 A000001

Attachment 1.
(FACE SHEET) ETHICAL REVIEW COMMITTEE, ICDDR,B.

Date 19 JUL 1992

Principal Investigator Darryl J. Holman Trainee Investigator (if any) _____

Application No. 92-021 Supporting Agency (if Non-ICDDR,B) NSF, MELLON, HU, Am. Inst. & Studies

Title of Study Demography of Fetal Loss in rural Bangladesh

Project status:
(X) 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 NA
   (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 abortus — 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 NA
   (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 NA
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 *

* 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.

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

Darryl J. Holman A-032053

Principal Investigator Trainee

8 AUG 1992

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Mr. A. M. Sarder, ICDDR,B

Mr. Mujuibur Rahman, ICDDR,B

3. TITLE OF PROJECT:

The Demography of Fetal Loss in Rural Bangladesh

4. STARTING DATE:

January 1993

5. DATE OF COMPLETION:

January 1994

6. TOTAL BUDGET:

US $36,757.00

7. FUNDING SOURCES:

Mellon Foundation Dissertation Grant
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8. HEAD OF THE PROGRAMME:

Dr. Michael A. Strong
Associate Director,
Population Science and Extension Division

Abstract

The effects of fetal loss on human reproduction will be examined in a one-year prospective endocrinological study of 375 women from Matlab Thana, Bangladesh. Twice-weekly urine samples will be collected and assayed for human chorionic gonadotrophin to detect conceptions as early as the time of implantation. Pregnancies will be monitored prospectively until they terminate in a live birth or a fetal death. Following a fetal death, pregnanediol-3-glucuronide and estrone-3-glucuronide will be assayed to monitor the time until return of normal ovulation. Hazards analyses of the survey results will be used to estimate (1) the distribution in the probability of fetal loss by a woman's age, (2) the distribution of gestational ages at a fetal loss, and (3) the gestational-age pattern of residual infecundability following a fetal loss. The overall effects of fetal loss and heterogeneity in the risk of fetal loss on birth spacing will be studied using the Wood-Weinstein model of age-specific effective fecundability combined with estimates from the endocrine survey. Simulation models will be used to generate distributions of birth intervals using the survey results. Simulation results will be tested for goodness-of-fit to the actual distribution of birth intervals from existing demographic data collected in Matlab.

9. PROJECT AIMS

a. General Aim

This project will examine the demographic effects of fetal loss on human birthspacing. Levels for several different components of fetal loss will be empirically found from a field survey and used to study mathematical models of human birthspacing

b. Specific Aims

The specific objectives of this research are:

1) To assess the overall probability of fetal loss in a near-natural fertility population.

2) To determine changes in the probability of fetal loss with age.

3) To estimate the distribution of gestational ages at the time of a fetal loss

4) To estimate the effect of gestational age on the duration of the infecund period following a fetal loss.

5) To estimate heterogeneity in the risk of fetal loss.

6) To assess the overall effects of fetal loss on human birth spacing patterns.

The research consists of two major components. The first is an eleven-month prospective endocrinological survey of fetal loss in a sample of women in a low contraceptive-prevalance population. This survey will use a highly sensitive and specific enzyme immunoassay for human chorionic gonadotrophin (hCG) that can detect pregnancies by at least seven days after conception (around the time of implantation). The second component will use results from the endocrinological survey to test theoretical models of age-specific effective fecundability and human birth spacing. Simulation models will be used to generate distributions of birth intervals using the results of the fetal loss study in conjunction with other, well-characterized, reproductive parameters. The results of the simulations will be tested for goodness of fit to the actual distribution of birth intervals using existing demographic data collected in Matlab Thana.

c. Significance

In the last few years, remarkable progress has been made in the understanding of human reproduction from contributions in the traditionally disjoint fields of anthropology, reproductive biology, and demography. Much of this progress reflects the development of theoretical models of human reproduction that are based on the underlying biology (Wood, 1989). With these models, demographers are able to disaggregate population level phenomena into biologically meaningful components. Biologists, on the other hand, can use these models with detailed information on individual components of fecundity to understand their effects on reproduction at the population level.

The proposed research uses both approaches to arrive at an understanding of the effects of fetal loss on variation in human birth spacing. The endocrinological survey of a near-natural fertility population will provide detailed information on (1) variation in the probability of fetal loss by a woman's age, (2) the distribution of gestational ages at fetal loss, and (3) the effects of gestational age at fetal loss on the duration of residual infecundability following the loss. These are the three components required for a complete characterization of the effects of fetal loss on human reproduction (Wood, 1989). When combined with other, better-characterized components of fecundity, the overall impact of fetal loss on human birth spacing can be studied.

The demographic impact of fetal loss can be understood in terms of the components it adds to a birth interval. A single fetal loss adds the time of the gestation leading to a fetal death, the residual period of infecundability, and a new fecund wait to the next conception (figure 1). At higher levels, fetal loss exerts a major impact on birth spacing because of the increased probability of multiple fetal losses within the same birth interval. In order to model human birth spacing effectively, fetal loss must be well characterized. It is possible that most of the increase in birth interval length in natural fertility populations is attributable to the increased risk of fetal loss by age rather than to age changes in the ability to conceive. The traditional demographic measure, *age distribution of the onset of permanent sterility*, may actually represent an age-related elevation in the probability of fetal loss censored by the onset of menopause (Wood, 1989).

This research will begin to answer some of these questions by empirical measurements of these components. Although some studies exist on the probability and the gestational age distribution of fetal loss, the resolution of these studies has been too coarse, the sample sizes have been too small, or the studies suffer from biases introduced by selecting a clinical sample. The hCG survey portion of the proposed research is designed to minimize these difficulties and provide more suitable estimates of the parameters for theoretical models.

In addition to the importance of this research to the fields of anthropology, human reproductive biology and demography, this project has an important biomedical implication. A definitive test of the heterogeneity/selectivity hypothesis (discussed below) would be of major importance to women who wish to delay childbearing until later ages. If the hypothesis is correct, the probability of carrying a pregnancy to term at older ages may be much higher than is currently believed.

10. ETHICAL IMPLICATIONS

This research will address questions about the effects of fetal loss on human birthspacing patterns using a detailed empirical study and mathematical models. As outlined in the proposal, this research has inportance to the field of human reproductive biology and demography. This project also has an important biomedical implication.

The data to be gathered in this study come from three sources: (1) private interviews, (2) urine assays, and (3) vital event histories previously collected by ICDDR,B. All interviews will be conducted by female Bangladeshi field assistants, who will initially be instructed in the interview and urine collection protocol and will be periodically examined and retrained. None of the questions asked will place the subjects at legal or financial risk. Some of the information from urine assays and interviews are potentially sensitive or embarrassing, and might be considered an invasion of privacy (e.g. detection of a conception during a period when the spouse has been absent). However sensitive information will not be made available for individuals. To maintain the privacy of the subjects, individual results of pregnancy tests will not be made available to anyone, including the field workers; only the labratory technician, data-entry person, and PI will know the individual results. As detailed below, I will do everything possible to safeguard the confidentiality of the subjects.

The following procedure will be used to acquire informed consent: upon first contact with the subject, the Bangladeshi field assistant will describe the study, purpose, and the procedures for interviews and urine collection. A Bangla copy of this *Explanation of Study* (see attached) will be offered and provided to each subject. The interviewer will then ask the subject to indicate her consent by either signing or providing a thumbprint on the consent form. All consent forms will be returned to the Pennsylvania State University and stored in the secure archives at the Office of Compliance.

The only individuals who will have access to the questionnaires will be the field worker who collected the information (and replacement field workers, if necessary), data entry personnel, and the PI. The questionnaires will be entered into the computer in Bangladesh. To ensure that the field workers properly

identify subjects, names will be used on the questionnaires. A subset of the data without the subjects name, using only ICDDR,B registration ID instead, will be used for all analyses. The master data set will be securely stored on my personal computer, which has hardware password protection at boot-up time.

The questionnaires will be secured by storing them in a locked room or file drawer while in the field. After questionnaires are returned to the US they will not be released to other researchers and will be protected as confidential documents (i.e. not left in an unlocked or unsupervised setting). Even so, there is almost no risk of individuals being identified without the cooperation of the ICDDR,B.

To ensure confidentiality of the subjects, as well as to avoid influencing any pregnancy outcome (via elective abortion, for example), the laboratory assay results will never be known to the field workers. Results of this study will be published in such a way that individuals will not be personally identifiable; names and ID numbers will never be used.

11. BACKGROUND

Fetal loss is a major determinant of birth spacing patterns in humans (Wood, 1989) and may be one of the most important factors influencing the age-pattern of reproduction in natural fertility populations (Wood and Weinstein, 1990). However, the overall probability of a fetal loss has proved difficult to measure. The problem is particularly elusive because early pregnancies have, until recently, been almost impossible to detect. Earlier population surveys of fetal loss, which underestimate early losses and thus grossly underestimate the overall rate, place the probability of fetal loss from 0.12 to 0.15 per conception (Kline et al., 1989). More recent surveys using hCG assays estimate probabilities from 0.31 to 0.62 (Wilcox et al., 1988; Miller et al., 1980; Edmonds et al., 1982); However, these studies must suffer from some selectivity bias due to their clinical nature, and in some cases to the non-specificity of the hCG assay used.

