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ABSTRACT

Previous studies of postpartum amenorrhea (PPA) have demonstrated distinct subgroups of women with short and long durations of amenorrhea. This phenomena has been attributed to cases where breastfeeding is absent because of pregnancy loss or infant death, or confusion of postpartum bleeding with resumption of menses. We explored these ideas using data from an eleven-month prospective study in Bangladesh in which 858 women provided twice-weekly interviews and urine specimens for up to 9 months; 300 women were observed while experiencing PPA. The resulting exact, interval-censored or right-censored durations were used to estimate parameters of two-component mixture models. A mixture of two Weibull distributions provided the best fit to the observations. The long-duration subgroup made up 84% (±4% SE) of the population with a mean duration of 457 (±31) days. The short-duration subgroup had a mean duration of 94 (±17). Three covariates were associated with the duration of PPA: women whose husbands had high-wage employment had a greater probability of falling in the short-duration subgroup; women whose husbands seasonally migrated had shorter periods of PPA, but only if they would otherwise fall into the long-duration subgroup; and mothers who gave birth during the monsoon season experienced a shortened duration of PPA, but only if they would otherwise fall into the short duration subgroup. We conclude that the bimodal distribution of PPA reflects biological or behavioral heterogeneity rather than shortcomings of data collection.

In societies that approximate conditions of natural fertility, breastfeeding behavior is a major determinant of birth spacing and completed fertility (Bongaarts and Potter, 1983; Wood, 1994). Breastfeeding lengthens birth intervals by delaying the resumption of ovarian cycles and ovulation. Intensive and sustained breastfeeding can result in years of postpartum amenorrhea (PPA), and in societies where intensive breastfeeding is the norm, couples tend to have long inter-birth intervals, and lower completed fertility (Howie and McNeilly, 1982; Short, 1984; Wood, 1994).

A frequent finding from studies of postpartum amenorrhea is that the distribution of times from parturition to resumption of menses is bimodal (Ford and Kim, 1987; Huffman et al., 1987; Potter and Kobrin, 1981). A small mode in the distribution of resumption of menses is frequently observed at three or four months postpartum, along with a later, more frequent mode. Ford and Kim (1987), building on the work of Potter and Kobrin (1981), developed a statistical method to identify subgroups within a distribution of PPA.[1] They reanalyzed four studies conducted in developing settings and found that the distribution of times to postpartum resumption of menses was made up of two statistically-identified subpopulations. For example, using data from Bangladesh, they estimated that three quarters of women made up one subgroup with a median duration of 19 months of PPA, and one quarter made up a second subgroup with a median duration of 4 months. Using data from Narangwal, India, they reported two thirds made up a subgroup with a median duration of 13 months of PPA and one third made up a subgroup with a median duration of 3 months.

Other studies have made similar findings using less formal methods. Henry (1961:90) observed that some breastfeeding women maintain long durations of PPA, whereas other mothers resume menses shortly after parturition. Rahman et al. (2002) observed a pattern consistent with a bimodal distribution in urban Bangladeshi mothers, where three quarters resumed menses at about 12 months and one quarter resumed menses at about 3 months. A bimodal distribution for resumption of menses and ovulation was empirically observed in a prospective study of 40 breastfeeding Filipino women, with the short-duration subgroup resuming menses around the fourth month postpartum (Eslami et al., 1990). Finally, a multicenter World Health Organization study found a bimodal distribution in which one subgroup had a mean time to resumption of menses from 3 to 4 months and the other subgroup had a mean time to resumption of menses of around 9 months (Le Strat and Thalabard, 2001).

The reasons that two subgroups arise—either identified statistically or observed as a bimodal distribution of time to resumption of menses—have not been fully identified. One proposed explanation is that some mothers never breastfeed at all, as happens in cases of stillbirths or late pregnancy loss. A related explanation is that perinatal deaths result in severely curtailed breastfeeding for some women. Women who never breastfeed their newborn typically resume menses within about two months of delivery (Jones, 1989; Leridon, 1977; Potter and Kobrin, 1981), so that a bimodal distribution would arise if a significant fraction of women never breastfeed or breastfeed for only a short time (Potter and Kobrin, 1981). Ford and Kim (1987) explicitly tested this, by excluding cases of known child death from their analyses. After doing

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Potter and Kobrin (1981) fit data on resumption of menses to a mixture model composed of a binomial distribution and a geometric distribution. The geometric component represents women who resume menses soon after parturition, perhaps because of infant mortality or early weaning and supplementation. The binomial component represents women who breastfeed exclusively to later postpartum ages. Ford and Kim (1987) pursued a related approach. They developed a two-component continuous mixture model that provided a good fit to data on postpartum amenorrhea. Each component was a Gumbel distribution, with an additional parameter estimating the fraction of women in each subgroup.

so, the fraction of women in the short-duration subgroup decreased from 26% to 24% in the Bangladesh sample and from 36% to 35% in the Narangwal sample. The quality of the data, however, did not allow the authors to exclude the possibility that the short-duration subgroup arose "due to errors in the data [from] undetected stillbirths, fetal losses, and infant deaths, and confusion with postpartum bleeding" (Ford and Kim, 1987:419). Nevertheless, it seems unlikely that these errors account for the one quarter to one third of all mother-infant dyads that made up the short-duration subgroups.

Huffman and colleagues (1987) went to great lengths to identify and remove cases of child mortality from their study of PPA in rural Bangladeshi women. Their distribution of times to resumption of amenorrhea included a small, early mode at 4 months postpartum, which they attributed to either confusion of post partum bleeding with first menses or "heterogeneity in breastfeeding practices or resumption of menses, or to undetected stillbirths or [undetected] infant deaths" (pg. 455). The study investigated covariates affecting the duration of PPA, but did not explicitly test for how heterogeneity in breastfeeding may have led to the early mode. A behavior closely related to breastfeeding—supplementary feeding within the first three months—led to a marginally significant decrease in time to resumption of menses. Jones (1988a) has also documented that early supplementary feeding is associated with an early resumption of menses.

There is other evidence that heterogeneity in breastfeeding behavior (maternal motivation to breastfeed) may be related to the bimodal distribution. A study of 42 women in New Mexico (NM) uncovered a bimodal distribution in which one subgroup of mothers resumed menses at about 10 months and the second subgroup resumed menses at about 4 months (Taylor et al., 1999). The same study investigated another sample of women who were highly motivated to breastfeed (members of the Couple to Couple League), and had a unimodal distribution with a median of 13 months. When the NM women were categorized into early and late subgroups, they found that the two subgroups differed by the pattern of breastfeeding and the timing of supplementation.

Breastfeeding disrupts ovarian cycles by suppressing pulsatile release of gonadotrophin releasing hormone from the hypothalamus, which, in turn, suppresses the production of the gonadotrophin hormones necessary to support ovarian activity (Howie and McNeilly, 1982). It is not clear if suckling directly inhibits the reproductive axis through direct neurological effects on the hypothalamus (Howie and McNeilly, 1982; Wood, 1994) or if this effect is mediated through the energetic cost of lactation (Ellison, 1995; Valeggia and Ellison, 2001). Whatever the mechanism, demographic studies have consistently reported a relationship between breastfeeding intensity (suckling frequency and duration) and long durations of PPA (Jones, 1988a, 1989; Vestermark et al., 1994; Vitzthum, 1989; Wood, 1994).

It is difficult to see how distinct subgroups would arise from differences in breastfeeding intensity and age at supplementation. Breastfeeding intensity, age at supplementation, and degree of supplementation probably differ among mother-infant dyads continuously rather than by falling into two discrete subgroups. If so, we would expect differences among mothers to increase the variance of a unimodal distribution of resumption of menses, rather than forming two distinct subgroups.

