

Declining fecundity and ovarian ageing in natural fertility populations

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Abstract

Worldwide, human fertility declines with increasing maternal age, after contraceptive-use patterns and behavioral factors are taken into consideration. Here, we summarize some of our theoretical and empirical work examining the biological factors contributing to this age pattern of fertility. We undertook an 11 month prospective endocrinological study in a natural fertility (non-contracepting) population (rural Bangladesh) to estimate the contributions of fetal loss and fecundability (the probability of conception) to declining fecundity with age. Prospective interviews and urine samples for pregnancy tests were collected twice weekly from up to 700 women. These data were used to test mathematical models of the underlying biological processes contributing to changing fecundability and fetal loss risk with maternal age. The results indicate that much of the decline in fecundity can be attributed to an increasing risk of fetal loss with maternal age. Much of this fetal loss is due to chromosomal abnormalities—a result of ageing oocytes. Fecundability, on the other hand, does not begin to decline until the early 40s. We hypothesize that this is also a result of ageing at the ovarian level, namely follicular atresia, in the years just prior to menopause. The irregularity of menstrual cycles—longer cycles and increasingly variable hormonal patterns—at these ages may be a direct result of the small and rapidly dwindling remaining pool of follicles. We present a simple mathematical model of this process, and some preliminary laboratory results that support the model. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

The reproductive years leading up to menopause are marked by two salient age-related

changes. The first is a steady decline in fertility, and the second is increased variability of menstrual cycle lengths. In this paper we summarize some of our research on age-related variation in human fecundity (the biological capacity to reproduce), and present evidence suggesting that it is primarily the result of ageing effects within the

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ovaries rather than the result of ageing of the uterus, hypothalamus, or pituitary.

Worldwide, fertility is observed to decline with maternal age [1,2]. Fig. 1 shows age-specific fertility rates observed among so-called natural fertility populations (left panel) and among populations that effectively use contraception (right panel). In contracepting populations the decline in fertility with age is shaped largely by contraceptive use patterns, which ultimately reflect an underlying distribution of desired family sizes [3]. In natural fertility populations, where effective birth control is absent, the pattern presumably reflects natural physiological changes in reproductive capacity. When we control for the overall levels of fertility by adjusting each population to the same rate at age 20 (Fig. 1, bottom left panel) the age-related decline is almost identical among populations, suggesting that there is a universal pattern of female reproductive ageing.

Control of fertility obscures this universal pattern (Fig. 1, bottom right panel), so that an undistorted view of reproductive ageing requires investigation among natural fertility populations. Additionally, by studying natural fertility populations, we avoid a serious selectivity bias: couples in contracepting populations who are still attempting to reproduce at later ages are more likely to be subfecund. Some fraction of couples at all ages are subfecund, but subfecund couples make up a larger fraction of the pool of those trying to reproduce at later ages.

Our approach to studying female reproductive ageing has been to decompose fertility into a series of proximate determinants (Table 1) [3], and examine how each determinant changes with age in natural fertility populations. These biological and behavioral factors must always be operating at some level for reproduction to occur. Any demographically measurable variation in fertility across the lifespan, or across populations, will operate through one or more of these determinants. One advantage of the proximate determinants framework is that we can build probability models that reflect how each determinant ought to act on reproductive processes. The models are based, whenever possible, on known underlying physiological and behavioral mechanisms. We can

then use data derived from field work and laboratory assays to estimate model parameters and test specific hypotheses derived from the models.

In this paper we examine a number of biological susceptibility factors that may account for the age-related decline in fertility. These include the probability of fetal loss, duration of the fecund waiting time to conception, and the length of ovarian cycles.

2. Fecundability and fetal loss in natural fertility populations

The fecund waiting time to conception is closely related to fecundability, which is defined as the monthly or cycle-wise probability of conception in couples exposed to risk of pregnancy. Fecundability is a fundamental measure of reproduction, but