Boklage (1990) developed a statistical model and used the results of previous hCG surveys to estimate the overall probability of loss (including those that are not currently detectable) at nearly 0.76. Wood (1989), considers the effect of the probability of fetal loss on the expected number of fetal deaths per birth interval, and finds that, with a probability of 0.7, 2.3 conceptions are lost in each birth interval. He also examines the expected time added to each birth interval by a fetal loss and finds that values of 0.7 and higher should add unrealistically long times to birth intervals. Thus, Boklage's estimate seems too high, yet it is unclear if this reflects the assumptions of his model, or weaknesses in the studies used to derive the estimate. Wilcox et al. (1988) published one of the most extensive and sensitive hCG surveys of fetal loss in which the probability of fetal loss was estimated to be 0.31. This corresponds well to the estimate of 0.29 in Hertig's (1959) study of fertilized eggs recovered from hysterectomized uteruses.

Given that a loss occurs, its effect on a birth interval results from, in part, the time added by the duration of gestation prior to the death. These waiting times define the age-specific probabilities of fetal loss. The time until a woman resumes ovulation following a fetal loss also contributes some time to the birth interval. The variability of this time and the distribution of post-loss times by gestational age are almost completely unknown. A simple model would predict little residual infecundity for a fetal loss immediately after conception, with longer residual times as the fetal loss occurs nearer the end of gestation. In the absence of any strong evidence, Wood (1989) uses a linear model in which zero time is added by losses at conception and some maximum time added by fetal deaths at nine months. However, a linear model might not be realistic; in part, the infecund period after a fetal loss might be affected by such things as the time for the expulsion of the fetus or by the time needed to clear elevated pregnancy hormones from circulation.

Wood (n.d.) shows patterns for three hypothetical models of the relationship between duration of gestation and the infecund period that follows a fetal death. Figure 2 shows the three models plotted with results from studies of ovulation following termination of pregnancies by live birth in the absence of breast feeding, fetal loss, and elective abortion. From these grouped data, it appears that a linear relationship does not adequately describe this relationship. The upper curve may more closely reflect the relationship. The

curvilinear relationship appears to be more consistent with a hormonal clearance model, in which ovulation resumes at some time following decay of hCG, prolactin, human placental lactogen, progesterone, and estrogen from the maternal circulation.

Theoretical models of human reproduction (Wood, 1989; Weinstein et al., 1990; Boklage, 1990) suggest that fetal loss has the potential for inducing substantial variability in patterns of human reproduction output. Much of this variability is captured by the observed j or u shaped pattern of fetal loss by mothers age. However, Santow and Bracher (1989), Wilcox and Gladen (1982), Casterline (1989), Resseguie (1977), and Leridon (1976) have suggested that the j or u shape distribution of fetal loss by mother's age is largely a statistical artifact at young and old ages, and exaggerated at the older ages. This statistical artifact, in part, results from sampling fetal loss from populations in which some individuals use contraception. If there is heterogeneity in risk of fetal loss among women in the population, then women who are still reproducing at older ages over-represent the subgroup of women who are at higher risk of fetal loss. This is because women with lower risk of fetal loss terminate reproduction after reaching their family size goals at a younger age. Thus, surveys of fetal loss in contraccpting populations tend to over-represent this selected subgroup of high-risk women at older ages. Santow and Bracher (1989) attempt to test this hypothesis using retrospective survey data, but to date, no hCG studies exist to test this heterogeneity/selectivity hypothesis.

The overall impact of fetal loss has been examined by Wood (1989) and Wood and Weinstein (1990) in order to study the behavior of theoretical models of human birth intervals and age-specific fecundability. The approach is to dissects birth intervals into separate components based on underlying biological processes as in figure 1. Figure 3 shows the distributions of lactational infecundability, waiting time to conception, time added by fetal loss, and length of gestation - the four components that determine the length of a single birth interval (the second level in figure 1). With the exception of fetal loss, each of these components have been estimated from reliable data (Wood, 1989; Wood et al., 1992). As shown in figure 1 (bottom), each conception might result in one or more fetal deaths. Given reliable estimates of these components, the total distribution of times added by fetal loss can be estimated from mathematical methods developed in Wood et al. (1992) and outlined in the *Analysis* section; however, estimates for the individual components of fetal loss are unreliable or unavailable. Wood et al.'s (1992) method allows for the incorporation of heterogenity in risk of fetal loss, which provides the basis for estimation of heterogenity from survey data.

Methods

a) Location of Research

This study will be conducted in Matlab Thana, Comilla District, a rural area of Bangladesh. Matlab is situated on the flat deltaic plain of the Meghna river, about 55 km south-east of the capitol city of Dhaka. The climate is subtropical with three major seasons: the monsoon from June to September, the cool-dry season from October to February, and the hot-dry season from March to May (Becker, 1981). Agriculture is the major economic activity, followed by fishing. The population density of this region exceeds 350 people per km^2 (2000 people per $mile^2$). The population of the region is about 85 percent Muslim and 15 percent Hindu.

A striking aspect of fertility in Bangladesh is the strong component of seasonality in conceptions and births (Becker, 1981; Chen et al., 1974). The pattern of conceptions shows a peak begining in March and lasting throught June. Over fifty percent of the conceptions in the Chen et al. (1974) study took place within these four months. This study will not address the issue of seasonality in risk of fetal loss, however the February starting date of the field survey portion of this study will cover the time of peak conceptions and permit the longest period of prospective pregnancy follow-up.

b) Population Survey

The sampling frame to be used consists of those women who are currently enrolled as subjects in the

International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B) demographic surveillance system (DSS) currently underway in Matlab Thana. The DSS has been in place in Matlab since 1963. Since 1966, the DSS has maintained a continuous registration of births, deaths, stillbirths, migration, marriage, divorce, and residence changes. The DSS interview area covers about 200,000 individuals, whose vital events are recorded every two weeks. Approximately 100,000 people are in a Maternal Child Health and Family Planning (MCH-FP) study area and are targeted for a family planning program. In the comparison area (the non-MCH-FP villages) contraception rates vary by village, but overall are lower and a majority of women do not use contraception. Subjects for the DSS survey were selected by the ICDDR,B because of physical access from Dhaka and their representativeness of the rural population.

The DSS comparison area will form the sampling frame for this study. A baseline survey will be conducted in villages with a low prevelance of contraception. A random sample of women who meet the selection criteria will then be draw and 375 women will be recruited into the fetal loss study. The women will be selected according to several criteria: they will be within the ages of 18 to 45, married, non-menopausal, and not contracepting. Furthermore, households in which the husband is a fisherman will not be included. Fishermen have a high prevalence of absence from the household that reaches a peak of about 60 percent and a minimum of about 20 percent over the year. Cultivators, on the other hand have a peak absence of 20 percent and a minimum of 10 percent (Chen et al., 1974). This selection by husband's occupation should increase the number of conceptions observed relative to the study of Chen et al. (1974), in which 25 percent of their sample cycled through out the two year study. This selection will probably result in a somewhat attenuated seasonal component to conceptions, as well.

The subjects will be visited twice a week by trained female field assistants. Approximately twelve field workers will be employed to conduct these interviews. Initially they will be trained in interview and urine collection techniques. Throughout the study, the field workers will periodically be examined and retrained. A "visit" will consist of urine collection and a short interview on the status of reproductive events of interest to this study (births, spontaneous and induced abortions, vaginal bleeding, breast-feeding status, death or absence of husband, and contraceptive use). The urines will be transported to a field laboratory at ICDDR,B for analysis and preservation. A portion of each urine specimen will be preserved with refridgeration and a preservative, or by drying it on filter paper for later quantitative laboratory analysis.

In order to assess the nutritional status of each subject, a one-time anthropometric survey will initially be conducted to measure height, weight, and mid-upper arm circumference. The anthropometric measures will be repeated when the mothers leave the study at the time of a fetal loss or birth.

c) Sample Size

The number of women required for this study has been estimated as the number of women required to find the distribution of fetal loss by mothers age. Although this is only one component of the research questions asked, the age distribution of fetal loss should be the most difficult component to significantly measure. This this estimated by computer simulations of fetal losses at rates estimated from population surveys from a population of women whose age distribution follows that of the Matlab area. The details of the simulation are found in the appendix.

I assume that about 54 percent of the subjects will provide information about conception and the remaining 46 percent will be left-censored (or truncated), extremely right-censored, or will show no conception. The study design is such that each subject contributes at most one conception. The 46 percent of non-informative subjects was estimated from distrubutions found in Chen, et al. (1974) modified for the different sampling scheme used here, and includes the following: 15 percent who will cycle throughout the study without a conception; 15 percent who are amenorrheic throughout the study; 11.25 percent who are amenorrheic for most of the study, resume menstruating, but do not conceive; and 5 percent who drop out of the study without providing any information.

Results from the simulations suggests that a sample of 200 conceptions (before adding in the 46 percent non-informative subjects) will be sufficient for estimating at $p < 0.05$ a two-parameter exponential distrubution for the age-pattern of fetal loss (discussed in the appendix) distribution about 70 percent of the time. Thus, the adjusted sample size is 375 when the non-informative subjects are added. Based on birth interval components from a similar prospective study in Bangladesh (Chen, et al., 1974), I calculate that this will result in approximately 202 detected pregnancies (some partly retrospective), of which 42 will terminate in a fetal death.

The sample size is admittedly marginal for estimating the age pattern of fetal loss, yet this sample size is at the limit of the funds currently available. There are several factors that may relieve this situation. First, there are two pending major funding sources. The sample size can be increased to, perhaps, 500 women if funding comes from both sources. In addition, I will likely have access to some some of the data currently being collected in the Turkana region of Kenya using an identical protocol. That research is part of the dissertation project of Michael Deluca, who is studying the effects of ecological stress on fecundability and fetal loss. Mr. Deluca and I have informally agreed to collect some information that we will share with each other. (Although our field methods are similar, we are addressing fundamentally different questions). The assay protocols that Dr. Kenneth Campbell and I developed this summer are being used for Mr. Deluca's research as well. Finally, with limited additional funds, I will be able to continuously recruit additional subjects as previous subjects drop out or are withdrawn from the study. For this reason, 375 women is the minimum sample that will be recruited, with an upper limit of about 500.

