Center for Studies in Demography and Ecology

Age-Dependent Decline of Female Fecundity is Caused by Early Foetal Loss

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Introduction

Changes in the pace of reproduction over the course of the reproductive lifespan are a complex outcome of physiological, cultural, economic and behavioural changes with age. In many industrialised countries, for example, age-specific fertility rates are largely dominated by the use of contraception for terminating or postponing reproduction as well as for the spacing of births. In these populations, physiological factors play a relatively weak role except at the beginning and end of the reproductive span. Consequently it is difficult to study age-related changes in fecundity (the biological capacity to reproduce) under conditions of widespread contraceptive use.

Demographers have addressed this difficulty by turning to populations in which birth control is rarely used. Age-related changes in fecundity can be fruitfully studied in these so-called natural fertility populations because these strong parity-related controls of fertility are not exercised. A universal finding from decades of demographic research on natural fertility populations is that female fecundity declines with the age of a women [1]. In examining the causes of this decline, fecundity can be decomposed into two meaningful components. The first component is a true age-related decline in the probability of conception. Demographers measure this component of fecundity as *fecundability*, which is defined as the monthly or cycle-wise probability that a cohabiting couple will experience a conception. The second component of fecundity is *foetal loss*, which is the probability that a pregnancy terminates prior to birth.

The problem with measuring fecundability and foetal loss is that pregnancies are almost impossible to detect non-invasively until well after conception. Sensitive pregnancy assays based on measurement of human chorionic gonadotropin (hCG) cannot detect a rise in hCG until about one week after conception [2][3]. To make matters worse, the risk of pregnancy loss appears to be highest early in pregnancy [4][5][6][7][8], so that with the best technology available today, some fraction of early pregnancies go unnoticed before terminating.

The difficulty of detecting early pregnancy has led demographers to define several types of fecundability. The term *total fecundability* refers to the probability that fertilisation occurs in a single month or cycle for a sexually active woman; this is what we would like to measure, but cannot. The term *apparent fecundability* is the monthly or cycle-wise probability of conception given that the pregnancy survives long enough to be detected using some particular method. In other words, apparent fecundability is a measure that encompasses both the probability of conception and the probability that the conceptus will survive until it can be detected. In the same spirit, we can define *total foetal loss* as the probability of losing a pregnancy between fertilisation and birth; *apparent foetal loss* is the probability that a recognised pregnancy does not survive to term.

This confounding of fecundability and foetal loss means that studies of either are sensitive to the technology used to detect pregnancy. A study that uses sensitive endocrine methods to detect pregnancies should yield higher estimates of apparent fecundability and apparent foetal loss than a similar study using mother's self-reports of pregnancy. The difference arises because many pregnancies that can be biochemically detected terminate before they can be recognised by the mothers. As a consequence, it is difficult to compare values of fecundability and foetal loss among different studies, unless the same method was used to detect pregnancies in each study. Ideally, estimates of apparent fecundability should include a description of the assay for detecting pregnancies along with the gestational age-specific sensitivity of the assay. This information is rarely provided so that it is difficult to make direct comparisons of apparent fecundability among studies.

Methodological difficulties aside, the general patterns of age-related changes in foetal loss and fecundability have been examined from studies among natural fertility populations [9]. Figure 1 shows a composite age pattern of apparent fecundability compiled from several natural fertility populations after maximum fecundability was rescaled to one at age 22. Apparent fecundability exhibits a steady decline from the early 20s until it approaches zero in the late 40s. This pattern of age-related changes in fecundability is well accepted, even though it is necessarily based on indirect or incomplete ascertainment of pregnancies.

Figure 1 also shows age-specific apparent foetal loss compiled from several studies after rescaling each study to a common rate of 150 pregnancy losses for 1000 conceptions [10]. Apparent foetal loss shows a steady rise with age approaching a peak probability of 40% toward the end of the reproductive span. Based on studies of early pregnancy loss we can conclude that the true overall rate of pregnancy loss is likely to be substantially higher at each maternal age [4][5][7][11], but the overall pattern that shows an increase in risk of pregnancy loss with age is probably correct.