A number of demographic and ecological factors are known to affect the duration of PPA. For example, maternal age increased the duration of PPA in some studies (Chen et al., 1974; Habicht et al., 1985; Heinig et al., 1994; Huffman et al., 1987; Jones, 1988b; Nath et al., 1993; Potter et al., 1965; Singh et al., 1993; Vestermark et al., 1994) but not others (Rahman et al., 2002). Income (Huffman et al., 1978; Nath et al., 1993), maternal anthropometric measures (Heinig et al., 1994; Huffman et al., 1978; Jones and Palloni, 1994; Kurz et al., 1993; but not

Rahman et al., 2002), and parity (Heinig et al., 1994; Liestøl et al., 1988), have sometimes been associated with the length of PPA. A child's sex sometimes affects PPA, usually by delaying the return of menses for male children (Rahman et al., 2002; Singh et al., 1993). Place of residence (urban versus rural) has been shown to affect the timing of the resumption of menses (Rahman et al., 2002; Salway et al., 1993). None of these factors, however, has been clearly identified as responsible for bimodal patterns for resumption of menses. In fact, most of these factors are continuous and unimodially distributed among women, so that differences among women in these factors would not be expected to result in a bimodal distribution of resumption of menses.

Henry (1961) proposed that unrecognized physiological differences among women result in a subgroup having shorter periods of PPA. This subgroup would have a reduced physiological response to breastfeeding or a lower threshold below which breastfeeding can maintain amenorrhea. If large differences existed among subgroups in the breastfeeding intensity required to maintain amenorrhea, a bimodal distribution with two discrete underlying subgroups could arise. This type of variation could result from a simple Mendelian trait, although there is no evidence that such a trait exists. Pennington (2004) predicts an evolved mechanism in humans with two different maternal investment strategies, based on a model derived from maternal-offspring conflict theory (Pennington and Harpending, 1988; Trivers, 1974, 1985).

In this paper, we explore the causes of bimodality in postpartum resumption of menses. Using data from a prospective microdemographic study conducted in rural Bangladesh, we examine whether resumption of menses is bimodal. We were able to minimize several sources of errors: (1) confusion of postpartum non-menstrual bleeding with menstrual bleeding, (2) resumption of menses following the death of an infant, and (3) resumption of menses following pregnancy losses and stillbirths. The prospective portion of the study included twice-weekly interviews and collection of urine specimens. Thus, for subjects with the earliest resumption of menses, we could rule out non-menstrual bleeding using serial endocrine markers and by demonstrating patterns of bleed length and cycle length consistent with menstrual bleeding.

The resulting observations from parturition to first menses were fit to a parametric mixture model for the distribution of postpartum resumption of menses. The model was modified from that proposed by Ford and Kim (1987) in three ways. First, we examined Weibull, lognormal and Gamma distributions as alternatives to the Gumbel distribution used to model the distribution of PPA in each subgroup. We selected the pair of distributions that resulted in the best statistical fit of the model to the data. Second, we modified the method to accommodate right-censored, interval-censored, and left-truncated observations that sometimes arise in prospective studies of breastfeeding. Third, we modeled the effects of covariates on the three parts of the mixture model (the two distributions and the mixture fraction). By doing so, we found covariates associated with subgroup membership as well as covariates affecting the duration of amenorrhea within each subgroup. After fitting the mixture models as well as nested single-distribution models to data from rural Bangladesh, we conclude that a mixture of two Weibull distributions most parsimoniously described the distribution of PPA. This bimodal distribution of the duration of PPA was found after minimizing errors suggested by Ford and Kim (1987), ruling out the possibility that the short-duration subgroup was an artifact of these errors. Covariates affected all three parts of the mixture model.

SUBJECTS AND METHODS

Field Site

The research was conducted in 28 villages within Matlab thana, a rural administrative unit in Bangladesh, located 50 km southeast of the capital city of Dhaka**.** Most of the thana is part of an ongoing large scale survey of demography, health, and disease conducted by the International Centre for Diarrhoeal Disease Research in Bangladesh (ICDDRB). The ICDDRB has maintained a demographic surveillance system (DSS) in the Matlab study area since 1966, including a continuous registration of pregnancy outcomes, deaths, marriages, divorces, and migrations. Currently, the DSS covers about 200,000 people in 143 villages. Half of the villages are part of a maternal, child health, and family planning (MCHFP) intervention area, and the other half are in a non-intervention area (van Ginneken et al., 1998). The subjects in this study all resided in villages within the non-intervention area.

The Matlab area is in a low-lying river delta, with a subtropical climate. Three seasons are recognized. The monsoon season is from June to September, the cool-dry season is from October to February, and the hot-dry season is from March to May (Becker, 1981). The primary economy of the region is subsistence farming of rice and jute; this is followed in economic importance by fishing (Bhuiya and Mostafa, 1993). Some employment involves seasonal migration of males. For example, some fishermen migrate seasonally, with a peak in household absences through July and August and a second peak in January (Chen et al., 1974). The society is religiously conservative; 85% of the population is Muslim and the remainder is primarily Hindu. Although the area is rural, population density is high (~1100 people/km^2) and the infectious disease load is high (Razzaque and Streatfield, 2002). Rural Bangladeshi women have a low standard of living and relatively little formal education (Buiya and Mostafa, 1993; Bhuiya and Streatfield, 1992). A majority of the residents of Matlab thana are chronically undernourished (Miller et al., 1994; Pebley et al., 1985).

Participants and Data

Data were collected in an eleven-month prospective study of birth spacing and fecundity conducted from February through December 1993 (Holman, 1996). The field study consisted of three components: a one-time baseline survey, a prospective follow-up survey administered twice-weekly for up to nine months, and a one-time exit interview. Participants gave informed consent prior to participation in the study, and the study protocol was reviewed and approved by the Pennsylvania State University Office for Regulatory Compliance and the International Centre for Diarrhoeal Disease Research, Bangladesh Research and Ethical Review Committees.

Baseline survey. From February to March 1993 a sample of 3,290 women were interviewed once as part of eligibility screening for the longitudinal component of the study. Interviews were given to all resident married women between the ages of eighteen and forty-five

who were present in the household during the survey period and agreed to participate. Interviews were conducted in Bengali by Bangladeshi female community health workers who resided in the Matlab area. Women of all reproductive statuses were included in the survey. The baseline interviews included questions on past fertility, current reproductive status, contraceptive use, current breastfeeding behavior including duration of breastfeeding, and length of postpartum amenorrhea.

Follow-up survey. In March 1993, eligible women who participated in the baseline survey were randomly selected for enrollment in the follow-up study. Twice-weekly interviews were collected for up to nine months. Women of all reproductive statuses were selected by the following criteria: married women living with their spouse in the study area who were not using contraceptive methods and had not reached menopause. Continuous recruitment was used, so that at any time during the follow-up survey, about 100 subjects were enrolled. After a subject dropped out of the study, became ineligible, or was otherwise lost to follow-up, a new subject was selected at random from the same village. Reasons for ineligibility included adoption of contraception, divorce or marital separation, or migration out of the study area. Additionally, women who gave birth during the follow-up study period were taken out of the follow-up survey within a month of delivery (although many were later given an exit interview where they reported menstrual status). The follow-up questionnaire contained information about menses, pregnancy status, pregnancy outcome, contraception, and breastfeeding behavior. At the first postpartum interview, subjects were queried as to the sex of the child, delivery complications, and death of the child.

Exit Survey. During November and December 1993, a one-time exit interview was conducted. An attempt was made to re-contact the subjects who provided interviews during the follow-up survey. The interview asked questions on reproductive status, the most recent start and end date of menses, and any pregnancy outcomes.

Demographic records. Additional data came from the DSS records of ICDDRB. These data included records on births, deaths, migration events, marriages, divorces, stillbirths, and spontaneous abortions collected from 1974 through 1993. The DSS records were used, in part, to verify birth dates for children born prior to the baseline interview, and the survival status of the index child for each mother.