Urines are being collected for four different segments of the reproductive span: (1) the period of exposure to conception, (2) the early fetal period, (3) the later stages of pregnancy, and (4) the period infecundability following a fetal loss. In addition, some proportion of women will be in (5) a state of lactational infecundability. To avoid selectivity bias, the sample of women will be selected without regard to pregnancy status, and thus some fraction of the women will be in each of the states at the start of the study. In addition, some women might be post-menopausal. Each subject will be followed until she has experienced a live birth, experienced a fetal loss, or the survey ends. In addition, subjects will be followed until the resumption of ovulation after a birth or a fetal loss. Some women will not complete segment four by the end of the study; however, the statistical methods outlined below eliminate biases caused by this censoring.

Field Protocols

Field assistants will make regular rounds of subjects, visiting each subject twice per week. They will fill out a interview form and then request a sample of urine from each subject. The subject will be given a 128 ml specimen cup (with a screw-on lid). The specimen cups will be reused after being throughly cleaned in water and bleach. The field assistant will use masking tape to label the specimen with the subject identification, time and date. The specimens will be temporarily stored in a portable plastic cooler containing a frozen blue-ice pack in it. At the end of her rounds for a day, the field assistant will deliver the specimens to the field station where they will be stored in larger coolers or refrigerators. All specimens will be picked up once or twice per week and delivered to the field laboratory by the field supervisor.

The volume of urine will be measured and specific gravity will be estimated using a refractometer. Urines that are too concentrated for the assay will be diluted. A portable meter will assess the PH of urines, and specimens that are too acidic for the assays will be buffered to an acceptable PH. A preservative will be added and the urines will be refridgerated until needed for assays. Urines will be assayed for pregnanediol-3-glucuronide (PdG) and estrone-3-glucuronide (E3G) to assess ovarian function and status, and hCG to detect conceptions and follow pregnancies. Solid-phase enzyme immunoassays will be used in the field laboratory. *RAMP ProgestURINE* will assay PdG. This is a competitive, enzyme-linked monoclonal immunospecific qualatative assay. For E3G, I will use a competitive immunoassay designed by Dr. Staben, Feld and Munro and modified for anthropological field settings by Dr. Kenneth L. Campbell. While at Dr. Campbell's lab, I tested the response of this assay using materials that were stored in suboptimal conditions for one year. The results indicated that the assay was still valid. The details of the assay are described in Appendix III.

The concentration of hCG in the urines will be assayed by Hybridtech, Incorporated's *Tandem ICON II HCG ImmunoConcentration* assay using a modified protocol to increase the sensitivity. This is a qualitative, monoclonal antibody-based, enzyme-linked immunoassay specific for intact β subunits of hCG. Appendix II describes tests and modifications to this kit so it will detect concentrations of hCG as low as 6 mIU/ml. The kit detects concentrations as low as 20 mIU/ml 100% time according to the manufacturers specifications. The assay is highly specific and shows no cross reaction with LH at 500 mIU hLH/ml, a level that is higher than that found in normal ovarian cycles. The modified level of 6 to 9 mIU/ml hCG means that pregnancies will be detected at about the time of implantation.

The proposed sampling rate of two urines per week is necessary to identify conceptions as early the time of implantation and to capture with sufficient resolution the duration of gestations that lead to early fetal loss (segment 2). This rate over-samples segments 1 and 4 above; however, this frequency is necessary so that subjects are not made aware of changes in their reproductive status by the visits themselves, as might be the case if collection frequency was increased after a pregnancy was detected. Although the collection of urines is high for segments 1 and 4, urines will be assayed in a way that will reduce the total number of assays conducted. Women in the fecund state (segment 1) will be assayed only for the two-week period (four samples) of each cycle when a conception might be identified. That is, assays will not be done over the late follicular phase and early luteal phase, with due allowance for the time required for implantation and the buildup of hCG to detectible levels. This two-week period will be timed according to self-reports of menses and will begin ten days before the onset of menses and last to four days after the onset of bleeding. Wilcox, et al. (1988) screened daily urine samples for 89 menstrual cycles and found that all early-loss pregnancies produced detectable hCG in the 15 day window that began ten days before the onset of menses.

The four unused urine samples each cycle will be preserved for future analyses should they be deemed necessary. Women in the early pregnant state will provide twice-weekly urine samples until they are no longer pregnant as assessed by a pregnancy loss, resumption of menses, or a live birth. Then, the subject's urines will be assayed in a binary search order. In this way, only $\log_2(n)+1$ (instead of n) assays of the urine series will be required to find the interval in which pregnancy ceased. All newly detected pregnancies will be assayed twice-weekly for three weeks past detection, so that fetal loss followed immediately by a another conception will be detected. For women in the post-loss state, urines will be collected and assayed for ovarian function four times a month. Women who resume menstruation following a period of lactational infecundability will have urines back assayed over the previous three months, one sample per week, to detect fetal losses prior to the first menses.

I estimate the number of assays required as follows. I assume that, out of the 375 women recruited into the study: (a) 20 percent (75) will be pregnant at the first visit and will be followed for an average of 30 weeks with 5 percent of them experiencing a fetal loss; (b) 30 percent (113) will be followed for an average of 12 weeks and conceive, and 70 percent of them will be followed another 30 weeks until birth or the study's conclusion, and the other 30 percent will be followed an average of 10 weeks and diagnosed with a loss; (c) 15 percent (56) will be followed for 45 weeks with no conception; and (d) five percent (19) will drop out after an average period of 12 weeks; (e) 30 percent (113) of the women will begin as amennorheic, and of those, 50 percent will remain amenorrheic for 48 weeks and 50 percent will resume menstruation and be followed for 24 weeks. These estimates are based on distributions and tabulations of censoring found in Lincoln et al. (1974), after adjusting for an increase in the number of conceptions by eliminating women whose husbands are fishermen and adjusting for the increased number of fetal loss that will be detected using current methods.

The total number of assays required is:

sub-group	Number of women	Weeks tested susceptible	1-3 Weeks post-concept.	4+ Weeks post concept.	Total assays
a	75	0	1	.05ln(30-6)+1	269
b	113	12	6	(1/2)ln(10-6)+1	2225
c	56	45	0	0	2520
d	19	12	0	0	228
e	113	0.5(24)	0.5(12)	0	2034
	Total				7276

The number of PdG and E3G assays required is based on (1) testing each woman twice after initially excluding those who are found to be pregnant (leaving 300), and (2) assuming 41 women with fetal loss are followed an average of 2.5 months to the resumption of ovulation and are assayed four times per month. This totals 1,010 assays.

c) DSS Demographic Data Collection

Pregnancy histories for each subject will be collected from the DSS demographic database at ICDDR,B in Dhaka. These will provide information on birthspacing and previously detected fetal wastage. Variables of interest are data on exposure to intercourse (marriage, divorce and spousal presence), pregnancies, births, stillbirths and elective and spontanious abortion, mother's age, and parity. A complete set of these data will be collected for each study subject.

A random sample of birth intervals from Matlab villages with a low prevalence of contraception will be collected in order to test the goodness-of-fit to simulation models. In addition, an extract of DSS data will be made of about 2000 women will be made from Matlab villages with high levels of contraception, as well as an extract from another region of Bangladesh (Teknaff) that has an extremely low prevalance of contraception. These two sets of data will be used test for the different age patterns of late fetal loss in contracepting and non-contracepting groups, as an additional test of the heterogenity/selectivity hypothesis. All of these data are prospectively-collected vital information.

Analyses

Estimation of the overall probability of fetal death will be done using multistate lifetables and hazards analysis. These methods are outlined in Namboodiri and Suchindran (1987:chapter 9) and Wood et al. (1992). Given this overall probability, the expected number of fetal deaths in each birth interval is a geometric random variable with mean $q(a)/(1\text{-}q(a))$ and variance $q(a)/(1\text{-}q(a)^2)$, where $q(a)$ is the observed probability of fetal loss at age a (Wood, 1989).

Despite the high quality of data collected using hCG assays and frequent sampling, statistical estimation of the distribution of the gestational ages for fetal death is complicated by several things. First, a conception is not detectible until a minimum of about the time of implantation. Thus, there is left-censoring for every observation. The second difficulty is the right-censoring of observations in which a conception is detected but follow-up to fetal loss or pregnancy is interrupted by the conclusion of the field survey or by the subject leaving the study. The third difficulty is the interval-censoring inherent in any follow-up study without continuous monitoring. The interval within which fetal losses are detected will be about three or four days.

Hazards analysis will be used to estimate the distribution of gestational ages at fetal loss. Let $f(t)$ be the probability density function of gestational ages at the time of fetal death, and $S(t)$ is one minus the cumulative density function of $f(x)$. Furthermore, I simplify by assuming that conceptions are always detected at some mean true gestational age t_η. Then, a solution for the parameters of $f(x)$ is found by maximizing the likelihood:

$$L = \prod_{i=1}^{n} \frac{S(t_{\delta_i} - t_\eta) - S(t_{\alpha_i} - t_\eta)}{S(t_\eta)},$$

where t_{α_i} is the last time at which the fetus is alive and t_{δ_i} is the first time the fetus has been detected as dead. For right-censored observations, t_{δ_i} becomes infinity.