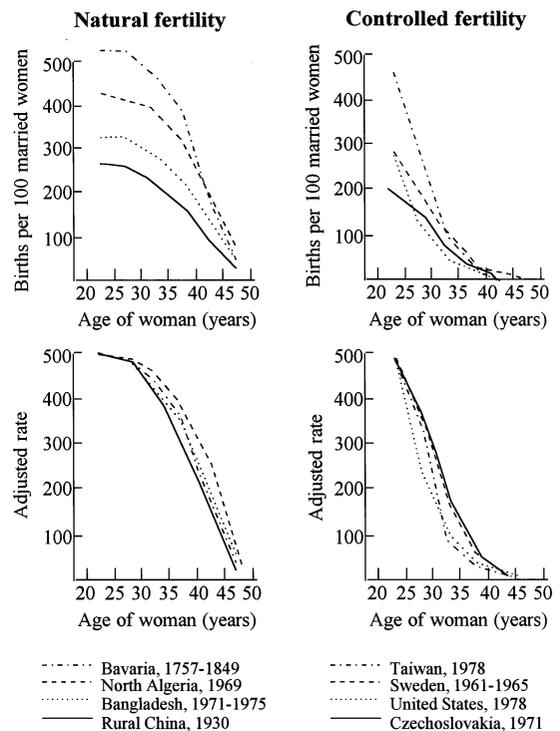


Fig. 1. Age-specific marital fertility rates for natural and controlled fertility populations. Adjusted rate is raw rate at each age divided by the rate for the 20–24 age group. Redrawn from Wood [1].

Table 1
The proximate determinants of natural fertility

I. Exposure factors
Age at marriage or entry into sexual union
Age at menarche
Age at menopause
Age at onset of pathological sterility
II. Susceptibility factors
Duration of lactational infecundability
Duration of the fecund waiting time to conception (determined by the following fecundability factors):
Frequency of insemination
Length of ovarian cycles
Proportion of cycles ovulatory
Duration of the fertile period, given ovulation
Probability of conception from a single insemination in the fertile period
Probability of fetal loss
Length of nonsusceptible period associated with each fetal loss
Length of gestation ending in live birth

Source: Wood [1].

has proven very difficult to measure. The problem has been that pregnancies are almost impossible to measure non-invasively until well after conception. The most sensitive pregnancy assays based on measurement of human chorionic gonadotropin (hCG) cannot detect a rise in hCG until about 6 or 7 days after conception [4,5]. Furthermore, risk of fetal loss appears to be highest early in pregnancy [6–8], so that our ability to measure fecundability is obscured by early fetal loss (Fig. 2). For this reason, most studies of fecundability report apparent fecundability, defined as the monthly or cycle-wise probability of conception given that the pregnancy survives long enough to produce a detectable pregnancy. Clearly, estimates of apparent fecundability are sensitive to whatever technology is used to detect pregnancies, making them difficult to compare among studies. For example, a study using self-reports of pregnancy will produce much lower estimates of apparent fecundability than will a prospective endocrine study such as that of Wilcox et al. [8]. The different estimates of apparent fecundability between the two studies is the fraction of fetal losses that occur before pregnancy is detected. If we could detect all pregnancies at fertilization, we could compute total fecundabil-

ity. So far, direct measurement of total fecundability has not been possible.

The age pattern of apparent fecundability compiled from several natural fertility populations (Fig. 3) shows a steady decline from the early 20s until the end of the reproductive span. A fundamental question is whether this decline represents a true drop in total fecundability with age, an increase in the probability of early fetal loss, or some combination of both. Fig. 2 suggests one way to address this issue. If we had a mathematical model of the risk of fetal loss across all of gestation, and if that model largely reflects the underlying biology, we could estimate parameters of the model from data on detected pregnancies, and project the risk back to conception. Additionally, this would provide the information needed to estimate total fecundability. This is the approach we adopted for a prospective study of fetal loss and total fecundability in a natural fertility population in rural Bangladesh.

2.1. A model of fecundability and fetal loss

In 1964 Bishop outlined the first modern form for an etiologic theory of fetal loss [9]. He proposed that, (1) the majority of fetal losses resulted from chromosomal abnormalities, (2) chromosomal abnormalities would increase with age of parents, and (3) many unobserved losses would occur early in gestation. Many of the basic elements of Bishop's theory were expressed mathematically by Wood [1,3] and independently by Boklage [10]. The premise of the model is that at the time of conception, a conceptus is either chromosomally abnormal or chromosomally normal. The risk of fetal loss in the chromosomally abnormal subgroup is modeled as high, and constant across gestation. Likewise, risk of loss in the normal subgroup is modeled as low, and constant across gestation. Even though the risk of loss in each subgroup is constant, the two subgroups combined show a declining risk of fetal loss with increasing gestational age (Fig. 4). The decline in risk occurs because, on average, abnormal conceptuses are lost earlier in gestation, leaving an increasingly larger fraction of normal conceptuses. Extensions to the model allow for age and