Specimen collection. Urine specimens were collected from participants in their homes by female Bangladeshi field workers. Urine specimens were 'spot specimens' collected at whatever time of the morning was convenient for the participants. The specimens were collected before noon in the majority of cases but they were occasionally collected in the afternoon. Immediately after collection, specimens were placed in coolers with ice packs and transported within two days to a research hospital (Holman, 1996). Specimens were kept at 4ºC for up to one week and then brought to room temperature to determine pH (Horiba C-1 pH meter) and specific gravity (Atago Uricon-N urine specific gravity refractometer). A 6.5 ml sample of each specimen was taken, preserved with 17 g/L boric acid solution, and stored at -20° C. The preserved specimens were transported via frozen air freight to the United States and stored at -20C until they were assayed for reproductive hormones in 1997. The specimens underwent from two to five freeze-thaw cycles, and variable times at refrigerated (never more than 2 weeks) or ambient temperatures (never more than 1 day). These collection and storage conditions are not likely to have significantly affected the stability of the urinary steroid metabolites (O'Connor et al., 2003).

Laboratory Methods

Urinary pregnanediol glucuronide (PDG) and estrone-3-glucuronide conjugates (E1C) concentrations were determined in microtiter plate-based enzyme immunoassays (EIAs). The assays are described in detail elsewhere (O'Connor et al., 2003). Briefly, the E1C EIA used the 155B3 monoclonal antibody which cross-reacts 100% with free estrone, estrone sulfate and estrone glucuronide. The PDG EIA used the Quidel 330 monclonal antibody, which cross-reacts 100% with pregnanediol-3-alpha-glucuronide and 119% with 20-alpha-hydroxy-4-pregnen-3-one. The limits of detection (mean +3 SE above the zero standard) were 21 nmol/L for the PDG EIA and 0.27 nmol/L for the E1C EIA. Inter- and intra-assay CVs for high concentration urine control pools were 10% and 9% for the PDG EIA, and 11% and 7% for the E1C EIA.

Urine specimens were assayed in duplicate, and were added to the assays neat, or pre-diluted for higher concentration specimens. Absorbance was measured with a Dynatech MR7000 Plate Reader (test wavelength 405 nm, reference wavelength 570 nm). Hormone concentrations were estimated from optical density using a four parameter logistic model (Robard, 1974) in Biolinx 1.0 Software (Dynex Laboratories, Inc., Chantilly, VA). Standards (5β-pregnane-3α, 20α-diol glucuronide, Sigma Catalog No. P3635; Estrone-ß-D-glucuronide, Sigma Catalog No. E1752; CR 127 provided by J. O'Connor, Columbia University) and in-house urine controls were run in duplicate. Hormone concentrations were normalized using specific gravity (Miller et al., 2004), with 1.015 as the target specific gravity.

Statistical Methods

Observations were used to estimate the parameters of a two component mixture model (Pearson et al., 1992). The two-component mixture model has the general form $f(t; \boldsymbol{\theta}, \boldsymbol{\phi}, p) = pf_1(t; \boldsymbol{\theta}) + (1 - p)f_2(t; \boldsymbol{\phi})$, where $f_1(t; \boldsymbol{\theta})$ is the probability density function (PDF), with parameters $\boldsymbol{\theta}$**,** for the long-duration subgroup. The second subgroup has PDF $f_2(t; \boldsymbol{\phi})$ with parameters $\boldsymbol{\phi}$, for the short-duration subgroup. The mixing parameter p ($0 \le p \le 1$) is the fraction of mothers in the long-duration subgroup; whereas, $1 - p$ are in the short-duration subgroup.

The particular PDFs used for $f_1(t; \boldsymbol{\theta})$ and $f_2(t; \boldsymbol{\phi})$ were determined empirically by examining a number of different distributions and evaluating the set of distributions that best fit the data. The candidate distributions were a lognormal, Weibull, gamma, and extreme value type 1 (or Gumbel) distribution for each component of the model. The criterion for selecting distributions is discussed below.

The observations. The observations of postpartum amenorrhea are specified as three durations from the date of delivery. The three times specify an interval within which menses resumed and an ascertainment time. Times t_o and t_c specify the minimum and maximum observed postpartum times between which first postpartum menses occurred—that is $[t_o, t_c)$ is the half-opened interval that surrounds resumption of menses. The third time, t_a, is the time from parturition to first ascertainment of PPA status. This time is when the subject is first observed postpartum. The ascertainment time is used to statistically left-truncate observations at the time since parturition the subject was first observed. Left truncation statistically removes the portion of risk prior to when the subject is under observation.

The meanings of these three times, and special cases, are shown in Figure 1. In observations 1 through 4, PPA status is first ascertained during the baseline survey some months postpartum. For observation 1, resumption of menses is observed down to the day, that is, the last time before resumption of menses and first time after resumption of amenorrhea are identical. For this observation $t_a < t_o = t_c$. For observation 2, a subject's amenorrhea status is ascertained at the baseline survey and some months later the subject is enrolled in the follow-up study after she has resumed menses. For this observation, $t_a = t_o < t_c$. For observation 3, the subject remains amenorrheic, and then drops out of the study or otherwise is unavailable for follow-up until a much later interview date or the exit interview. An exact date of resumption of menses is not known. Hence, the observation is interval censored over a large interval, and $t_a < t_o < t_c$. For observation 4, the subject remains amenorrheic until her last interview (usually the exit interview). Here, t_o is the duration until the last observation, and t_c is, in effect, infinity. For observations 5 and 6, the subject is pregnant for the baseline interview, and perhaps into the follow-up study. Parturition occurs during the prospective study so that ascertainment occurs at birth (time $t_a = 0$). For observation 5, an exact time to resumption of menses is observed during the longitudinal portion of the study so that $t_o = t_c$. Finally, observation 6 is like observation 5 except that the subject is right censored at her last observation (usually the exit interview), so t_o is the duration from birth to the last interview and t_c is infinity.

Covariates. Covariates were collected either from the baseline survey or the ICDDRB DHS records. *Mother's age* and *years married* were taken from the ICDDDRB marriage records; *wants more children* is 1 if the subject reported that she wants additional children, and 0 if not; *desired number of children* is her self-report of the number of desired additional children; *child's sex* is the sex of the index child; *parity*, *living children*, and *pregnancy loss* are taken from the ICDDRB DSS records; *low-wage occupation* is coded as 1 for subjects who report their husband's occupation included unemployed categories and low-wage income, such as farm laborer and other unskilled laborers, and 1 for higher wage occupations; *husband migrates* is coded as 1 if the subject's husband migrates away from the household for more than one month per year for seasonal employment; *cool dry*, *hot dry*, and *monsoon* is a dummy variable for the season in which the index child was born.

The likelihood. Maximum likelihood was used to estimate model parameters from left truncated, interval-, and right-censored observations (Wood et al., 1992). The likelihood for a sample of N observations is constructed as

$$L = \prod_{i=1}^{N} \frac{\left[f(t_{o_i};\boldsymbol{\theta},\boldsymbol{\phi},p) \right]^{1-\delta_i} \left[S(t_{o_i};\boldsymbol{\theta},\boldsymbol{\phi},p) - S(t_{c_i};\boldsymbol{\theta},\boldsymbol{\phi},p) \right]^{\delta_i}}{S(t_{a_i};\boldsymbol{\theta},\boldsymbol{\phi},p)}, \tag{1}$$

where $S(t; \boldsymbol{\theta}, \boldsymbol{\phi}, p)$ is the survival function for the mixture model, which can be found from the survival distribution of each of the two component distributions as $S(t; \boldsymbol{\theta}, \boldsymbol{\phi}, p) = pS_1(t; \boldsymbol{\theta}) + (1 - p)S_2(t; \boldsymbol{\phi})$, and $f(t; \boldsymbol{\theta}, \boldsymbol{\phi}, p)$ is the PDF found as $f(t; \boldsymbol{\theta}, \boldsymbol{\phi}, p) = pf_1(t; \boldsymbol{\theta}) + (1 - p)f_2(t; \boldsymbol{\phi})$. The indicator variable δ_i is 1 if the observation was interval or right censored, and 0 if resumption of menses was known down to the day.