Note that left-censoring is accounted for using an estimate of the mean time to detection of a conception (t_η). If the actual distribution of times from conception to detection of hCG is known, then the method outlined in Appendix 1 provides a better treatment of the left-censoring problem.

Estimation of the duration of infecundability following fetal loss requires that right-censoring be accommodated. Standard hazards analysis will be used to estimate this distribution with duration of conception as a time-varying covariate. Wood (n.d.) derives the probability density function of times added to the birth interval by a fetal loss, allowing for heterogeneity in the risk of loss among women:

$$f(t) = \int_0^1 \sum_{k=0}^{\infty} <g^* h(t)>_k * g(t) q^k (1 - q) s(q) dq$$

where $g(t)$ is the PDF of time to next conception regardless of outcome, $h(t)$ is the PDF for length of gestations ending in fetal loss, $s(q)$ is the PDF describing variation in the risk of loss among women, and $<g^* h(t)>_k$ is the k-fold convolution of g and h. This equation is not solvable analytically, but can be solved numerically. It provides the basis for studying the overall impact of fetal loss on human birth spacing with simulation studies using the estimated parameters of fetal loss in conjunction with components of human fecundity (Wood and Weinstein, 1988; Weinstein et al. 1990; Wood, 1990).

The hazards analyses will be done using software I have written to do general-purpose maximum likelihood estimation. The software can perform hazards analyses that takes into account right-, left-, and interval-censored and left truncated observations. I am currently developing a simulation program to perform the discrete-time simulations required for this research.

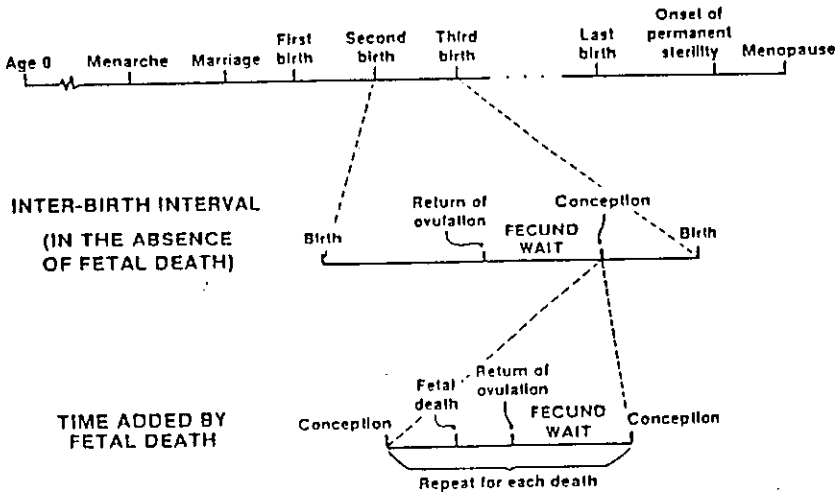

Figure 1. A telescopic view of the female reproductive life life course viewed as a series of time intervals. From Wood, 1990; adapted from Bongaarts and Potter, 1983:4.

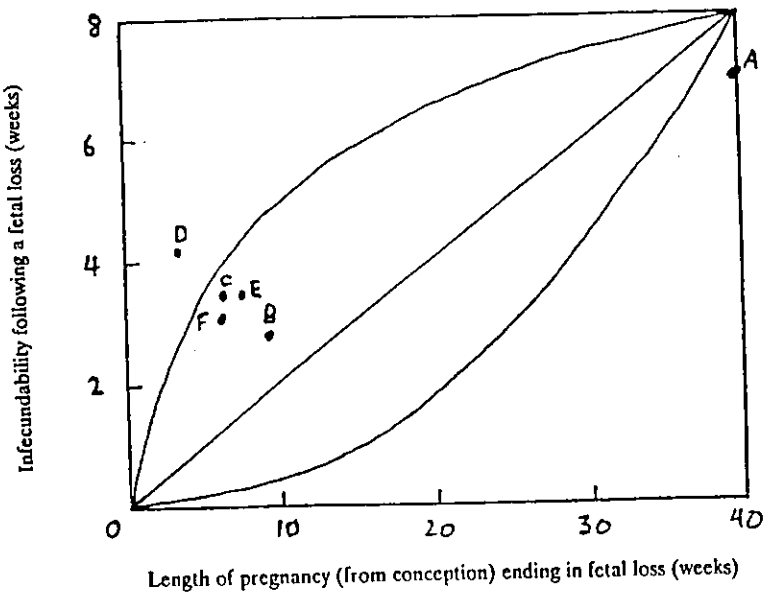

Figure 2. Three hypothetical models between the length of a pregnancy ending in fetal loss and the duration of residual infecundability following the loss (from Wood, n.d.). The points are group medians (mean for A) of times to ovulation following termination of pregnancies. Group A is from Blazar et al's (1980) study of 18 non-breastfeeding women following normal delivery. Group B is from Donnett et al's (1990) study of ovulation following fetal loss in 17 women. Groups C and D are from Cameron and Baird's study of ovulation following elective abortions by vacuum aspiration (14 women, group C) and prostaglandin pessaries (18 women, group D). Groups E and F are from Blazar, et al's (1980) study of hormone profiles following elective abortions by vacuum aspiration (8 women, group E) and prostaglandin pessaries (7 women, group F).

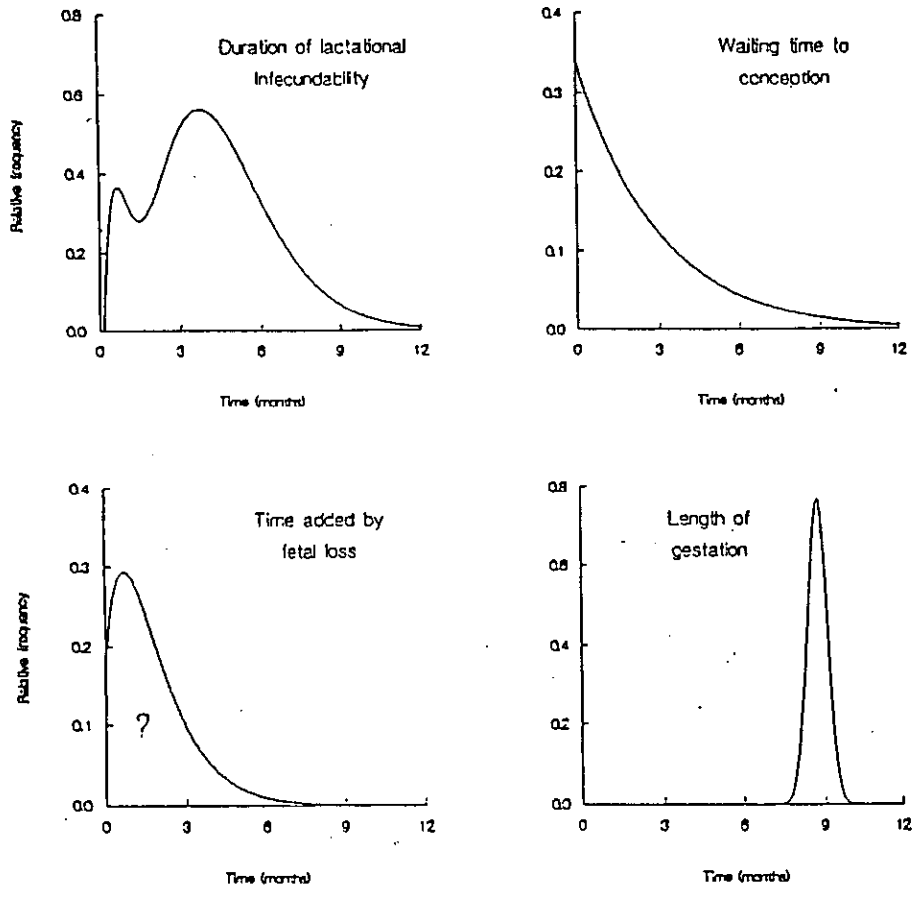

Figure 3. Distributions of the individual components that make up a completed birth interval. Each complete interval between successive live births must include a time to postpartum ovulation, a fecund waiting time to the next conception, and gestation. In addition, there is some distribution of times added for each fetal loss weighted by the probability of 1, 2, 3, ..., deaths in a single birth interval. An overall rough distribution of the effects of fetal loss is estimated from equation 2 and is shown in the lower left panel. The overall distribution of birth intervals is formed from the convolution of the four component distributions shown. From Wood et al., 1992.

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Papers

Wood, JW, KM Weiss, AV Buchanan, DJ Holman, Maxine Weinstein and Ming-chen Chang, submitted, Joint estimation of effective fecundity and proportion of couples sterile from censored waiting time data, *Demography*.

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Wood, JW, DJ Holman, KM Weiss and AV Buchanan, in press, Hazards models for human biology, *Yearbook of Physical Anthropology*. Vol. 35.

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Holman, DJ, A Mukherjee, AC Connor and RE Jones, 1992, *Fluctuating asymmetry in the timing of deciduous tooth emergence*, Human Biology Council 17th annual meeting, March 31 (Abst: *American Journal of Human Biology*, Vol. 4(1):143-4, 1992).

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13. SCHEDULE OF TASKS

Present-Aug 1992	Continue development and debugging of the reproductive simulation and the maximum likelihood estimation programs.
Jun 18-Aug 1, 1992	Preliminary trip to Dhaka, Bangladesh to organize field project, get research permissions, and recruit field assistants.
Jan-Dec 1993	Field and lab work in Bangladesh
Jan 1993	Train field assistants.
Jan 1993	Set up field laboratory.
Jan 1993	Select and recruit sample.
Feb-Dec 1993	Conduct survey and do lab work.
Dec 1993	Collect DSS birth interval extracts.
Jan 1994-Aug 1995	Analysis and writing
Aug 1995	Dissertation defense

14. SPECIFIC TAKSK

Essentially, I will corrdinate the field work, set up the field laboratory, do the laboratory analyses, and do the statistical analyses for this project.

