অকে পারসনের সাথে নিচের কোন নম্বরে যে কোন সময় যোগাযোগ করতে পারেনঃ ৯৮৮-৫১৫৫ (ঢাকা)

আপনার কি কোন প্রশ্ন আছে ?

হ্যাঁ

না

আপনি কি এই গবেষণায় অংশগ্রহণে সন্মত আছেন ?

হ্যাঁ

না

স্বাক্ষর
(প্যারামেডিক)

মায়ের স্বাক্ষর / টিপসই

তারিখ :



BIRDEM

An enterprise of the
Diabetic Association of
Bangladesh

Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders



WHO Collaborating Centre
for Prevention and Control
of Diabetes

June 25, 2003

To: Prof. A K M Nurul Anwar
Chairperson, ERC

From: Prof. Hajera Mahtab
Member, ERC

Sub: Review and comments

Dear Prof. Anwar,

Please find enclosed herewith my review and comments on (i) research protocol # 2003-021 entitled "Antimalarial drug resistance in Bangladesh" (ii) proposal for an addendum to research protocol #2000-025 entitled "Combined interventions to Promote Maternal and Infant Health Study." (Sub-title: Measurement of breast milk intake using stable isotope methods in infants born in MINIMAT study.)

Best wishes & regards.

Hajera Mahtab

Prof. Hajera Mahtab

Director

Clinical Services, Research & Academy

BIRDEM

REVIEW

Subject : Addendum to "Combined Interventions to Promote Maternal and Infant Health' Study." (Sub-title: Measurement of breast milk intake using stable isotope methods in infants born in MINIMat study.)

The MINIMat study is being conducted among a single group of pregnant women who live in Matlab upazilla. In this study 4 combined interventions are being evaluated.

1. Randomly assigning women to receive advice to begin food supplementation programme, either a) immediately after diagnosis of Pregnancy (early care), or b) at the time of choosing (usual care)
2. With each of these groups, women are randomly assigned to receive a pill that contains a) 30 mg Fe and 400 mg folic acid or b) 60 mg Fe and 400 mg folic acid (usual care) or c) 30 mg Fe, 400 mg folic acid and 13 additional micronutrients
3. All women are screened for Bacterial Vaginosis (BV). Within each of the groups mentioned above, asymptomatic BV-positive women are randomly assigned to a) 250 mg metronidazole orally thrice daily for 7 days or b) lactose tablets given with the same dose frequency.
4. All women are randomly assigned to receive either a) counseling for exclusive breast feeding or b) a different health message of equal intensity.

This is an ongoing surveillance programme. Pregnant women are identified within 6-8 weeks of conception. Follow-up of enrolled pregnant women continues throughout pregnancy. Birth weights are measured by home visits within 72 hours of delivery. Approximately 20-30% of births occur in health facilities where they are weighed. Infants are then followed up until they are 24 months of age. Infant nutritional status and morbidity is measured by monthly home visits.

- ✓ The aim of the proposed addendum is to measure actual breast milk intake in infants born during the MINIMat study, using the dose to the mother deuterium dilution technique. In addition to quantifying the actual breast milk intake, this technique significantly allows for the estimation of non-breast milk water intake, and so it can be used to assess breast-feeding and complementary feeding practices. ✓

In the MINIMat study, all mothers are randomly assigned to receive either (a) counseling for exclusive breast feeding (EBF) or (b) a different health education message of equal intensity.

Questionnaires administered to the mothers during the infant follow up will help ascertain both actual breast-feeding practices, and practices according to participation in the two intervention groups. The use of the dose to the mother deuterium dilution technique will help describe actual patterns. The specific objectives of this addendum will be to use the deuterium dilution technique for measuring breast milk intake in order to:

1. Quantify breast milk intakes in infants
2. Assess the impact of counseling for exclusive breast feeding on breast milk intake compared to health education counseling only; and
3. Validate mother's reports on breast-feeding patterns

100 mother-infant pairs will be enrolled into the proposed addendum when the infants are 13 to 15 weeks of age. Breast milk intake will be measured by the deuterium dilution technique. A baseline sample of saliva (2 ml) from the mother and a urine sample (2 ml) from the child will be collected at baseline (day 0) following which the mother will receive an oral dose of deuterium (10 g). A further 3 samples of saliva from the mother, and 5 urine samples from the baby will then be collected over a 14 day period.

Deuterium enrichment in the saliva and urine samples will be analysed using isotope ratio mass spectrometry (IRMS). Intake of breast milk and water from non-milk sources will be calculated by fitting the isotopic (tracer) data to a model for water (tracee) turnover in mothers and infants and the transfer of milk from mother to the baby.

Deuterium is a naturally occurring non-radioactive isotope of hydrogen, which has a natural abundance of about 150 ppm. For measurements in this study mothers will be asked to drink a small amount of deuterium (10g) enriched water that will enrich body water by about an additional 150 ppm. As a result of the infant receiving tracer from mother's milk, enrichment in the infant will increase to about 75 ppm above baseline values.

After this the isotope will disappear from the mothers and infants with biological half-lives of about 5 to 6 and 3 to 4 days, respectively. Similar amounts have been used worldwide in many studies and there are no known risks.

Comment:

The Voluntary Consent form does not follow the standard ICDDR,B format for English and Bengali consent forms.

The study can otherwise be approved.