Covariate effects are modeled on the p, the proportion of subjects in the long-duration subgroup. The effects on p are specified by logistic regression. An $M + 1$ array of parameters, $\boldsymbol{\beta}_\mathbf{p} = (\beta_{p0}, \beta_{p1}, \beta_{p2}, ..., \beta_{pM})$, quantifies the effects of M covariates, $\mathbf{x}_i = (x_{1i}, x_{2i}, ..., x_{Mi})^T$, for the i-th subject as $p_i = [1 + \exp(\mathbf{x}_i\boldsymbol{\beta}_\mathbf{p})]^{-1}$, and p_i replaces p in likelihood (1).

Covariate effects are also modeled on the hazard of resumption of menses for each

subgroup. For the first subgroup, $\mathbf{x}_i\boldsymbol{\beta}_1 = x_{1i}\beta_{11} + x_{2i}\beta_{12} + ... + x_{Mi}\beta_{1M}$ is a vector formed by M covariates $\mathbf{x}_i$ for the i-th subject. Likewise, parameters for the second subgroup are $\mathbf{x}_i\boldsymbol{\beta}_2 = x_{1i}\beta_{21} + x_{2i}\beta_{22} + ... + x_{Mi}\beta_{2M}$. A proportional hazards specification is used to model $\mathbf{x}_i\boldsymbol{\beta}_1$ on the first component and $\mathbf{x}_i\boldsymbol{\beta}_2$ on the second component. Under the proportional hazards model, the survival function is specified as $S_{1i}(t_i; \mathbf{x}_i, \boldsymbol{\theta}, \boldsymbol{\beta}_1) = S_1(t_i; \boldsymbol{\theta})^{\exp(\mathbf{x}_i\boldsymbol{\beta}_1)}$, and on the PDF as $f_{1i}(t_i; \mathbf{x}_i, \boldsymbol{\theta}, \boldsymbol{\beta}_1) = f_1(t_i; \boldsymbol{\theta})S_1(t_i; \boldsymbol{\theta})^{\exp(\mathbf{x}_i\boldsymbol{\beta}_1) - 1}\exp(\mathbf{x}_i\boldsymbol{\beta}_1)$.

The maximum likelihood estimates are those values of $\boldsymbol{\theta}$, $\boldsymbol{\phi}$, p, $\boldsymbol{\beta}_1$, $\boldsymbol{\beta}_2$, and $\boldsymbol{\beta}_p$ that maximize the likelihood. Parameter estimates were found numerically using the *mle* version 2.2 programming language (Holman, 2003).

Model selection. We examined a number of different distributions for $f_1()$ and $f_2()$, and models with different numbers of parameters. The Akaike Information Criterion (AIC) was used to select the model that most parsimoniously approximates the true (but unknown) model from which the data were drawn (Akaike, 1973, 1992; Burnham and Anderson, 1998). The AIC for a model is computed as $-2\ln(\hat{L}) - 2M$, where M is the number of parameters estimated for the model, and $\hat{L}$ is the maximized likelihood for that model. The model with the lowest AIC is taken as the most parsimonious among the candidate models.

RESULTS

A total of 300 subjects, aged 17 to 48 (mean 28.3) were followed while experiencing PPA. This total excludes all miscarriages (6), stillbirths (4), and induced abortions (3) observed during the study. One infant died within hours of delivery, and the mother was excluded from further analyses; she resumed menses at 69 days postpartum. Questionnaire answers, demographic records, and menstrual calendars were examined for each subject to determine the end of PPA, or the interval within which menses resumed. Reported menstrual bleeding was usually sufficient to determine the end of PPA. Figure 2 shows the durations and pattern of censoring for all observations. The observations included 47 times to resumption of menses observed down to the day, 65 interval censored observations, and 187 right-censored observations. Of the 112 exact and interval censored observations, 91 mothers continued to breastfeed beyond resumption of menses, and for 21 mothers, post-PPA breastfeeding status could not be determined.

In order to rule out confusion of postpartum bleeding with menstrual bleeding, we examined postpartum and menstrual bleeding reported by subjects, and we examined hormone values for evidence of follicular development and luteal function. Hormone profiles for the four shortest non-censored durations are show in Figure 3. Comparative examples of profiles for women with later times to resumption of menses are given in Figure 4. It is difficult to ascertain individual ovarian cycles and day of ovulation from these data because urine specimens were only collected twice weekly; nevertheless, the profiles can provide evidence of ovarian or luteal function. Subjects with later (unambiguous) or no resumption of menses during the study tended to remain amenorrheic until (1) E1C concentrations exceed about 20,000 pg/ml, providing evidence of follicular activity and (2) PDG concentrations exceed 700 ng/ml, providing evidence for luteal function. By these criteria, the four subjects with early resumption of menses (Figure 3) exhibited evidence of follicular activity or luteal function prior to resumption of menses. Additionally, each subject in Figure 3 prospectively reported no bleeding for a week, and usually

much longer, prior to reporting menstrual bleeding. In this way, we were able to exclude postpartum bleeding for all subjects by examining reports of postpartum bleeding (retrospectively reported and prospectively reported as in Figure 3, panel a), menses following an extended period with no reports of bleeding (e.g. Figure 3, panels b and c), or endocrine evidence of ovarian or luteal activity.

Combinations of lognormal, Weibull, gamma, and Gumbel distributions were fit to observations of duration from birth to the first postpartum menses. Of the distributions examined, a mixture of two Weibull distributions provided the best fit to the data (Table 1). The first subgroup made up 84.0% (±4.0% SE) of the population with a mean PPA duration of 456.5 (±30.6) days and a standard deviation of 174.3 (±11.8) days. The second subgroup, 16% (4.0%) of the population, had a mean PPA duration of 93.9 (±17.1) days and a standard deviation of 37.3 (±8.0) days. The overall mean for the combined subgroups was 398.2 (±21.5) days. We also examined reduced models with only a single subgroup and models with mixtures of three subgroups. None of these alternatives fit the data as parsimoniously (assessed by AIC) as the two-subgroup model. Figure 5 shows the best-fitting parametric probability density function, and the two component distributions. The corresponding survival distribution is given in Figure 6, showing an overall median time to resumption of menses of 409 (±30) days.

Individual covariates were entered into the model, one at a time, to assess univariate effects on time to resumption of menses (Table 2). The three covariates *low wage occupation*, *monsoon*, and *husband migrates* were significant in five of the univariate models. All five of these covariate effects were examined in a multivariate model and AIC was used to find the most parsimonious final model (Table 3). The covariate "*husband migrates*" reduced the duration of PPA within the long-duration subgroup. The effect of being born during the *monsoon* season was to reduce the duration within the short-duration subgroup. Finally, *low wage occupation* affected the probability of being in each subgroup: subject's whose husbands had low wage occupations (or unemployed) were in the long-duration subgroup with a probability of 0.90, whereas subjects whose husbands had high-wage occupations were in the long-duration subgroup with a probability of 0.74. The relative effects of combinations of covariates on the expected distribution of PPA are shown in Figure 7. For most combinations of covariates, a high degree of separation remains between the two component distributions, so that the overall appearance is bimodal. Mothers who give birth outside the monsoon season and whose husbands migrate seasonally have a greatly reduced duration of amenorrhea, and the modes of the two underlying distributions cannot be discerned.

DISCUSSION

This study provides new evidence that the distribution of postpartum amenorrhea in rural Bangladesh women follows a bimodal distribution composed of two distinct subgroups. Similar results have previously been observed in Bangladesh (Ford and Kim, 1987; Huffman et al. 1987; Rahman et al., 2002) and other populations (Eslami et al., 1990; Ford and Kim, 1987; Le Strat and Thalabard, 2001; Potter and Kobrin, 1981; Taylor et al., 1999).

Previous researchers have suggested that the subgroup with early resumption of menses might reflect shortcomings in data collection so that a subgroup of non-breastfeeding women (those who experienced a miscarriage, stillbirth, or whose infant died) were included with breastfeeding women, or that some women reported non-menstrual postpartum bleeding as

resumption of menses (Ford and Kim, 1987; Huffman et al., 1987; Potter and Kobrin, 1981). We were able to rule out these possibilities in our study because we could exclude all cases of infant death and pregnancy loss, and we examined hormone profiles for evidence of ovarian activity for subjects who resumed menses at early postpartum ages.