15. BUDGET

TRAVEL:	
Round trip airfare, Chicago, Dhaka (Jan 1993)	0.00[a]
PERSONNEL:	
Per diem, 350 days @ $6/day	0.00[a]
PhD registration fee, 2 semesters @ $570	1,140.00
Matlab field assistants, 12 x 8 months ave. @ $100/month	9,600.00
Matlab field supervisor, 1 x 12 months @ $175/month	2,100.00
Field labratory assistant, 1 x 12 months @ $150/month	1,800.00
Data entry assistant, 1 x 12 months @ $100/month	1,200.00
MATERIALS:	
RAMP hCG assay kits, 7,300 @ $67.00/50	9,782.00
LH assay kits, 1,010 @ $60.00/50	1,212.00
PdG assay kits, 1,010 @ $65.00/50	1,313.00
Specimen containers, 22,000 @ $63.00/500	2,772.00
Collection bottles, 750 @ $0.57	427.50
Specimen paper, 600'x3", 3 rolls @ $44.85	134.55
Vinyl gloves, 600 @ $16.00/100	96.00
Pipette tips 30,000 @ $38.90/1,000	1,167.00
"Blue Ice" Packs, 96 @ $1.25	120.00
3.5" diskettes, 50 @ $13.50/10	68.00
Air Freight	1,800.00
Questionnaire printing costs	400.00
EQUIPMENT:	
Large Coolers, 16 @ $14.00	224.00
Small Field Coolers, $12 @ 10.00	120.00
Refridgerator	500.00
Sigma adjustable pipette 2 @ $178	356.00
PH meter/thermometer	125.00
Refractometer	0.00[b]
COMPUTER:	
ICDDR,B Mainframe Computer costs	300.00
Notebook computer	0.00[c]
TOTAL:	36,757.05

16. BUDGET NOTES

Travel: [a]Airfare to and from Bangladesh will be paid by a fellowship from the American Institute of Bangladesh Studies.

Personnel: Twelve female Bangladeshi field assistants will be hired on a temporary basis and trained for collecting urine samples and reproductive events from the study subjects. The field lab assistant and data entry assistant will assist with the peak rate of 750 questionnaires and laboratory sample processing per month.

Materials and Equipment: Estimation of the number of assay kits required is described in the Methods

section. The refridgeration unit will be purchased for the Matlab field hospital to store urines in the field. It will be left with the hospital at the completion of the project. [b]The refractometer will be supplied by the Penn State's Anthropology Department. US $1800 is allocated for shipment of assay kits and supplies to Dhaka and transport to the field laboratory and return shipment of questionnaires, field notes, and urine samples.

Computer: [c] The computer will be supplied by Penn State's Population Research Institute.

Funding

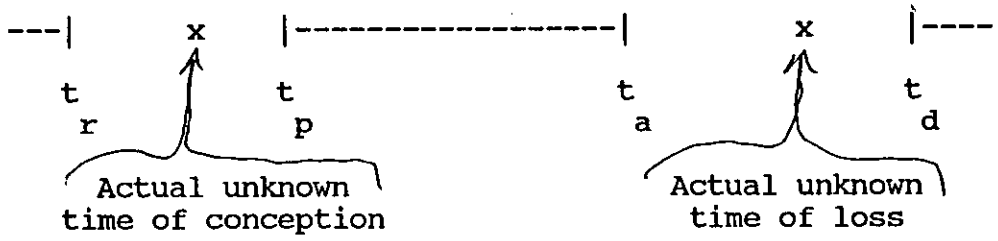
The total cost of the field project will be shared among several sources of funding. Currently, I have committments of funds from the Hill Foundation ($4,150) and Mellon Foundation ($10,000). Also, I have been awarded a fellowship from the American Institute of Bangladesh Studies (AIBS), which will cover travel and living expenses as well as some field worker expenses.

Additional funding has been sought from the National Science Foundation ($12,000) and the Wenner-Gren Foundation for Anthropological Research ($12,000). As of 27 Jul 1992, the proposal has been approved by the NSF anthropology panel, but is awaiting funding approval by NSF.

Appendix 1

Estimates of Fetal Loss from Doubly Interval-Censored Observations

Estimation of the parameters for the duration of a gestation ending in a fetal loss is complicated by the practical reality that pregnancies are only detectible some unknown time after actual conception. Thus, there is left-censoring of the observations of a limited type because, at some point before conception, one knows that conception has not occurred. This is the case just before ovulation. Thus, observations of this type have interval-censoring on both the right and left, and possibly right-censoring. The figure shows a conception and fetal death surrounded by intervals of observation:



where,

t_r is some time before conception occurs (perhaps ovulation).
t_p is the time conception is first detected (this is the zero reference point).
t_a is the last time the fetus was known to be alive.
t_d is the first time the fetus was known to be dead.

$f(x)$ is the pdf for ages from actual conception to a fetal loss.
$g(x)$ is the pdf for ages from actual conception to when hCG is actually detected.

Then, the overall likelihood of the observations on the distributions is

$$L = \prod_{i=1}^{n} \int_{t_r}^{t_p} \int_{t_a}^{t_d} g(x - \mu) f(y - \mu)\, dx\, dy$$

where,

$$\mu = \int_{t_r}^{t_p} g(t)\, t\, dt$$

When an observation is right-censored, t_d is ∞.

Appendix 2

Laboratory Test of the Tandem ICON II hCG Assay

The Tandem ICON II hCG assay for human chorionic gonadotropin (hCG) is a simple monoclonal chromatographic assay that works with urine. The product is extremely easy to use. According to the manufacturer's instructions, the assay is robust under some of the environmental conditions that are likely to be encountered in the field. The purpose of this report is to (1) document the specifications of this assay, (2) describe laboratory tests of this assay and (3) describe simple modifications of the assay to adopt it for anthropological field settings.

Anthropological field settings provide a number of environmental challenges for the use of immunoassay systems. Two major problems are temperature extremes that the components are likely to be exposed to and storage periods that exceed expiration dates of the kits. Also, anthropological studies of fetal loss require sensitivities much greater than the relatively conservative sensitivities used for clinical diagnosis of pregnancy. It appears that some simple modifications to the instructions for these kits allows much greater sensitivity than the manufacturer claims.

Specifications

The directions for the ICON assay specify a 250 ul sample of urine to be placed into an absorbent cup which has an assay table in it. Two cycles of adding reagents and wash solution follow. The urine and reagents filter through the table by means of a wick. Each assay contains a positive control area as well as a negative control area. This assay system is designed to be stored in temperatures from 15 to 30 degrees C. The kits should not be frozen. Each kit (which contains multiple assays) includes an expiration date.

Sensitivity

The claimed sensitivity of the ICON product is 20 mIU/ml hCG. This is based on 20 tests of urine at 10, 20, 30, 40, 50, 60 and 100 mIU/ml each. All urines above 20 mIU/ml produced a discernible indication. The specification sheet included results from the manufacturer's sensitivity tests with urine. With 400 positive and negative specimens, all individuals tested identically to the TANDEM ICON I assay.

Specificity

The manufacturer tested the product against urines spiked with 21 potentially interfering substances. All tests with no hCG were negative and tests with 50 mIU/ml hCG were positive. Specifically, the assay has tested successfully against high levels of several drugs, urinary analytes and hCG homologous hormones. Homologous hormone tests gave negative readings against 1 mIU/ml of hTSH, 500 mIU/ml of hLH and 1000 mIU/ml hFSH.

Laboratory Sensitivity Tests

I tested the sensitivity of the ICON assay for different combinations of urine volumes and hCG concentrations. The tests were performed on 12 June 1992 at 19 C room temperature. Test were performed in Dr. Kenneth L. Campbell's lab using a new, unexpired kit.

Methods

Young male urine was filtered through a C18 column to strip out thimersol preservative. Five aloquots of urine were set up with concentrations of hCG of 0 mIU/ml, 3 mIU/ml, 10 mIU/ml, 30 mIU/ml, and 100 mIU/ml. Two sets of tests were run on each concentration. Manufacturer's instructions were followed, except for (1) the volume of urine supplied to one set was 500 mIU/ml, (2) reagent A waiting time was increased to three minutes before washing, and (3) reagent B (substrate solution) waiting time was increased

to five minutes before washing . The first row had 250 ml of urine (as per instructions), and the second row had 500 ml of urine (Table 1).

The results were read at the time of completion, after drying for one day, and 1.5 months after completion. insertion of the absorbent device. The assay plate was removed from the cup for drying and storage.

	0 mIU/ml hCG	3 mIU/ml hCG	10mIU/ml hCG	30 mIU/ml hCG	100 mIU/ml
250 ul urine	0.00 mIU	.------	2.50 mIU	7.50 mIU	25.0 mIU
500 ul urine	.------	1.50 mIU	5.00 mIU	15.0 mIU	.------

TABLE 1 - The matrix of hCG concentrations and volumes of urines placed into conjugate tubes. The amount of hCG in IUs is shown for each tube. Three tests (----) were not done.

Results

The results of the first reading are shown in table 2. When the indicator is read while wet, the test has a minimum sensitivity of 10 mIU/ml hCG in 250 ul of urine. "Dark" indicates that the test spot was darker than the control spot.