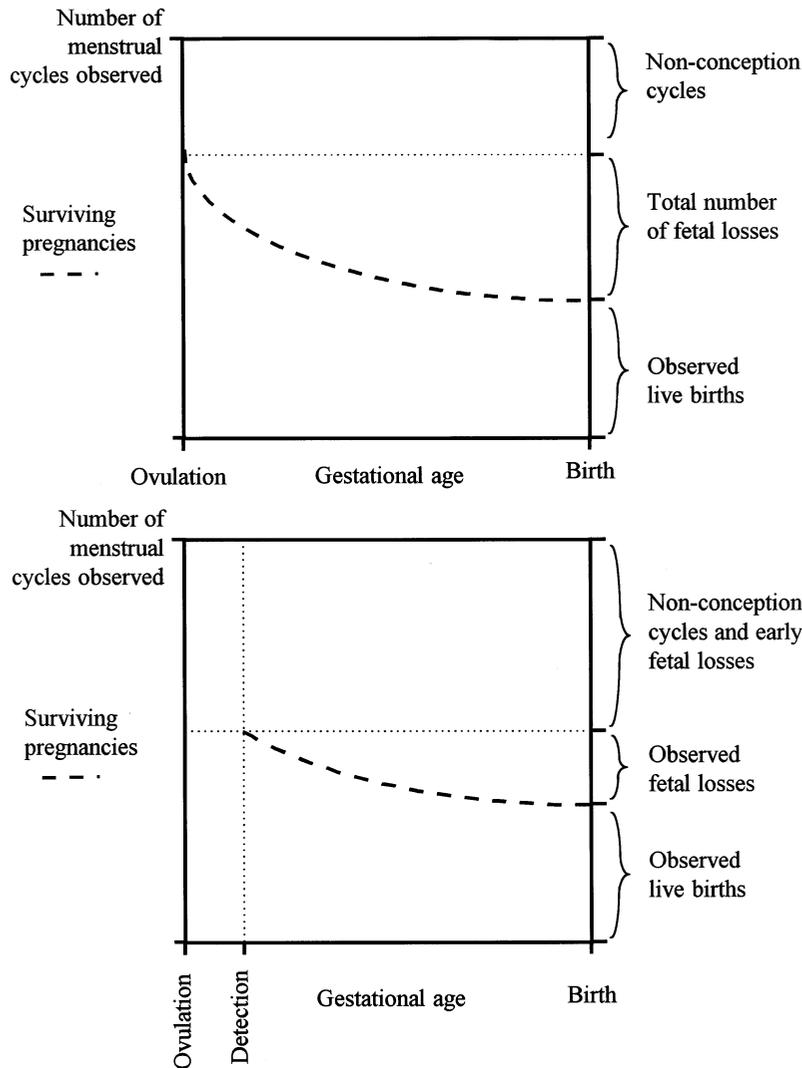


Fig. 2. The probability of fetal loss and fecundability are confounded by incomplete sensitivity of pregnancy assays. The y axis represents the number of menstrual cycles under study, and the x axis is time from ovulation to birth. The upper panel shows classification of each cycle if exact information were known. Fecundability is computed as (fetal losses plus births)/(number of menstrual cycles), and fetal loss as (number of fetal losses)/(fetal losses plus births). The bottom panel shows what happens when all pregnancies cannot be detected. The mean gestational age at which the assay can detect pregnancy is shown by the line 'detection'. Note that the earliest fetal 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 fetal loss are not known. Source: Holman [7].

other variables to act on parameters of the model (Fig. 4).

Finally, we used the Wood–Boklage model of fetal loss as the basis of a new model designed to estimate total fecundability [7]. The model, based on the logic underlying Fig. 2, uses pregnancy

assay results on a series of menstrual cycles to estimate age-specific total fetal loss and age-specific total fecundability. The model incorporates the effects of both assay sensitivity and assay specificity, and incorporates statistically interval-censored and right-censored observations.



Fig. 3. Composite age pattern of fecundability compiled from birth interval data from several natural fertility populations. Redrawn from Wood [1].