Clearly, the increase in apparent foetal loss and decrease in apparent fecundability constitute two important aspects of reproductive ageing in women. A fundamental issue for our understanding of human reproductive biology is whether the age-specific decline in fecundability represents a true drop in total fecundability with age or whether it reflects an age-related increase in the probability of early pregnancy losses. Similarly, we would like to ascertain the magnitude of pregnancy loss and the way in which it changes by maternal age.

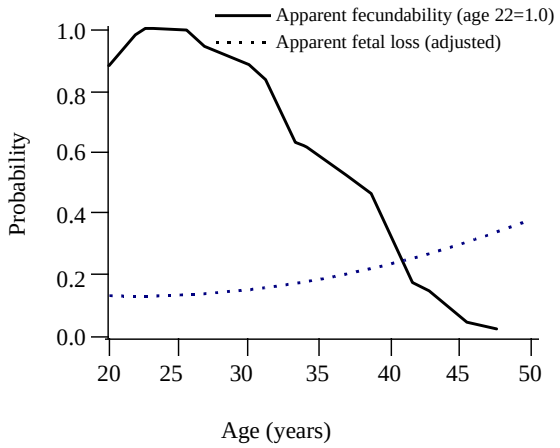

Figure 1. Composite age patterns of apparent fecundability and apparent foetal loss compiled from several natural fertility populations. Apparent fecundability is adjusted so that the maximum value is 1.0 at age 22 (redrawn from [9]). Apparent foetal loss from an analysis of nine population, each adjusted for an overall rate of 150 pregnancy losses per 1000 conceptions (redrawn from [10]).

One way to disentangle total fecundability and foetal loss is to examine the way in which pregnancy losses are distributed across gestation. The top panel of Figure 2 represent a hypothetical study in which some number of menstrual cycles are observed (*y*-axis) and those that result in a pregnancy are followed to term (*x*-axis). The horizontal line indicates the number of cycles in which fertilisation occurred, and the dashed curve represents the numbers of ongoing pregnancies that survive to each gestational age. After all pregnancies have terminated in this fictitious study, each menstrual cycle can be classified as a non-conception cycle, a cycle that ended in the loss of the pregnancy, or a cycle that resulted in a livebirth. We could then directly compute total fecundability as the number of pregnancies over the total number of cycles, and the probability of foetal loss as the number of pregnancy losses over the total number of pregnancies. Since we cannot detect pregnancies at fertilisation, a more realistic portrayal of what we measured in the fictitious study is shown in the lower panel of Figure 2. The vertical line labelled "detection" represents the sensitivity limit of the method used to diagnose pregnancies. We cannot detect any pregnancy before this point in gestation. Now each menstrual cycle must be classified in one of three ways: cycles in which a pregnancy was not detected, cycles that end in the loss of a detected pregnancy, and cycles that end in a livebirth. Clearly, we do not know the proper denominator for computing either total fecundability or the probability of foetal loss; neither do we know the proper numerator for computing the probability of foetal loss.

One possibility of getting around this problem, as suggest by Figure 2, is to use a parametric mathematical model for the gestational age-specific risk of foetal loss. Given the model we could, in principle, use observations from the point of detection forward to estimate the entire distribution of pregnancy loss. If so, the resulting estimated distribution could be used to compute total foetal loss, after which estimation of total fecundability is simple. This procedure involves "back projecting" the distribution of foetal loss to the earliest portion of pregnancy, a method that will be convincing only to the extent that the mathematical model of foetal loss reflects the actual biological mechanisms underlying pregnancy loss. The validity of the foetal loss model will strongly shape our confidence in the resulting estimates of total fecundability and total foetal loss.

The model

An etiologic theory of foetal loss was proposed by Marcus Bishop in 1964 [12]. Based on cytogenetic studies of human abortuses and his own work on the cytogenetics of bull sperm, Bishop proposed that most pregnancy losses result from chromosomal abnormalities arising from defects in the gametes, and that many of these pregnancies are lost so early in gestation that they are never observed as pregnancies. Furthermore, he postulated that chromosomal abnormalities should increase by parental age.