The results of the second (dry) reading are shown in table 3. These results suggest that a dry reading of the indicator plat one day after the test provides a sensitivity of down to 3 mIU/ml in 250 ul of urine. The positive tests are clearer when the plates are dry. There was no degeneration of the spots after 1.5 months of storage at room temperature (one month of which was in Bangladesh).

	0 mIU/ml hCG	3 mIU/ml hCG	10mIU/ml hCG	30 mIU/ml hCG	100 mIU/ml
250 ul urine	Neg	---	Pos	Pos	Dark
500 ul urine	---	Neg	Pos	Dark	---

TABLE 2 - Wet results.

	0 mIU/ml hCG	3 mIU/ml hCG	10mIU/ml hCG	30 mIU/ml hCG	100 mIU/ml
250 ul urine	Neg	---	Pos	Pos	Dark
500 ul urine	---	Pos	Pos	Dark	---

TABLE 3 - Dry results.

Discussion

These results suggest that levels as low as 3 mIU/ml hCG in 500 ul and 10 mIU/ml hCG in 250 ul of urine are detectable. This corresponds to about the time of implantation, thus making the tests viable for studies of fetal loss. Although none of the controls in this and other tests with 0 mIU/ml hCG read positive, further tests might need to be done to determine the rate of false positives when more urine is added into the tube or

the plates are read after drying.

The optimal amount of urine that should be added to the tubes seems to be 500 ul. This will detect hCG in urines with 3 uIU/ml hCG concentration (or 6 uIU/ml hCG in 250 ul of urine). The additional time between washing probably increased the sensitivity, although this should be tested. The assay device is easy to take apart with a screwdriver, and air drying the plate enhanced the readability of the test spots. A quantation of the indicator can be made relative to the control spot on the plate.

Appendix 3

Notes for the Estrone-3-Glucuronide Assays

This is a competitive immunoassay that uses rabit anti estrone-3-glucuronide (R522) produced by the University of California at Davis. The indicator is a conjugate of E3G and horseradish peroxidase (HRP). The assay is set up so that pre-made test tubes and dry reagents are brought to the field. Reagents are reconstituted as needed for assaying. E3G is quantified by comparing the color to tubes containing a control urine with a known quantity of E3G. Since this is a competitive assay, the HRP indicator becomes darker with lower concentrations of E3G in the sample.

Manufacturing the Tubes

Materials:

Stock rabbit anti E3G:Bovine serum albumen
Bicarbonate coating buffer
Polystyrene test tubes (Nunc Immuno-tube Polysorp StarTube 12x75mm)

1) Take 200 ul stock, add to 1.8 ml coating buffer (bicarbonate). This gives a 10:1 dilution. Aliquots of 100 ul can be frozen.

2) Dilute a 10:1 100 ul aliquot to a 1:2500 dilution in coating buffer and coat each clean tube with 50 ul/tube. The tubes should be kept cold.

Processing the E3G-HRP Conjugate

1) Add 50 ul concentrated conjugate solution to 4.95 ml EIA buffer to give a 1:100 dilution. This is the "working" stock and is stored at 4 degrees C. Refreeze the undiluted conjugate.

2) For assay use, dilute sufficient 1:100 stock to 1:10000 in EIA buffer to give just enough volume for each assay in a day. e.g. for 10.0 ml 1:10000 conjugate, add 100 ul 1:100 to 10.0 ml EIA buffer.

Field Use of the Assay

1) Count the number of samples to be assayed plus 5 standard tubes plus 1 blank tube (double the materials for parallel assays). Dilute an appropriate amount of EIA buffer solution with distilled water.

2) Add 200 ul of EIA buffer to the tubes about 10 minutes prior to adding the sample and conjugate.

3) Add 2.2 ml of EIA buffer to each tube of E1G-HRP conjugate to be used. Each tube is enough for 40-42 tubes. Cap and mix well.

4) Add 2.5 ul of urine to each tube.

3) The conjugate has previously been freeze-dried. Dilute the dried contents of each cryovial to 2.2 ml with EIA buffer and mix thoroughly just prior to doing the assays. Add 50 ul of the conjugate to each tube.

5) Agitate the tubes to mix, and incubate 2 hours at room temperature. A bit longer is permissible, but not shorter.

5) Empty the tubes and then rinse them as follows: add EIA buffer to the tubes to about 1/3 their

volume, wait 1 minute, and empty the tube. Repeat this for a total of four rinses.

6) Make up substrate solution in a clean bottle (phosophate citrate buffer and TMB*2HCL) from powder form shortly before use. Dilute 1 TMB tablet for each 10 ml of substrate required (about 20 tubes per tablet) along with 1/20 of a phosophate citrate buffer capsule (or 10 ml from the 100 ml generated by an entire capsule) in a total of 10 ml of water for each TMB tablet. Mix well.

6) Add 450 ul of substrate to each tube.

7) Examine and record the color at 5 and 15 minutes after the addition of substrate. The reaction can be stopped by adding 8 drops of maleic acid solution (which will change the color to yellow).

Appendix 4

Age Distribution of Fetal Loss: Estimation and Sample Size Simulation

The purpose of this appendix is to develop a method for estimating the distribution of risk of fetal loss by mother's age from longitudinal survey data. In addition, the power of the method is tested for particular sample sizes by simulation studies. The results are presented here.

The distribution of fetal loss by mother's age is believed to follow a U-shaped, or, perhaps, a J-shaped distribution. Wood and Weinstein (1988) fit a quadratic regression to pooled data from nine populations and estimated the equation as $\hat{y} = 0.279 - 0.013x + 0.0003x^2$, where x is age. This curve is troublesome for two reasons. First, the curve is based on retrospective data, so it almost certainly underestimates the magnitude of fetal loss, and so must be compensated for by adjusting the constant from 0.279 to some value like 0.40. The second point is that the model is simply descriptive and the parameters provide little insight into what process is responsible for this pattern of fetal loss. It is desirable to develop a more etiologic model to describe the age pattern of fetal loss.

One such model would break the age pattern of fetal loss into three components (much like the Siler model of human mortality): an overall age-independent level of fetal loss shared by all fecund women; an age-dependent component that applies to very young mothers; and an age-dependent component that increases risk with maternal age. I will largely ignore the second component - that of young mothers - because this research focuses on mothers between 18 and 45 years old.

It appears that much of the age pattern of increased risk of early fetal loss can be attributed to increasing incidence of chromosomal defects (Jacobs, 1982), perhaps a result of senesence of oocytes. This aging process can be described as an exponential increase in the risk of a fetal loss by mother's age. Thus, a model for the constant and age-specific increase of fetal loss would is $\hat{y} = A + B\exp(BX)$, where x is age, A is the constant level, and the B term describes an exponential increase in fetal loss with age. I fitted the data used by Wood and Weinstein (1988) to this two parameter exponential model, after adjusting the overall level up to an overall higher probability of fetal loss. This resulted in a model with $A = 0.08$, and $B = 0.04566$.

To determine if the sample size is large enough to estimate this distribution, I developed a simulation model and ran simulations with different size samples. The simulation model requests as inputs the number of simulations to run and the number of conceptions to simulate per run. For each run, a cohort of women are generated in the age range 18 through 24, weighted according to the age distrubution of women in the Matlab region in 1985 (ICDDR,B, 1992). Each woman has the outcome of her conception randomly assigned and weighted by the probably of a women at her age experiencing a fetal loss drawn from the distribution fitted above. Thus, out of N women spanning the age range 18 through 45, some fraction (25 to 30 percent) will experience a loss.

After each cohort of N women and their pregnancy outcomes have been simulated, the two parameters of the exponential model are estimated by finding the parameters that maximize the likelihood of the simulated observations. At each age A, the likelihood of n women experiencing d fetal deaths is:

$$\text{lik}(A) = p(A)^{d}(1-p(A))^{(n-d)}$$

where $p(A)$ is the distribution of age specific probabilities of fetal losses, $\hat{p}(A) = \hat{B}_1 + \hat{B}_2\exp(\hat{B}_2X)$, and B_1 and B_2 are the parameters being estimated. Thus, across all ages, the overall likelihood to be maximized is:

$$\mathcal{L} = \prod_{i=18}^{45} p(i)^{d(i)}(1-p(i))^{(n(i)-d(i))}$$

For each simulated cohort, two tests are performed. First, a likelihood ratio test is performed between the full model, and a reduced model with B_2 held constant at zero. This tests the two parameter model against a model with a constant component but no age-specific component of fetal loss. The second test is a likelihood

ratio test of the full model (two free parameters) and a reduced model with both parameters set to the values of the source distribution (B_1=0.08 and B_2=0.04566). This tests whether the simulated cohort produced a distribution of fetal loss that is significantly different from the distribution used to produce fetal loss in the cohort. For each cohort size, 100 simulations were run and the results of the two tests were tallied. These overall results are shown in table one.

Number of Simulations	Number of Conceptions	mean age	mean losses	Percent significant at p=.025	p=.05	p=0.1	Percent different
100	100	29.03	26.1	29.0	41.0	55.0	4.0
100	125	29.03	33.1	30.0	34.0	47.0	2.0
100	150	28.95	39.5	36.0	45.0	60.0	5.0
100	175	28.95	45.6	41.0	53.0	66.0	5.0
100	200	28.97	54.1	57.0	70.0	76.0	3.0
100	225	28.96	59.4	56.0	74.0	77.0	2.0
100	250	29.06	65.7	62.0	76.0	83.0	6.0

TABLE 1 -- Results of simulations for the age-pattern of probability of fetal loss using the two-parameter exponential model discussed in the text. For a given number of simululations, the "percent significant" column shows the percentage of simulations that were significant for three levels of significance using a likelihood ratio test of the two-parameter exponential model and the reduced one-parameter (intercept only) model discussed in the text. The "percent different" column is the percent of simulations in which the maximum likelihood estimate of the two-parameter exponential model is significantly different (at p < 0.05) than the original distribution of the age-pattern of fetal loss used in the simulation.