2.2. Fecundability and fetal loss in Bangladesh

To estimate fecundability and fetal loss with the model, we collected new data in a near-natural fertility population in rural Bangladesh [7] and used a highly sensitive and specific hCG assay to detect pregnancies at early gestational ages [4]. The sample was drawn from the general rural population and included married women of all reproductive statuses, including those who were

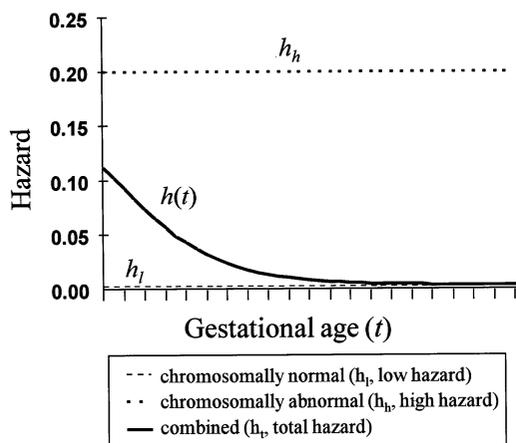


Fig. 4. Distribution of fetal loss across gestation under the Wood–Boklage model. The hazard (or risk) of loss is constant within each subgroup, but the combined hazard declines with age. A third parameter of the model is p , the proportion of abnormal conceptuses at fertilization. Details of the model can be found in Wood [3] and Holman [7].

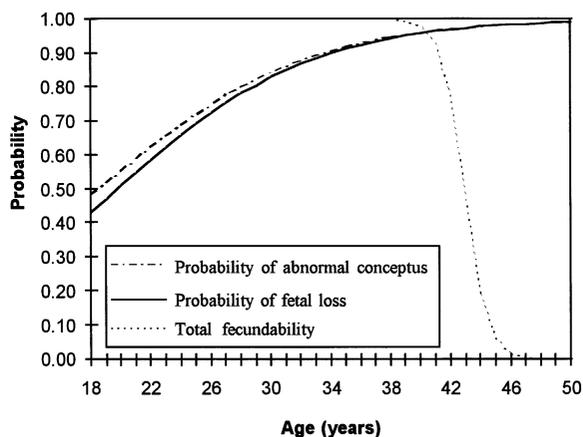


Fig. 5. Maternal age-specific total fecundability and fetal loss in Bangladeshi women. Source: Holman [7].

pregnant or breastfeeding at the start of the study, so that we did not select for subfecundity by eliminating those of proven fecundity. Twice weekly interviews and urine specimens were collected over the 9-month prospective study. By the end of the field study, 19000 paired interview and urine specimens were collected from 708 women. A total of 494 subjects were in the study for at least 1 month and up to 9 months. After each complete menstrual cycle for a subject, urines from the last 1/3 of the cycle were assayed for chorionic gonadotrophin [4]. A total of 329 pregnancies were followed for some portion of gestation. 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, ten were later pregnancy losses, and three were induced abortions [7].

Parameters for the fetal-loss–fecundability model were estimated from a sample of 1561 menstrual cycles. Effects of age were modeled on the risk of fetal loss in three ways: affecting the initial fraction of abnormal conceptuses, changing the risk of loss for abnormal conceptuses, and changing the risk of loss for normal conceptuses.

The results for age-specific probability of fetal loss and fecundability are shown in Fig. 5. Holman [7] found that the only significant effect of age was to increase the fraction of abnormal

conceptuses. The probability of fetal loss showed a steady increase from about 45% at age 18 to 92% by age 38. In contrast, total fecundability was high and nearly constant until age 40, when it dropped rapidly to nearly zero by age 46.

These results support the idea underlying the model that the mechanism of fetal loss appears to be an age-related increase in the fraction of chromosomally abnormal conceptuses. Furthermore, the results suggest that the observed decline in apparent fecundability from the early 20s to the early 40s is not caused by a decline in total fecundability; rather, it is a result of an increasing risk of fetal loss with maternal age.

Finally, the observed rapid decline in total fecundability in the early forties was unexpected. This decline begins well before the mean age at menopause in Bangladeshi women (D. Holman, unpublished data). This result led us to examine some of the other proximate determinates that contribute to the fecund waiting time to conception.

3. Modeling the biology of the peri-menopause and menopause

The decline in total fecundability observed in Bangladesh coincides with the years preceding menopause, when some qualities of menstrual cycles begin to change. Specifically, cycles are more likely to be anovulatory [11] and there is a substantial increase in the variability of menstrual cycle lengths (Fig. 6) [12]. At the same time, the pool of ovarian follicles is dwindling [13,14]. We hypothesize that one major component of increased cycle-length variability during the peri-menopause, specifically the increased proportion of long menstrual cycles, is a direct result of the process of follicular depletion, and this may be an important factor contributing to declining fecundability during the peri-menopausal years.