Many of the basic elements of Bishop's theory were expressed mathematically by Wood [1][9] and independently by Boklage [13]. The underlying logic of the Wood-Boklage model is that at fertilisation a conceptus can be classified as chromosomally normal or abnormal. The risk of pregnancy loss in the chromosomally abnormal subgroup is modeled as high and constant across gestation. The risk of pregnancy loss in the normal subgroup is modelled as low and constant across gestation. Even though the risk of loss in each subgroup is constant, the combined subgroups show a declining risk of foetal loss with increasing gestational ages (Figure 3). The decline in risk occurs because, on average, abnormal conceptuses are lost earlier in gestation, leaving an increasingly larger fraction of normal conceptuses at later gestational ages.

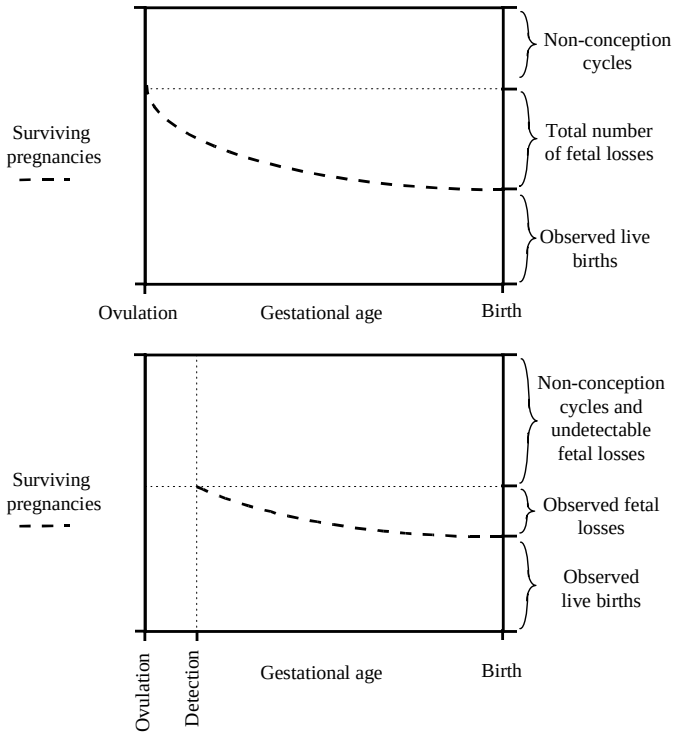

Figure 2. The probability of foetal loss and fecundability are confounded by incomplete sensitivity of pregnancy assays. The y axis represents some number of menstrual cycles under study, and the x axis is time from ovulation to birth. The upper panel shows classification of each cycle if exact information were known. Fecundability is computed as (foetal losses plus births)/(number of menstrual cycles), and foetal loss is the number of (foetal losses)/(foetal losses plus births). The bottom panel shows what happens when not all pregnancies can be detected. The average gestational age at which the assay can detect pregnancy is shown by the line "detection". Now, the earliest foetal losses and the non-conception cycles cannot be differentiated. The proper numerator for fecundability is not known, and the proper numerator and denominator for estimating the total probability of foetal loss are not known [14].

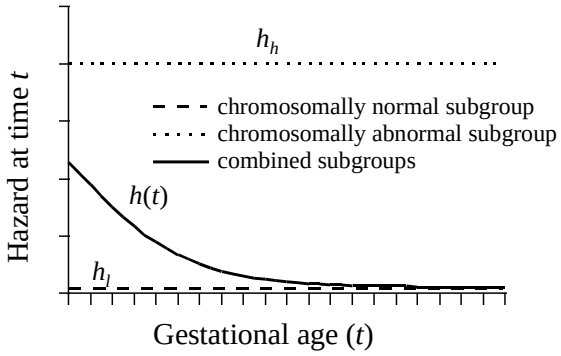

Figure 3. An example of the distribution of foetal loss across pregnancy under the Wood-Boklage model. The hazard (or risk) of loss is constant within each subgroup, but the combined hazard declines with age. A third parameter of the model is p, the proportion of abnormal conceptuses at fertilisation. Details of the model can be found in [1][9][13][14].