We found that a subgroup, making up 16% of the population, was characterized by a short duration to resumption of menses, with a mean duration of 3.1 months. Ford and Kim (1987) found that 24% of Bangladeshi subjects made up a short-duration subgroup with a mean of 4.4 months, and 35% of the subjects from Narangwal, India made up a short duration subgroup with a mean of 3.5 months. The higher fraction of individuals in the short-duration subgroup found by Ford and Kim (1987) may reflect data quality. If so, the higher mean duration of PPA for the short-duration subgroups in Ford and Kim (1987) argues against confusion of postpartum bleeding with menses in their study. We found that postpartum bleeding in our Bangladesh sample lasted, on average, 0.81 (±0.04) months and with a 99% upper confidence interval of 2.1 (±0.1) months (Holman et al., n.d.). Thus, it is unlikely that mistaking postpartum bleeding for resumption of menses could account for the later mean time to resumption of menses found in Ford and Kim (1987). The data analyzed by Ford and Kim (1987) may have included some cases of unreported child mortality, which could increase both the fractions in the short-duration subgroup and the mean duration for that subgroup. Alternatively, the differences between our findings and those of Ford and Kim (1987) may reflect valid differences in patterns of feeding, supplementation, or maternal health, rather than differences in data quality.

Past studies have uncovered variables affecting the duration of PPA, such as breastfeeding intensity and duration, maternal age, anthropometrics of the mother, and parity (e.g. Heinig et al., 1994; Huffman et al., 1978; Jones, 1988b; Nath et al., 1993; Singh, et al., 1993; Vestermark et al., 1994). On the surface these variables do not easily explain a *bimodal* distribution of PPA, since they tend to vary continuously (or ordinally in the case of parity). Similarly, maternal energy availability or status would likely vary continuously among women. The breastfeeding behavior of the mother and infant is central to any discussion of this topic. A more intense pattern of breastfeeding (exclusive breastfeeding with frequent feeding episodes) results in a longer duration of PPA (Howie et al., 1981, Howie and McNeilly, 1982, Jones, 1990), but behavioral factors that would result in two distinct subgroups have not been clearly identified.

We examined the effects of a number of economic, attitudinal, and demographic variables on the three parts of the mixture model of PPA. Ten of thirteen covariates had no significant effect on time to resumption of menses. Covariates that reflect a woman's desire for more children had no affect on length of PPA. A child's sex had no effect on PPA, suggesting that, in this setting, mothers invested in daughters and sons equally through breastfeeding. Likewise, maternal age and birth history had no effect on the duration of PPA. The variables that affected the distribution of PPA were related to the husband's occupation and the season of birth.

Only one covariate affected subgroup membership. Women whose husbands had higher-wage employment had a greater probability of falling in the short duration subgroup. There are at least two ways this finding can be interpreted. First, this finding may indicate that in high-wage households, women had the financial means to acquire breast milk substitutes, such as a commercial formula, and therefore provided supplementary feeding earlier. Alternatively, this finding may reflect increased caloric energy availability in wealthier households. Women in Matlab are small, thin, and chronically undernourished by Western standards (Miller et al., 1994;

Pebley et al., 1985), as evidenced by an average body mass index (BMI) for a large random sample of non-pregnant reproductive-aged women from Matlab, Bangladesh of 18.8 ±1.9 (Ahmed et al., 1998). The role of caloric energy availability in determining bimodiality in the distribution of PPA is however difficult to explain. One possibility is that women who interrupted breastfeeding for whatever reason (because of mother or child illness, or difficulties breastfeeding) are unable to resume breastfeeding because of their generally low energy status. Unfortunately, detailed information on supplementation and maternal energetic status were not collected as part of this study, so we could not further explore these ideas. Alternatively, the short-duration subgroup may be composed of women whose energy intake exceeded their combined energetic demands at all postpartum ages. This is consistent with the hypothesis that amenorrhea is maintained by a high metabolic load, rather than suckling stimulus (Ellison, 1995; Valeggia and Ellison, 2001).

Women whose husbands were absent from the household for at least one month a year for employment had shorter periods of PPA, but only if they otherwise fell into the long-duration subgroup. This finding, like the previous one, suggests an effect of resources on breastfeeding behavior, perhaps through supplementation, or on the nutritional status of the mother. This finding does not suggest the origin of the short-duration subgroup, as only the long-duration subgroup is affected.

The effect of season of birth was to shorten the duration of PPA for women who delivered during the monsoon season, but only for the subgroup expected to experience an early resumption of menses. This might reflect seasonality in labor, the timing and availability of weaning foods, seasonal changes in disease patterns, or seasonal changes in maternal nutritional status. In Matlab, Bangladesh, strong seasonal patterns are found for conception, live births and resumption of menses (Becker et al., 1986), and maternal weight (Miller et al., 1994). We were not able to analyze seasonality of resumption of menses because many subjects were right-censored or interval censored. The season of birth may interact with seasonal availability of nutrition, resulting in a shorter duration of PPA during the monsoon season for the short-duration subset of women. Nutritional status of women in Matlab is poorest during the monsoon season in September, and peak food availability follows the major rice harvest in November (Becker et al., 1986). Births during the monsoon season (June through September) may increase the hazard of resumption of menses for the short-duration subgroup because of the increased energy available to mothers at early postpartum ages during the harvest, or because resources are available for earlier supplementation.

CONCLUSIONS

In rural Bangladesh, two distinct patterns for resumption of menses were statistically identified. This finding is consistent with previous work in Bangladesh (Ford and Kim, 1987; Huffman et al., 1987; Rahman et al., 2002). Previous authors (Ford and Kim, 1987; Huffman et al., 1987; Potter and Kobrin, 1981) have not been able to rule out that the short-duration subgroup arises from data quality errors; specifically, from including cases of pregnancy loss or perinatal mortality, or from attributing non-menstrual postpartum bleeding to menstrual bleeding following reestablishment of ovarian function. We were able to control for these data quality errors, and still found a subgroup of 16% who had an early resumption of menses. We conclude that this subgroup is not an artifact of bad data or improper statistical analyses, but reflects true

physiological or behavioral differences for some mother-infant dyads.

Economic and seasonal covariates affected the time to resumption of menses, and may reflect either a nutritional or food availability cause for this heterogeneity. Taken together, the covariates explained only part of the bimodal pattern of PPA. A more complete understanding of the bimodal distribution of postpartum amenorrhea will require additional investigation into how the reproductive axis is affected by postpartum changes in breastfeeding behavior, supplementation, maternal workload, maternal energy availability and maternal-infant health.

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Table 1. Parameter estimates for the best fitting mixture model.

Parameter	Estimate (SE)		t
p	0.84	(0.04)	20.88
θ_1	512.4	(32.7)	15.7
θ_2	2.84	(0.32)	8.82
ϕ_1	102.6	(18.9)	5.4
ϕ_2	2.72	(0.63)	4.28

[1] p is the baseline logistic parameter for the fraction in each subgroup; θ_1 and θ_2 are parameters for a Weibull distribution for the subgroup with a long time to resumption of amenorrhea; ϕ_1 and ϕ_2 are parameters for a Weibull distribution for the subgroup with a short time to resumption of amenorrhea.

Table 2. Univariate effects of each covariates on each part of the model.