Thus, with a sample of 200 conceptions followed, 70 percent of the simulations were significant at $p < 0.05$ value, and 3 percent of the simulations produced distributions that were significantly different (at $p < 0.05$) from the original distribution.

Reproductive Status Questionnaire

ubject Name _____ Regist No |__|__|__|__|__|__|__|__|__|__|

ate |__|__|__|__|__|__| Interviewer ID |__|__|
day month year

ast Interview Date |__|__|__|__|__|__| Interview Serial Number |__|__|__|
day month year

as the subject dropped from the study? Yes ____ No ____
If yes, the reason for leaving:
Death ____
Migrated out ____
Refused to Participitate ____
Loss of elidgibility ____

Date of leaving |__|__|__|__|__|__|
day month year

Notes on leaving: _____

urrent Reproductive Status:
Menstruating ____
Ammenorrhoea for unknown reason ____
Lactational Ammenorrhoea ____
Pregnant ____

f menstruating, date menstrual bleeding started? |__|__|__|__|__|__|
day month year

as menstrual bleeding stopped since the last interview? No ___ Yes ___

If yes, Date menstrual bleeding stopped? |__|__|__|__|__|__|
day month year

Was this last period normal? No __ Yes __
If No, why? _____

f currently pregnent, how do you know _____

Have you had any pregnancy illness: No ____ Yes ____

If yes, describe: _____

ny outcome of pregnancy since last interview? No ___ Yes ___

If yes, type: ____ Live birth ____ Stillbirth
____ Miscarriage ____ Induced abortion

Date of pregnancy outcome? |__|__|__|__|__|__|
day month year

Duration of gestation? (Unknown = 99) |__|__| months

The baby was: Male ___ Female ___ Unknown ___

Pregnancy complications: _____

Delivery complications: _____

How many hours before the child was breastfed _____ Hours

Is the infant still alive? Yes ___ No ___

If no, date of death |__|__|__|__|__|__| Cause _____
day month year

nce last visit, how many times were you away from your husband? _____ Times
f one or more times:

Date |__|__|__|__|__|__| Number days away _____ Cause: __1 __2 __3 __4 __5
Date |__|__|__|__|__|__| Number days away _____ Cause: __1 __2 __3 __4 __5
Date |__|__|__|__|__|__| Number days away _____ Cause: __1 __2 __3 __4 __5

Causes of Separation: 1=loss of partner 2=Temporary absense
3=Temporary seperation 4=Divorced 5=Severe sickness

If your husband is currently gone, when do you expect him to return
___ 1 to 7 days ___ 7 to 14 days ___ 2 to 4 weeks ___ 1 to 2 months
___ 2 to 4 Months ___ 4 to 8 months ___ 8 or more months ___ Never

ve you use any contraception since the last interview? No ___ Yes ___
If yes, what type: Oral contraceptive pill ___ Injection ___ IUD ___
Condom ___ Rhythm ___ Breastfeeding ___ Withdrawl ___
Herbs ___ Abstinence ___ Other _____

d you breastfeed since your last interview? No ___ Yes ___

About how many days did you breastfeed since the last interview? _____
About how many feedings in the? morning _____ afternoon _____ night _____
About how many minutes per feeding? morning _____ afternoon _____ night _____

Any problems breastfeeding? No ___ Yes ___ What _____

ve you gotten sick since your last interview: No ___ Yes ___

		Details
Fever	__Yes __No	_____
Vomiting	__Yes __No	_____
Diarrhea	__Yes __No	_____
Stomach Pains	__Yes __No	_____
Urinary	__Yes __No	_____
Vaginal	__Yes __No	_____
Other _____	__Yes __No	_____
Other _____	__Yes __No	_____

eline Questionnaire

E: THIS QUESTIONNAIRE IS UNDER REVISION, BUT THIS REPRESENTS
TYPE OF QUESTIONS THAT WILL BE ASKED

ject Name _____ Regist No |__|__|__|__|__|__|__|__|__|

e |__|__|__|__|__|__| Current ID No |__|__|__|__|__|__|__|__|__|
day month year Village Family Indiv

jects Birth Date |__|__|__|__|__|__| Interviewer ID |__|__|
day month year

this the household you usually live in? No___ Yes___

you married? No ___ Yes ___

no, do not fill out the remainder of the questionnaire.

yes, how long have you been married _____

In the last 2 weeks, how many times were you away from your husband? __Time
If one or more times:

Date |__|__|__|__|__|__| Number days away ____ Cause: __1 __2 __3 __4 __5
Date |__|__|__|__|__|__| Number days away ____ Cause: __1 __2 __3 __4 __5
Date |__|__|__|__|__|__| Number days away ____ Cause: __1 __2 __3 __4 __5
Date |__|__|__|__|__|__| Number days away ____ Cause: __1 __2 __3 __4 __5
Date |__|__|__|__|__|__| Number days away ____ Cause: __1 __2 __3 __4 __5

Causes of Separation: 1=loss of partner 2=Temporary absense
3=Temporary seperation 4=Divorced 5=Severe sickness

If your husband is currently gone, when do you expect him to return:
___ 1 to 7 days ___ 7 to 14 days ___ 2 to 4 weeks ___ 1 to 2 months
___ 2 to 4 Months ___ 4 to 8 months ___ 8 or more months ___ Never

What is your husbands occupation?
___None (no occupation)
___Farmer/agriculturist
___Fisherman/Fish Business
___Agricultural Labour/daily labour (kurani, chilli & paddy cooli)
___Mill/Factory Worker (jute mill, rice mill worker)
___Unskilled Labour (hotel boy cowboy, tea stall boy, helper, etc)
___Skilled labour (driver, barber, carpenter, mason, tailor, rickshaw puller, black-smith, mechanic)
___Boatman
___Cottage industry (hogla making)
___Service (salesman, cook, imam, member, chairman, clerk collector, choiwkider, dofader, teacher, etc)
___Businessman (peddlar, ice-crean seller, hawker, milk seller)
___Beggar
___Student
___Disabled (blind, dumb, old age, mad)
___Unemployed (seeking job, vegabond)
___Others (village doctor, nurse, compunder, singer)

Does you husband temporarily migrate away from this household for more than one month in a year? __no __yes
if yes, how many months each year ____

Do you temporarily migrate away from this household more than one month in a year? __no __yes
if yes, how many months each year ____

Over the next year, how many months will you and your husband be seperated? ___months

Are you and your husband fecund (that is, capable of bearing children if you want)? No ___ Yes ___

If not, what is the cause? You, yourself _____

Your husband _____

If menopausal, since when? |__|__|__|__|__|__|
day month year

o you want to bear (more) children? No ___ Yes ___
How many? _____

ow many livebirths have you ever had? _____

ow many children are still alive? _____

ow many children born dead have you had _____

ow many spontanious abortions have you had _____

re you currently doing anything to avoid or delay pregnancy (either using a device or technique, modern or traditional) No ___ Yes ___

If yes, what type: Oral contraceptive pill ___ Injection ___ IUD ___
Condom ___ Rhythm ___ Breastfeeding ___ Withdrawl ___
Herbs ___ Abstinence ___ None ___ Other _____

ave you ever used a method of birth control? No ___ Yes ___

If yes, what was the last type:
Oral contraceptive pill ___ Injection ___ IUD ___
Condom ___ Rhythm ___ Breastfeeding ___ Withdrawl ___
Herbs ___ Abstinence ___ Other _____

If yes, what did you use before that:
Oral contraceptive pill ___ Injection ___ IUD ___
Condom ___ Rhythm ___ Breastfeeding ___ Withdrawl ___
Herbs ___ Abstinence ___ None ___ Other _____

f you are not currently using birth control, when will you start?
____ When I have as many children as I want ---> ___ more children
____ Within one month ____ Within one year
____ Within five years ____ Never _____ Other

ent Reproductive Status:

Menstruating ____
Ammenorrhoea for unknown reason ____
Lactational Ammenorrhoea ____
Pregnant ____

ou are currently pregnant,
how do you know _____

ve you had any pregnancy illness: No ____ Yes ____

If yes, describe: _____

On what date did you find out you were pregnant? |__|__|__|__|__|__|
day month year

What date are you expecting to give birth? |__|__|__|__|__|__|

ou are currently breastfeeding, for how many weeks have you been _____

out how many feedings in the? morning ____ afternoon ____ night ____
out how many minutes per feeding? morning ____ afternoon ____ night ____

y problems breastfeeding? No ___ Yes ___ What _____

long has it been since your last menstrual period ended? _____ days

NNSTATE

(814) 865-2509
FAX: (814) 863-1474

Department of Anthropology
College of Liberal Arts

The Pennsylvania State University
409 Carpenter Building
University Park, PA 16802-3404

July 16, 1992

TO WHOM IT MAY CONCERN:

As supervisor of Mr. Darryl J. Holman's doctoral research, I wish to confirm that his research proposal on "Effects of Fetal Loss on Birthspacing in Rural Bangladesh" as been approved unanimously and without reservation by his dissertation committee.

James W. Wood

James W. Wood, Ph.D.
Associate Professor

STATE

Senior Vice President for Research and
Dean of the Graduate School
Office for Regulatory Compliance

(814) 865-1775
The Pennsylvania State University
115 Kern Graduate Building
University Park, PA 16802-3300

Date: April 24, 1992

From: Lorraine M. Mulfinger, Compliance Coordinator

To: Darryl Jon Holman

Subject: Results of Review of Proposal

"The Demography of Fetal Loss in Rural Bangladesh"

The Biomedical Committee of the Institutional Review Board has reviewed and approved your proposal for use of human subjects in your research. You may proceed with your study.