Mathematically, we parameterize the model by defining h_h as the hazard for the abnormal (high-risk) subgroup and h_l as the hazard for the normal (low-risk) subgroup. These hazards define a constant risk of foetal loss across gestation. Under these assumptions, the fraction of abnormal conceptuses surviving to gestational age t is $\exp(-h_h t)$. Likewise, the fraction of normal conceptuses surviving to t is $\exp(-h_l t)$.

At fertilisation, a certain fraction of conceptuses are chromosomally abnormalities. This fraction is denoted p_h. Because the abnormal conceptuses are lost at a greater rate than the normal conceptuses, the surviving proportion of high risk conceptuses will decline over the course of gestation. The proportion of abnormal conceptuses at gestational age t, denoted $p(t)$, is

$$p(t) = \frac{p_h \exp(-h_h t)}{p_h \exp(-h_h t) + (1 - p_h)\exp(-h_l t)}.$$

The hazard for the combined subgroups at some gestational age t is $h(t) = p(t)h_h + [1–p(t)]h_l$. The distribution of surviving conceptuses at gestational age t is derived directly from the $h(t)$ as

$$S(t) = 1 - \exp\left[-\int_0^t h(u)du\right] = p_h \exp(-h_h t) + (1 - p_h)\exp(-h_l t).$$

This survival distribution is analogous to the conceptus survival curve in Figure 2. Other variables such as maternal age can be modelled as affecting one or more of the three parameters of the basic model. Under this model, we are not required to know whether individual pregnancies are normal or abnormal; rather, parameters for the combined-subgroup distribution can be estimated directly from observations.

The Wood-Boklage model of foetal loss was used as the basis of a new model for estimating age-specific total fecundability. The model, which is based on the logic underlying Figure 2, uses pregnancy assay results on a series of menstrual cycles to estimate age-specific total foetal loss and age-specific total fecundability. The mathematical details of the model are given elsewhere [14]; only an overview of the model can be given here. The model incorporates the effects of both assay sensitivity and assay specificity, and incorporates statistically interval-censored and right-censored observations. Additionally, the model statistically controls for a non-susceptible fraction of women (i.e. those who are sterile, sexually inactive, or otherwise not at risk of getting pregnant), so that estimates of age-specific fecundability will be one for at least one age, and less than one for all other ages.

The sensitivity of the pregnancy assay refers to the gestational age-specific probability (from fertilisation on) that the hCG assay will detect a true pregnancy. The types of hCG assays used for foetal loss studies have sigmoid-shaped distributions of sensitivity that are zero at fertilisation, increases to about 0.5 by about 10 days into the pregnancy, and approach one by day 25 (see [2] for an example). Determination of the sensitivity of a pregnancy assay involves the same difficulties as measuring early pregnancy loss itself. For this reason, our model simultaneously estimates assay sensitivity along with parameters for foetal loss and fecundability.

The specificity of the pregnancy assay is defined as one minus the probability that the assay will falsely diagnose pregnancy in a non-pregnant woman. Pregnancy assays that are more sensitive tend to have a lower specificity. This is because the most sensitive assays are able to detect low levels of background hCG found in non-pregnant women[15] [16]. The specificity of a particular assay can be easily determined by quantifying the number of false pregnancies diagnosed in a pool of women who have undergone tubal ligations. For hCG-based pregnancy assays, specificity is likely to be independent of cycle day, so we treat specificity as a constant probability.