3.1. Follicular depletion and the peri-menopause

Menopause in human and non-human primates is the ultimate exhaustion of the follicular reserve. The human peri-menopausal transition, which

may last up to 5 or 6 years, begins when approximately 1000 follicles are left in each ovary [14,15]. We suggest that the highly variable bleeding patterns and the hormonal patterns characteristic of the peri-menopause are caused by low numbers of follicles remaining in the ovaries.

Compared to hormonal patterns in cycling women, menopausal women show almost no production of ovarian steroids. Likewise, levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) are high and variable as a result of loss of negative feedback from ovarian steroids. The peri-menopausal transition seems to contain elements of both cycling and menopausal hormone patterns. Fig. 7 shows two urinary hormone profiles from individuals undergoing the peri-menopausal transition. Beginning at about cycle day 3 of the third menstrual cycle in the upper figures (from our laboratory), there is period of about 10 days with no evidence of ovarian steroidogenesis, but high levels of gonadotropins, especially FSH. The same pattern can be seen in the boxed section in the bottom pair of graphs from Santoro et al. [16] (see also [17,18] for additional examples of this pattern). The boxed-in portions of these graphs look much like a menopausal hormone profile; however, neither of the women profiled here could be considered menopausal. In both cases the women experienced

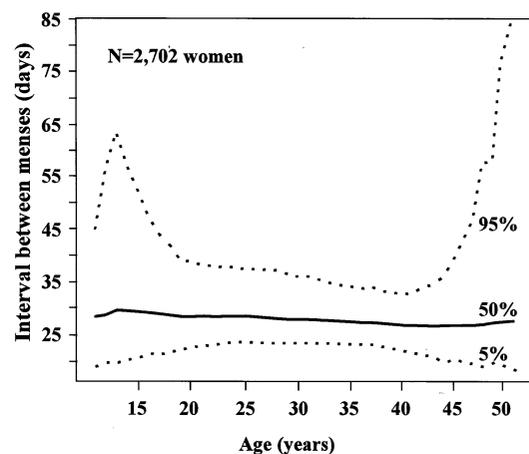


Fig. 6. Percentiles of menstrual cycle length by age, from Treloar's prospective study of menstrual cycles across the reproductive span. Source: Redrawn from Treloar et al. [12].