Approval for use of human subjects in this research is given for a period covering one year from today. If your study extends beyond this approval period, you must contact this office to request an annual review of this research.

Attached are mailing labels you can use to forward to 114 Kern Graduate Building the original, signed informed consent forms obtained from the subjects of your study. Contact this office if you need more labels.

Subjects must receive a copy of the informed consent form and the written explanation of your study that was submitted to the Compliance Office for review.

By accepting this decision you agree to notify the Compliance Office of (1) any additions or changes in procedures for your study that modify the subjects' risks in any way and (2) any events that affect the safety or well-being of subjects.

On behalf of the committee and the University, I thank you for your efforts to conduct your research in compliance with the federal regulations that have been established for the protection of humar. subjects.

LMM/cdv

Attachments

cc: J. W. Wood
K. M. Weiss
D. S. Palermo

Juniata
COLLEGE

Department of Political Science
Huntingdon
PA 16652-2119
814-643-4310

June 8, 1992

Mr. Darryl J. Holman
409 Carpenter Building
Department of Anthropology
Pennsylvania State University
University Park PA 16802

Dear Mr. Holman:

I am pleased to inform you that the selection committee of the American Institute of Bangladesh Studies has approved your application for a pre-doctoral grant to study "The Demography of Fetal Loss in Rural Bangladesh." As Penn State is a member of AIBS there will be no application processing fee to be paid.

Your application will be forwarded to the Government of Bangladesh for final approval.

Please let me know at your earliest convenience if you accept this award. I will be in touch with you about other details after the approval of the Government is received.

Sincerely yours,

Craig Baxter
President, AIBS

(1)

Title: Demography of fetal loss in rural Bangladesh

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of Project		✓	
Adequacy of Project Design	✓		
Suitability of Methodology		✓	
Feasibility within time period		✓	
Appropriateness of budget		✓	
Potential value of field of knowledge	✓		

CONCLUSIONS

I support the application:

a) without qualification ☐

b) with qualification ☑

- on technical grounds ☑
- on level of financial support ☑

I do not support the application ☐

Title: Demography of Fetal Loss in rural Bangladesh

Conducting such research in the rural community of Bangladesh is not easy for social factors but still it may be possible with good planning, developing good relation with the subjects through a well trained interviewer team, explaining the benefit of the research for human kind.

Objectives :

Within the objectives it is not clear that, from the proposed research how the individual / community in general will be benefited.

Ethical implication :

The interviewer must be female.

For the young mothers they may feel shy not only to give the answer to the personal questions also to give the specimen.

Confidentiality must be maintain very strictly. Accept PI no body should know the individual laboratory result. All reference to be dealt with ID numbers. Knowing the result to husband and other family members may create a great problem for the subject.

Methods :

The frequency of interviewing **twice a week for a period of eleventh months** this to be reviewed carefully, this may be to frequent, the subjects may feel tired and board and they may not like to exposed themselves to the community as a participants of such study.

Sample size :

To continue a cohort of 375 subject the following factors may be review for dropping out during the study period.

- migration Out
- long and frequent absence
- refusal to participate in the study any time
- starting of contraceptive use any time
- change of husband status (go on long absence)
- change of marital status

A base line survey may be needed to screen the subject following the criteria of subject character i.e. current marital status, contraceptive use, current reproductive status, husband away from home etc.

Budget :

1 standby interviewer may be needed to cover any emergency leave of 12 regular interviewer.

1 more field laboratory assistant may be needed to allow one's week ends /leave.

A category of daily wager will be needed (in monsoon country-boat, to transport the specimen - porter)

- 60 men month of country-boat @ 1500 taka/ month
- 44 men month of porter @ 1500 taka/month

Local travel - Speedboat use for supervisor and interviewer daily 2 hrs for eleventh months

Stationary and office Supplies -

Furnitures/ filing cabinet - for office/ taking care of records

Refrigerators
Specimen cold carriers

Incentives to the subjects : something to be given to the subjects, it may be in form of medical management for any sickness for the subject and her children.

Time period: Eleventh months time period may not be enough because the observation period of the study subject is eleven months. Initially a one/two months may be needed for identifying the project staff, trained them, pre-testing of questionnaire, setup of lab., organize the community, conduct baseline survey, identify subject, counselling.

(2)

Title: Demography of Fetal Loss in Rural Bangladesh.

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of Project	✓		
Adequacy of Project Design	✓		
Suitability of Methodology	✓		
Feasibility within time period	✓		
Appropriateness of budget	✓		
Potential value of field of knowledge	✓		

CONCLUSIONS

I support the application:

a) without qualification [✓]

b) with qualification [ ]

- on technical grounds [ ]
- on level of financial support [ ]

I do not support the application [ ]

This protocol proposes to investigate the effects of fetal loss on reproduction. Fetal loss will be detected by endocrinological assays of urine sample to be collected twice in a week from 375 married women aged 18-45 for a year from Matlab. Perhaps, this will be the second study (first study, a very crude one, was done by Chen et al at this Centre more than 20 years ago) of its kind in Bangladesh. Conducting such a study, which involves urine collection from women, will be very difficult in any place in the country other than Matlab where ICDDR,B workers have good rapport and easy access to the community. It may be better if Matlab CHWs instead of new workers as suggested in page 19 collect urine samples.

The objectives are clearly specified, the literature review is adequate, study design and statistical analysis including the procedure for determination of sample size are sound. However, I am not qualified to make any comment on the endocrinological assays proposed, but feel this study will be helpful to set up a laboratory procedure for this type of assays at the ICDDR,B.

contd.../

Contd. comments on "Demography in Fetal Loss in Rural Bangladesh.

In short, it will be a very important study and if it is completed successfully, the results will be useful to clarify many basic scientific queries such as what proportion of birth interval, which is quite large in Bangladesh, is attributable to fetal loss. However, as the lower age limit is set at 18 years, this study will be of little help to know how much of the subfertility during two years after menarche (estimated by Ann Riley et al in Matlab) is attributable to fetal loss. The study will also not be helpful to answer the query whether low nutritional status is a factor of fetal loss which in turn may make birth interval longer in malnourished population such as in Bangladesh in comparison with other natural fertility and lactating population such as Hutterite. I can appreciate the problem involved if lower age limit of study subjects is reduced to the age at menarche. Yet, it will be important if urine test can be done for a handful of purposively selected married women (say 20) from the onset of menarche and nutritional component such as weight and height of all study women (even one time measure) can be collected to have some answers to the two important queries mentioned above.

Response to reviews

Reviewer 1

Objectives: The benefits from this study are primarily in answering some basic scientific questions about very early fetal loss and its impact on birthspacing. However, as outlined below, there is the possibility for a medical management program to piggyback on this project. Also, in respones to reviews, I have revised the proposal to include an anthropometric assessment to look at the impact of nutrition on early fetal loss.

Ethical Implication: The interviews/urine collection will be done by females.

Subjects will be at least 18 years old. Even so, some may feel shy about providing urines and answering questions. I welcome any suggestions to avoid this problem. Recently, I asked a fieldworker in the MCH area if urine was difficult to collect (for glucose tests). She told me that she did not have difficulties collecting urine.

Individual assay results will only be known to myself and, in some cases, the laboratory assistant who does the assay. However, the specimens will be coded by ID number. Individual results will *never* be revealed to anyone else.

Methods: The twice-weekly interviews/collections are necessary to address these questions of very early fetal loss. Less frequent collection frequency will miss a large number of fetal losses because of the time frame within which hCG levels decay from maternal circulation.

Sample Size: As suggested by the reviewer, I will construct a baseline survey to screen subjects for expected husband absence, desire to contracept (note: women will never be discouraged from using contraceptives should they choose), expected migration, etc. This will alleviate part of this problem. Even so, I estimated based on the Chen, et al. (1974) study, that 46 percent of the subjects will not get pregnant. This includes women who drop out and cycle throughout the study, possibly because of contraception. However, another approach is to recruit a second cohort after the sample size in the first cohort drops off. Many of the subjects will be followed less than four months because of the seasonality of conceptions and the very high rate of early fetal loss.

Budget The budget was revised after being sent out for review, but before I received these comments. Some of the items listed here have already been included, and the budget has gone up by about US $4000. I will seek additional funds for other items listed. Some items can be paid by my fellowship.

Incentives to the subjects: This project will include frequent visits to subjects and urine collection; therefore, it provides a good opportunity for some types of medical management or screening programs. I am certainly willing to cooperate it this type of project; however, I am a biological anthropologist, and not qualified to design medical programs. Therefore, I solicit suggestions, and if a medical program is piggybacked, I can attempt to provide funds beyond the data collection. Since the grants I've received are restricted to dissertation research, I will have to get permission and seek additional funds.

Time Period: The survey period is eleven months, but it follows a one month period for organization, training, etc. If possible, I will pretest the survey and collection protocol before I leave Bangladesh in August.

Reviewer 2

As suggested by this reviewer, I will include an initial anthropometric survey and an exit anthropometric survey so nutritional effects on fetal loss can be investigated. The two reasons for excluding women below the age of 18 are: (1) It is currently very difficult to obtain research permission from my home institution (Pennsylvania State University Human Subjects Committee) for research that involves collection of bodily fluids from children, and (2) the sample size would have to be increased to incorporate this sample, and I do not have funds to support the many additional assays that would be required to monitor ovarian function and pregnancy status in this sub-fecund subpopulation.