In our prospective study, we collected twice weekly urine samples that were then assayed for the presence of hCG [2]. The start of each "potential pregnancy cycle" was taken as the estimated day of ovulation. The ending day of the cycle was either the day of the next menses (which includes both non-conception cycles and undetected pregnancies that terminated early), the day on which a detected pregnancy ended, or the day of a livebirth. Within each type of cycle, one or more pregnancy tests were made. From this collection of observations, the statistical model was used to find parameter estimates of total fecundability (ρ), total foetal loss (p, h_h, and h_l), the gestational-age-specific sensitivity of the pregnancy assay, and maternal age effects on fecundability and foetal loss.

The underlying logic of the method is seen in Figure 4. The basic idea is that events are occurring from one observation (pregnancy assay) to the next. The events occur with probabilities that follow the branches of this tree, yet all we observe are positive or negative assay results at the end of the tree.

At ovulation (time t_0), some fraction of women will be pregnant with probability ρ_0. This is parameter will be the estimate of total fecundability. For the sample of women who are not pregnant (probability $1–\rho_0$), the left branch of the tree is traversed. A pregnancy assay given at the first observation after ovulation (time t_1) will give a true negative diagnosis with specificity q, and a false positive diagnosis with probability $(1\text{-}q)$. For the women who are pregnant, some fraction $1\text{-}P_1$ will experience a pregnancy loss in the interval $[t_0, t_1]$. The probability P_k arises directly from the Wood-Boklage model as $P_k = S(t_k)/S(t_{k-1})$, and incorporates parameters p, h_h, and h_l. Again specificity q will probabilistically change the result of the pregnancy assays. For women who do not experience pregnancy losses (with probability P_1), some fraction of their pregnancies will be detected according to the sensitivity, with probability D_1. Likewise, fraction $1\text{-}D_1$ pregnancies will not be detected; specificity q probabilistically changes the result.

As this tree shows, there are four ways of getting a positive assay result, and three ways of getting a negative result for the first pregnancy assay at time t_1. For all women who have positive results, we can compute the probability that they are truly pregnant at time t_1 as the sum of the two pregnant branches that yield positive results

divided by the sum of all four positive branches, which simplifies to $\rho_1 = \rho_0 P_1[1-q(1-D_1)]/[1-q(1-\rho_0 D_1 P_1)]$. The probability that these women are not pregnant given a positive assay is $1-\rho_1$. For women who have negative assay results, the probability that they are pregnant (but the assay could not detect it) is $\rho_1 = \rho_0 P_1(1-D_1)/(1-\rho_0 D_1 P_1)$.

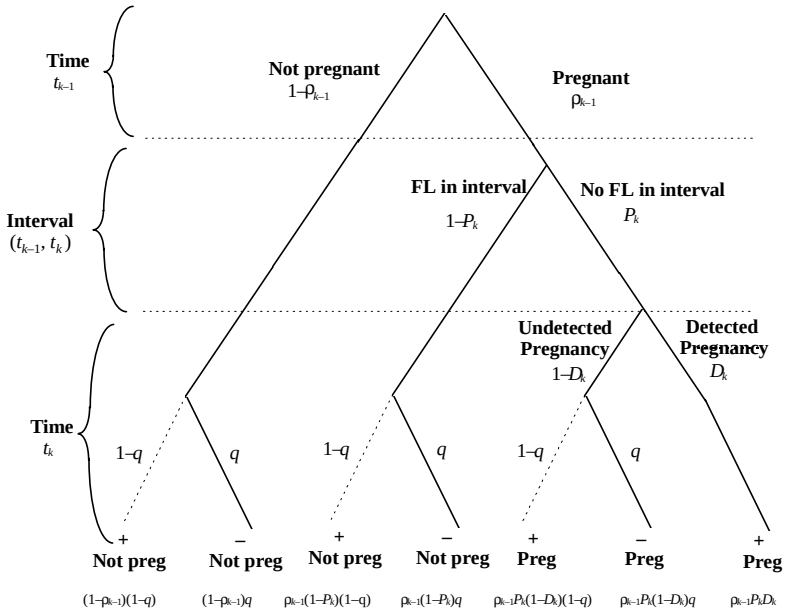

Figure 4. Probability tree showing the relationship among pregnancy assay results, characteristics of the assays, and probability of foetal losses across the interval (t_{k-1}, t_k). Symbols: ρ_k is the probability of pregnancy at time k; P_k is the probability of no foetal loss occurring in the interval; D_k is the sensitivity of the test at time k; q is the specificity of the test. At the terminal branches, + and – indicate whether the result of the pregnancy test is positive or negative; *Preg* and *Not preg* refer to whether the individual is actually pregnant or not. Below each terminus is an unconditional probability for arriving at that point.