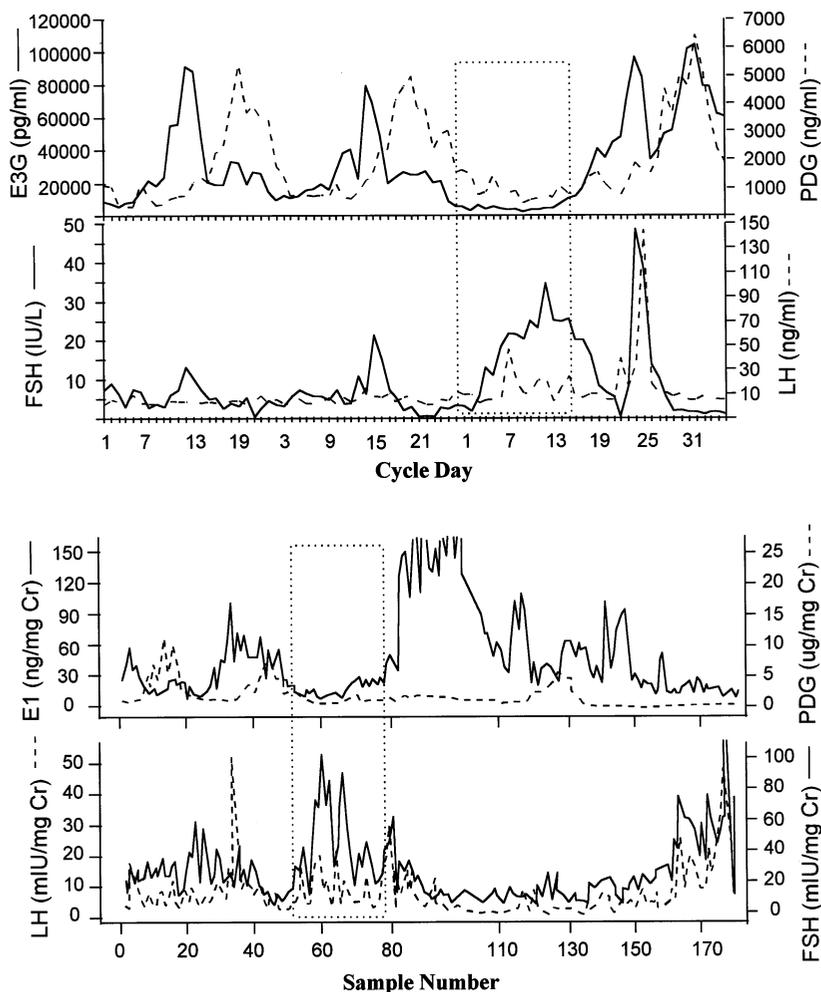


Fig. 7. Urinary steroid and gonadotropin profiles in perimenopausal women from our laboratory (top panel) and from the literature (bottom panel; Source: Redrawn from Santoro et al. [16]). E1 and E3G, oestrone-3 α -glucuronide. PDG, pregnanediol-3 α -glucuronide. LH, luteinizing hormone. FSH, follicle-stimulating hormone. Hormone values for the top panel are corrected by specific gravity.

a brief period of ‘false menopause’ or ovarian quiescence followed by ovulation, albeit after a period of hyperestrogenism in the lower case. The period of ovarian quiescence at the beginning of both cycles is responsible for increasing the total length of the cycles.

3.2. The inactive phase of the menstrual cycle

These periods of ovarian quiescence with loss of feedback control on LH and FSH can be thought

of as ‘inactive phases’ of the menstrual cycle. We suggest that these phases are direct reflections of the very small pool of remaining follicles in the peri-menopause. When the pool of primordial follicles is large, as at young reproductive ages, there will nearly always be a sizeable cohort of follicles initiating development at the start of any given menstrual cycle, even if the individual per-follicle probability of doing so is small. Oestradiol produced by a cohort of developing follicles keeps LH and FSH at relatively low levels. At older

ages, however, when the follicle reserve is low, there will, by chance, be periods when no follicles have initiated growth, and hence no steroidogenesis.

If this hypothesis is correct, the distribution of inactive phases by age should be predictable from the underlying rate at which follicles initiate growth, which is about the same rate as follicular depletion. The mean length of inactive phases should increase with age as the follicular reserve approaches exhaustion, until menopause, which can be viewed as a permanent inactive phase.

We have developed a statistical model in which menstrual cycle length is the sum of three phases: the inactive phase, the follicular phase, and the luteal phase. The inactive phase is the period of time in which no follicles are growing or matured to the point of producing oestradiol. At younger ages, this phase is effectively of length zero. The follicular phase is the time from when a cohort of follicles first excretes oestradiol until ovulation. The luteal phase is the waiting time from ovulation until menses. With probability models of these three phases, we can estimate age-specific parameters of the time spent in each phase based on observations of hormone profiles over menstrual cycles.

We use a simplified version of the probability model to illustrate the stochastic behavior of the model, and what the model predicts about cycle length variability. Assume that women begin with n_0 follicles at birth, that follicles initiate growth at any given time with a constant hazard λ per follicle (roughly corresponding to the rate of follicular atresia seen in [14]), all follicles eventually initiate growth, and all but a small fraction of follicles undergo atresia. We also assume that λ does not vary across follicles, and that there is no variation in n_0 among women (most of these assumptions are relaxed in more complicated versions of the model). The number of follicles remaining in the ovaries at age a is then $n_a = n_0 \exp(-\lambda a)$. This gives rise to the distribution of times for a woman age a to be in the inactive phase as a function of the number of surviving follicles and the probability that no follicle begins growing: $\Pr(T > t | \lambda, n_0) = \exp(-t\lambda n_0 e^{-\lambda a})$. The mean time in the inactive phase at

age a is $E(T|a) = (\lambda n_0 e^{-\lambda a})^{-1}$ and the variance is $V(T|a) = (\lambda n_0 e^{-\lambda a})^{-2}$.

Fig. 8 illustrates hypothetical examples of the time spent in the inactive phase by age. The top panel in the figure shows the probability of remaining in the inactive state for a given number of days at five different ages. Clearly, a 30 year old woman will almost always have extremely short inactive phases. By age 40, the median length is still quite short but has some probability of being up to 12 days long. By 50 years of age, many cycles should have long inactive phases. The bottom panel shows the age-specific mean lengths spent in the inactive phase and the upper 95% confidence interval for mean length. Finally, the model predicts that we should expect to see, albeit rarely, inactive phases in younger women.