Covariate	β_p (SE)[1]		β_θ (SE)		β_ϕ (SE)	
Mother's age	-0.0427	(0.0390)	-0.0230	(0.0248)	-0.0410	(0.0826)
Years married	-0.00779	(0.0304)	-0.0218	(0.0215)	-0.0343	(0.0705)
Wants more children	0.419	(0.636)	0.0264	(0.296)	0.901	(1.10)
Desired number of children	0.176	(0.152)	0.505	(0.369)	0.190	(0.401)
Child's sex	-0.285	(0.442)	-0.0185	(0.253)	-0.380	(0.757)
Parity	-0.0307	(0.101)	-0.0648	(0.0715)	-0.129	(0.243)
Living children	-0.0241	(0.123)	-0.0417	(0.0757)	-0.178	(0.263)
Pregnancy loss	0.0771	(0.261)	-0.108	(0.207)	-0.160	(2.06)
Low wage occupation	-1.44	(0.570)*	-0.360	(0.234)	-2.98	(0.956)*
Husband migrates	2.95	(1.22)*	2.67	(0.706)*	1.78	(8.47)
Cool dry	-0.336	(0.496)	-0.226	(0.241)	-0.753	(0.798)
Hot dry	0.390	(0.518)	0.462	(0.341)	-0.792	(1.21)
Monsoon	0.0429	(0.511)	-0.0356	(0.255)	2.37	(1.06)*

* Significant univariate effect (assessed by AIC) compared to the reduced model without the covariate.

[1] β_p is a logistic effect on the fraction of subjects in the long-duration subgroup; β_θ is an effect on the hazard for the long-duration subgroup, and β_ϕ is an effect on the hazard for the short-duration subgroup.

Table 3. Most parsimonious covariate model (assessed by AIC) for the distribution of postpartum amenorrhea.

Name[1]	Estimate	(SE)	t
θ_1	524.5	(31.5)	16.6
θ_2	3.00	(0.33)	9.01
$\beta_{\theta\_husband\_migrates}$	2.59	(0.88)	2.93
ϕ_1	131.2	(22.3)	5.9
ϕ_2	3.43	(0.74)	4.64
$\beta_{\phi\_monsoon}$	1.99	(1.31)	1.52
p	-1.06	(0.33)	3.20
$\beta_{p\_low\_SES\_occupation}$	-1.10	(0.51)	2.17

[1] θ_1 and θ_2 are parameters for a Weibull distribution for the subgroup with a long time to resumption of amenorrhea; ϕ_1 and ϕ_2 are parameters for a Weibull distribution for the subgroup with a short time to resumption of amenorrhea. p is the baseline logistic parameter for the fraction in each subgroup.

Figure 1. Types of observations made during the study. Symbols: *B* represents the birth of the index child, *A* is the time of ascertainment (when postpartum amenorrhea state is first observed), *[* is the last observation prior to resumption of menses, *]* is the first observation following resumption of menses, and *P* denotes the start of pregnancy. The dashed lines are periods during which the subject is not observed (prior to the baseline survey). The dates for these events are used to determine three durations: t_a, the duration from parturition until ascertainment; t_o, the duration from parturition until the last observation prior to resumption of menses; and t_c the duration from birth until the first observation following resumption of menses. Each observation type (1 to 6) is discussed in the text.

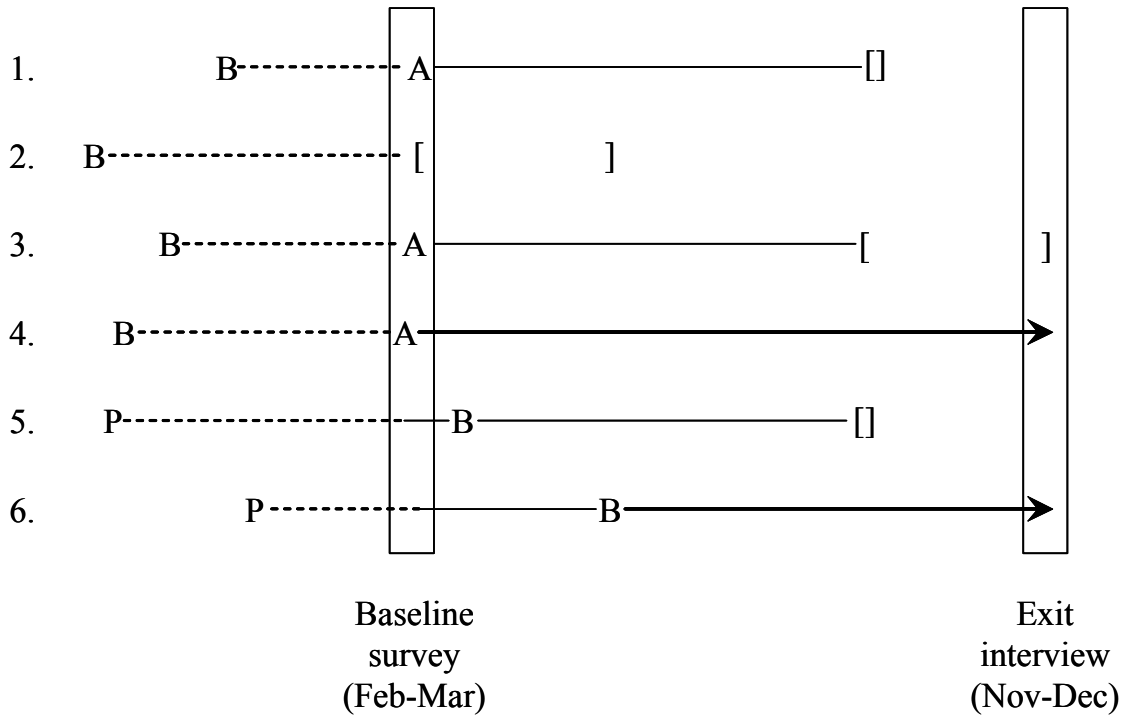

Figure 2. Individual observations of postpartum amenorrhea. The solid lines to the left are the periods of observation in the study. Lines ending in a solid circle denote exact times to resumption of menses. Dashed lines between open circles are interval censored observations within which menses resumed. Lines ending with + are right censored observations of amenorrhea (also shown on the right border).