Probability ρ_1 now weights the left and right branch of the tree between the first pregnancy assay and the second pregnancy assay. The tree is probabilistically traversed again and an assay result is used to compute ρ_2 given ρ_1. Likewise, we recursively traverse the tree for each assay, always computing the value of ρ_k from ρ_{k-1}.

The preceding description sounds as though values for p, h_h, h_l, ρ_0, D, and q are known when, in fact, the goal is to estimate these quantities from observations. Because the model was specified using only probabilities, maximum likelihood could be used to estimate the parameters of the model from observations. Parameter confidence intervals were found as the central 95 percent of bootstrapped parameter estimates. Details of the model, estimation methods, statistical validations, and complete results are described elsewhere [14].

Subjects

To estimate the fecundability and foetal loss model, we collected new data from a near-natural fertility population in rural Bangladesh [14] and used a highly sensitive and specific assay to detect pregnancies at early gestational ages [2]. The field study was conducted from February through December 1993 in the rural sub-district of Matlab thana, about 50 km southeast of Dhaka. At the start of the study, female field workers conducted one-time interviews of nearly all married women (N=3,290) ages 18 to 48, who permanently resided with their husbands in 28 villages in the region. The 17 villages with the lowest contraceptive prevalence were selected for the nine-month prospective study. The sample included married women of all reproductive statuses, including those who were pregnant or breastfeeding at the start of the study. In this way, we did not select for subfecundity by eliminating those of proven fecundity. Women who were using any form of contraception were excluded from the pool of potential participants. At the start of the study, 320 subjects were randomly drawn from the pool of eligible subjects. The subjects were interviewed twice each week and at the same time a urine sample was collected. The interviews included questions on menses, breastfeeding, contraception, and pregnancy. In the event a subject dropped out of the study or became ineligible (e.g. because of divorce), she was replaced by new subject randomly selected from the subject pool in the same village.

By the end of the field study, about 19,000 paired interview and urine specimens were collected. A total of 494 subjects had provided a month or more of urine samples. After each complete menstrual cycle for a subject, urine samples from the last one-third of the cycle were assayed for hCG [2].

Results

The final set of observations consisted of about 4,400 pregnancy assays in 1,561 menstrual cycles. Some portion of 329 pregnancies were followed. Of these, 81 pregnancies were ongoing at the end of the study (statistically right censored), 151 ended in live births, 84 biochemically detected pregnancies ended early in the pregnancy, 10 were later pregnancy losses, and 3 were induced abortions [14].

Effects of age were modelled on the risk of foetal loss in three ways: as affecting the initial fraction of abnormal conceptuses, as changing the risk of loss for abnormal conceptuses, and as changing the risk of loss for normal conceptuses. The only significant effect of age, as assessed by likelihood ratio tests, was to increase the fraction of abnormal conceptuses. In other words, maternal age did not significantly influence the risk with which pregnancies of either normal or abnormal conceptuses would terminate. Instead, the effect of age was to increase the probability that a conceptus was abnormal, corresponding to Bishop's prediction[12].

Maternal age significantly affected both fecundability and foetal loss simultaneously. These age effects are summarised in Figure 5**.** The probability of pregnancy loss steadily increases with maternal age, beginning at about 0.55 for a 20 year old and increasing to 0.96 by age 40. The age-related increase in risk of foetal loss closely parallels the increasing proportion of abnormal conceptuses by maternal age.