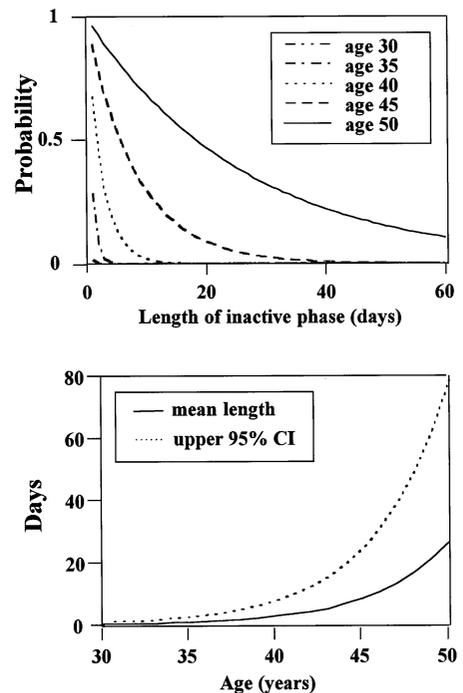


Fig. 8. Hypothetical example of times spent in inactive phase, under the simplified model, assuming an initial follicle pool (at birth) of one million follicles and a daily hazard of a follicle entering the growing state of $\lambda = 0.00065$ (based on data from Block [13] and Gougeon et al. [14]). The top panel shows the probability of no follicles initiating growth at five ages. The bottom panel shows the mean and variance in inactive phase length by age of a woman.

The few cases of inactive phases that we have found from our laboratory studies or from the literature are broadly consistent with our theoretical model. To critically test the theory and predictions we are conducting a five year prospective study, collecting daily urine samples and menstrual calendars, from a large cohort of US women. If the model holds with these data, then the process of follicular depletion by itself would account for much of the age-pattern of variability in menstrual cycle length, as well as the higher LH and especially FSH levels observed in perimenopausal women [19,20,16,21]. Finally, these age-related disruptions of the reproductive axis suggest that the process of follicular depletion is an important mechanism contributing to declining fecundability and fertility at the older female reproductive years.

4. Conclusions: declining fecundity and ovarian ageing

We consider both the increased risk of fetal loss, and increasing variability in menstrual cycle length and hormone patterns, to be direct results of ageing occurring at the level of the oocyte. Though both are a result of ageing at the oocyte level, the ageing mechanisms are different.

Most of declining fecundity with age is a function of an increase in early fetal loss. The bulk of early fetal loss appears to be a result of meiotic nondisjunction—probably due to spindle malfunction—which results in lethal chromosomal aberrations in aged oocytes [22–24]. The causes of spindle malfunction and non-disjunction are not well known, but may be related to a common ageing mechanism—accumulating mutations with age, particularly in DNA repair/maintenance, and cell cycle regulatory genes [25]. Oocytes may be subject to these mechanisms; however, ovarian follicles may be more susceptible than most cells as they have the unique distinction amongst human cells of being held for very long periods of time (up to 40 years or more) in the diplotene stage of meiosis I prophase [25].

Changes in menstrual cycle features are also a result of ovarian ageing; in particular, they may

be a direct reflection of the small and rapidly dwindling pool of follicles in the ovaries at older reproductive ages. Apoptosis—genetically programmed cell death—is the mechanism by which follicular atresia occurs [26,27], and is thus responsible for the loss of the vast majority of the finite pool of follicles. Here again, human oocytes are unusual cells—they are one of the few cell types which are steadily and purposefully depleted across the lifespan. The model given above assumed a constant rate of atresia across the lifespan and across women, but more complicated versions of the model incorporate the effects of covariates on the rate of atresia and the timing of menopause, including genetic and lifestyle factors (e.g. cigarette smoking, parity, age at menarche).

In summary, we believe that declining fecundity across the human female lifespan is primarily a result of ageing effects at the level of the ovaries. Increasing risk of fetal loss, probably caused by ageing oocytes, appears to be the main cause of declining fecundity throughout the female reproductive years. In the peri-menopausal years declining fecundity is a function of both an increasing risk of fetal loss as well as declining fecundability, with the latter also attributable to ovarian ageing, in particular, the approaching end of the stock of primordial follicles.

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