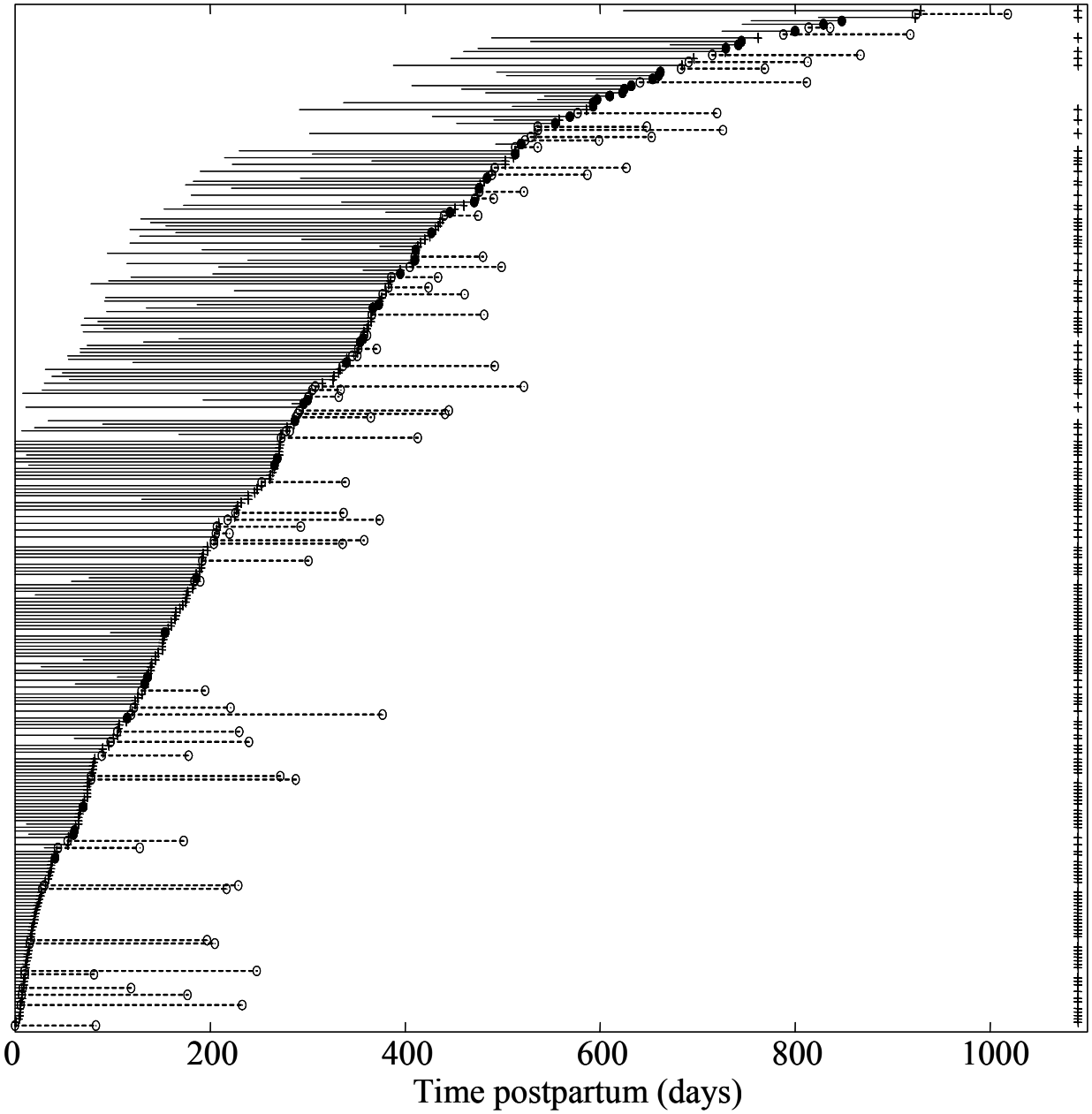

Figure 3. Hormone profiles for subjects with the shortest non-censored times to resumption of menses. Boxed areas show menses, and dashed box shows the extent of postpartum bleeding. Each tic on the *x*-axis is one week.

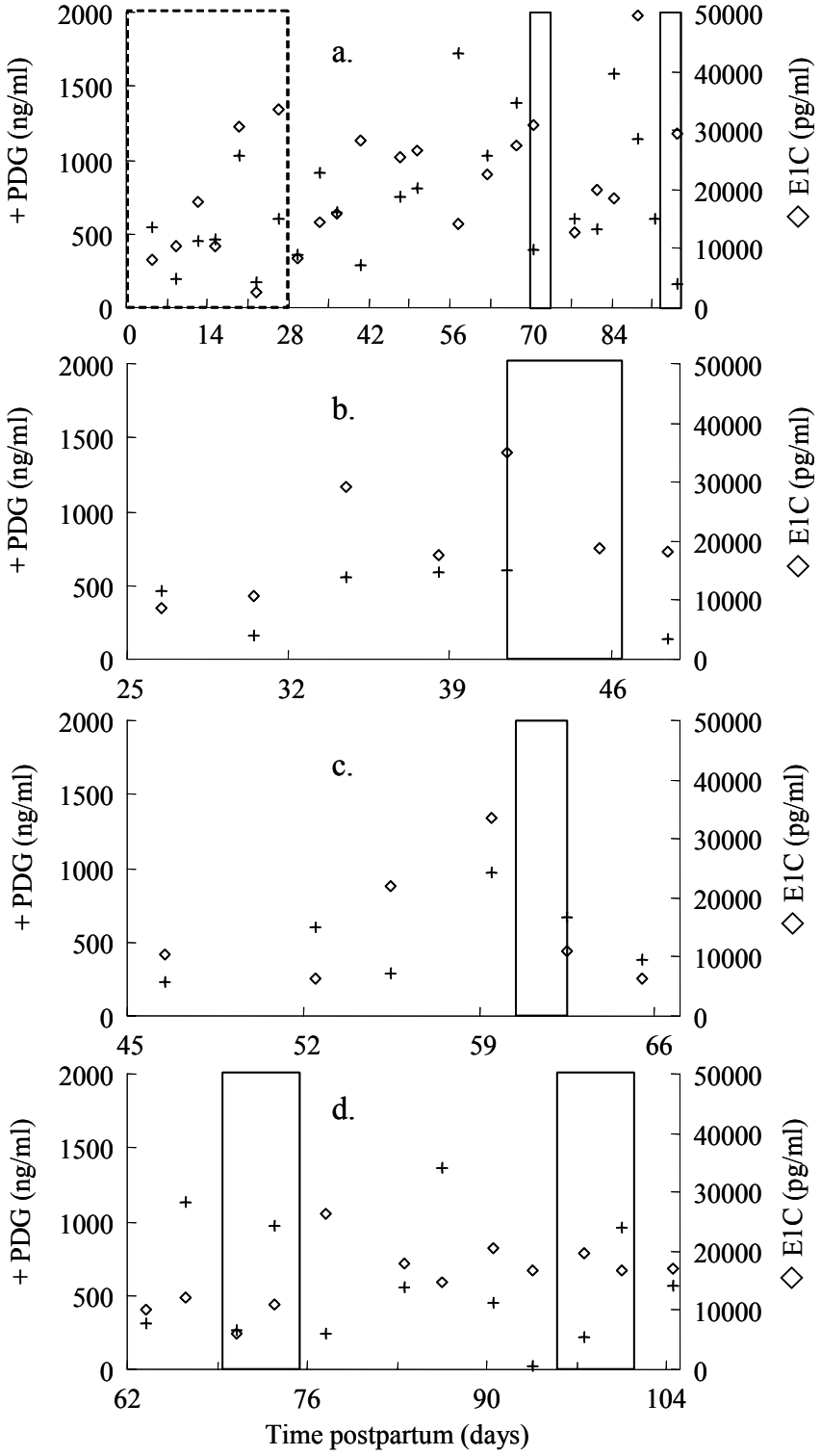

Figure 4. Hormone profiles for two subjects who remained amenorrheic throughout the study (panel a and b), and two subjects who resumed menses during the study at later postpartum ages (panel c and d). Boxed areas show menses. Each tic on the *x*-axis is one week.