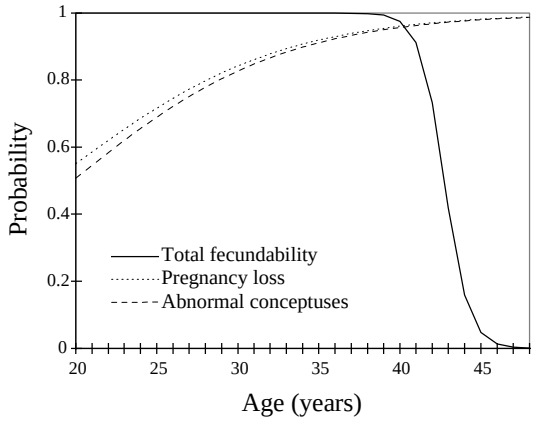

Figure 5. Maternal age-specific total fecundability and foetal loss in Bangladeshi women [14].

Total fecundability was remarkably constant across most of the reproductive lifespan. Fecundability was at its maximum of one at age 20 and declined very slowly until the late 30s. From the late 30s on, fecundability declined rapidly and reached zero by age 48 (Figure 5).

Discussion

The probability of pregnancy loss was surprisingly high; a twenty year old woman would be expected to lose about half of all her pregnancies. Most of the lost pregnancies would never be detectable, so that they would appear to be non-conception cycles. Boklage [13] previously used the same mathematical model along with results from an hCG-based studies of natural conceptions, IVF studies, and twin studies. He estimated an overall probability of pregnancy loss of 0.73. Our results confirm and refine those of Boklage by providing for maternal age-related effects. The best fitting model of pregnancy loss was one in which maternal age acted by increasing the fraction of conceptuses in the high-risk subgroup. This finding strengthens Bishop's theory of foetal loss and supports his idea that the primary mechanism acting over the reproductive life course is an age-related increase in the proportion of chromosomally abnormal conceptuses. It has long been recognised that many later pregnancy losses result from lethal chromosomal aberrations [17]. More recent investigations have begun to uncover the mechanisms by which maternal age increases the probability of nondisjunction during the resumption of meiosis in the oocyte [18][19][20].

The most surprising result from this work is the age pattern of fecundability, which was high and unaffected by age over much of the reproductive lifespan. This finding is consistent with the modelling results of Wood and Weinstein [10] in which the known biological components of fecundability predicted a total fecundability that is high and constant over much of the reproductive lifespan. We mathematically re-confounded the total fecundability and foetal loss results by generating expected age-related apparent fecundability curves for five different assay sensitivities from zero to 28 days (Figure 6). Clearly, *apparent* fecundability takes on a strong age-related decline even when pregnancy can be detected as early as day seven. From ages twenty to forty, the decline in fecundability is an artefact of foetal loss and incomplete sensitivity of the pregnancy assay.

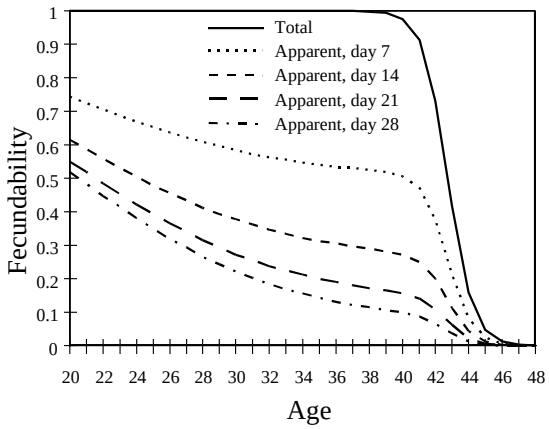

Figure 6. Total fecundability and early foetal loss were mathematically confounded using parameter estimates from the fecundability-foetal loss model. Age-specific apparent fecundabilities were computed given pregnancy detection on days 0, 7, 14, 21, and 28 [14].

Taken together, our results suggest that the observed age-related decline in apparent fecundability over most of the reproductive span is not caused by an age-related decline in total fecundability. Rather, it results from an age-related increase in early foetal loss that is masked by our inability to detect early pregnancies.

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