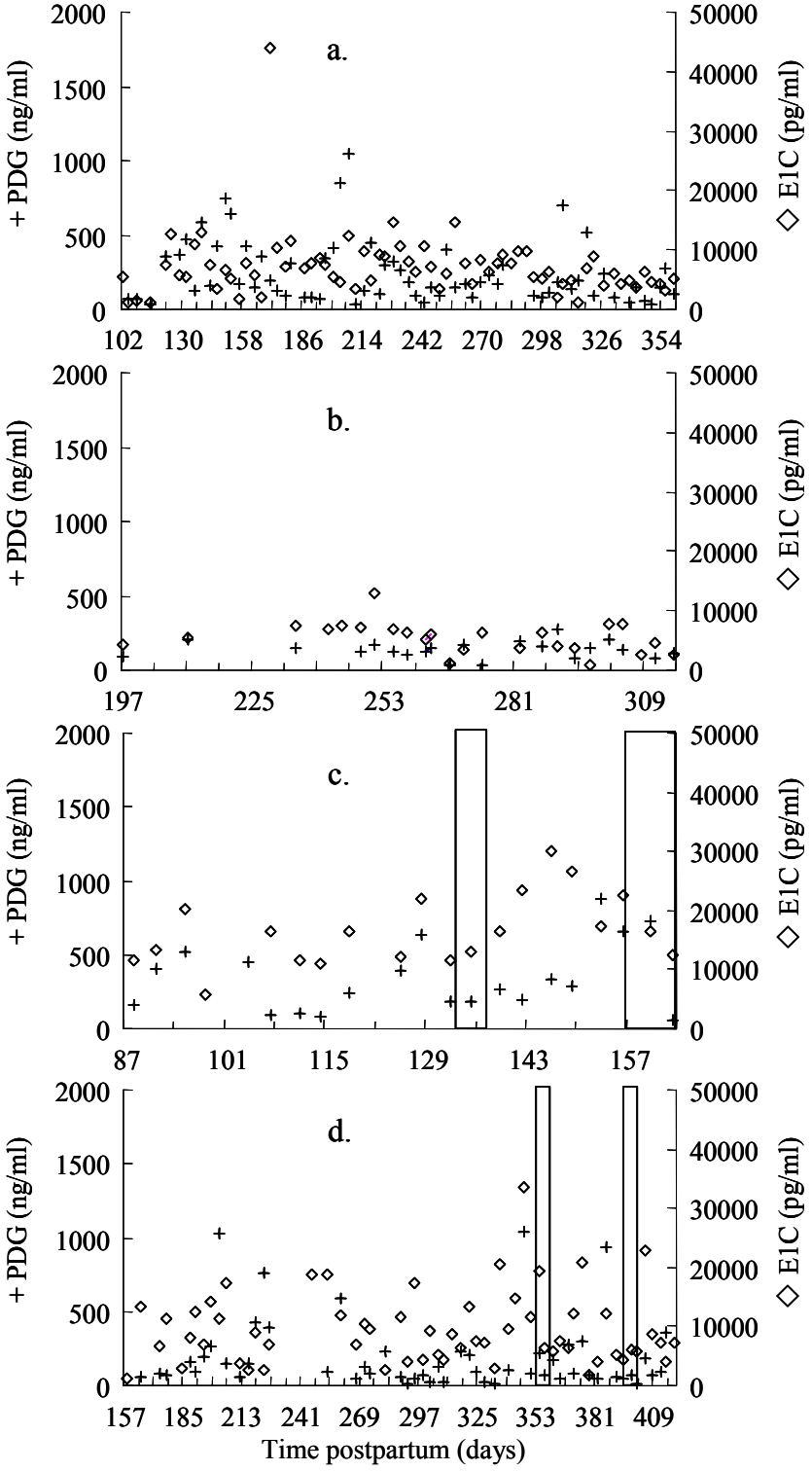

Figure 5. The fitted distribution of postpartum amenorrhea for each subgroup and the combined subgroups, based on parameter estimates in Table 1.

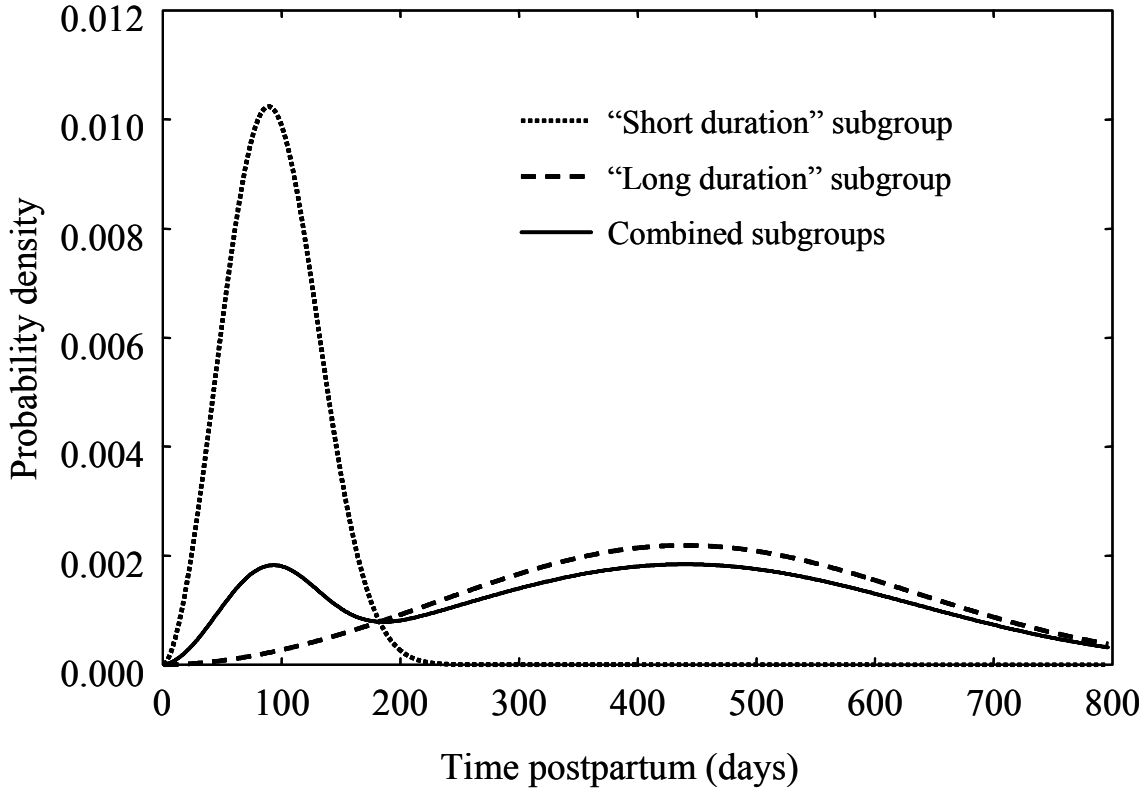

Figure 6. Survival distribution (±SE) for postpartum amenorrhea, based on parameter estimates in Table 1.

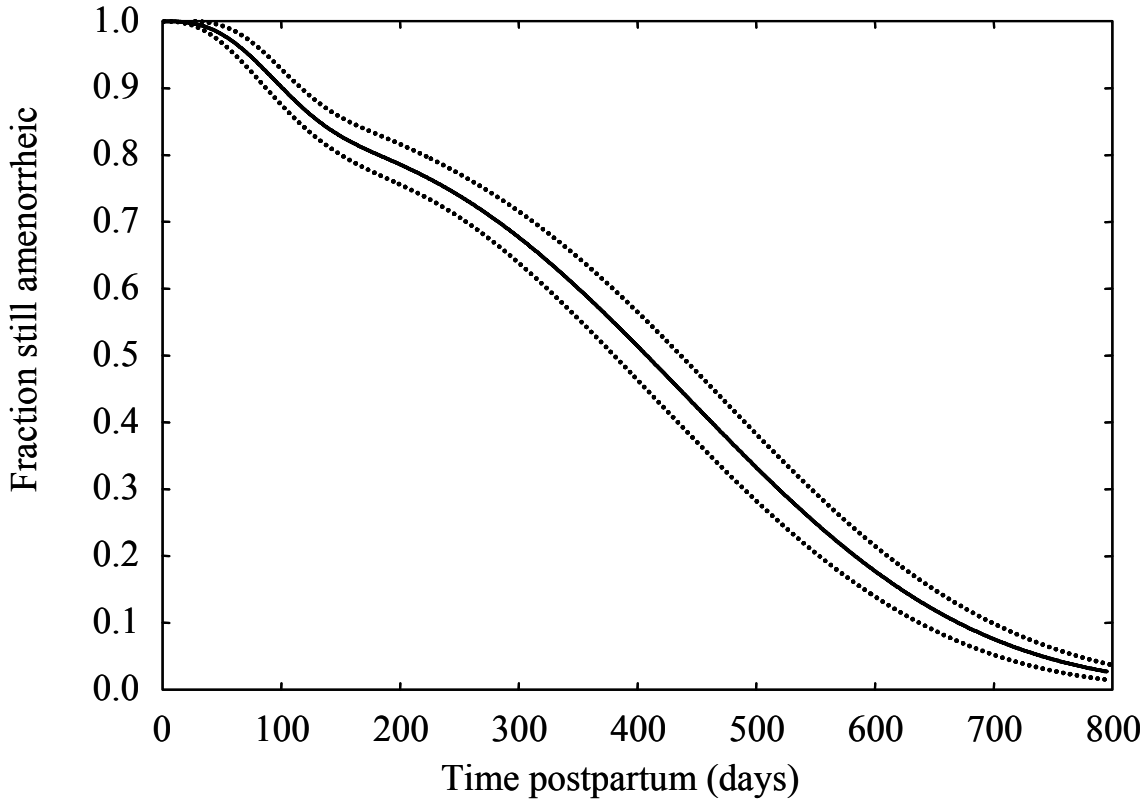

Figure 7. Expected distributions of postpartum amenorrhea for different combinations of three covariates, based on parameter estimates in Table 3.