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A model conforming the decline in follicle numbers to the age of menopause in women

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The store of primordial follicles in the ovary is fixed before birth and dwindles with age until it is unable to provide enough Graafian stages to sustain menstrual cyclicity. According to a simple bi-exponential model of ageing, the rate of follicle disappearance increases at age 37.5 years (or when 25 000 follicles remain) so that the numbers fall to approximately 1000 at 51 years, the median age of menopause in the population. This study attempts to produce a biologically more realistic model of follicle disappearance and harmonizes follicle dynamics with the distribution of menopausal ages from an American survey. The step-change in the rate of follicle attrition was replaced by a model which assumed that this rate changes more gradually with the size of the follicle store. This produced a distribution of predicted menopausal ages (based on an assumed threshold of 1000 follicles) which was closer to observed data. The fit further improved when the model was modified by having a threshold that varied across the population. Using such a stochastic threshold model for menopause, the number of fertile years remaining could be forecast with an acceptable margin of uncertainty if it ever becomes possible to estimate the size of the follicle store *in vivo*.

Key words: age/follicle/menopause/ovary

Introduction

Extinction of the oogonial stem cell population before birth and, hence, fixing the size of the primordial follicle store in the ovary, sets an upper limit on the reproductive lifespan. The rate of attrition of the store by follicle growth and atresia normally causes menstrual cycles to cease permanently at 45–54 years in women in well-nourished societies (Gosden, 1987). An onset of sterility in mid-life in humans is premature by the standards of wild animals and most domesticated species. The explanation is evidently not an under-endowed ovary because the follicle store at puberty is larger than in most species that have been studied and is commensurate with body mass (Gosden and Telfer, 1987). Rather it seems to be due to prodigal wastage of follicles and exceptional longevity. After approximately 37.5 years of age, the rate of follicle loss increases more than two-fold, causing the store to become prematurely exhausted (Faddy *et al.*, 1992). A faster rate of follicle growth initiation resulting from superstimulation with follicle stimulating hormone (FSH) during the premenopausal decade (Gougeon *et al.*, 1994) and/or excess atresia at primordial stages presumably underlie this wastage (Faddy and Gosden, 1995).

Menopause is triggered before the follicle store becomes completely barren and perimenopausal ovaries still contain several hundred small follicles, though far fewer than age-matched individuals with regular cycles (Richardson *et al.*, 1987). Extrapolating the bi-exponential decline in follicle numbers indicated that a threshold of approximately 1000 follicles remains at 51 years of age when 50% of women have reached the menopause (Faddy *et al.*, 1992). Why this residue is unable to develop to maturity is still a mystery. Our first model assumed that aggravated follicle loss occurs when a critical number of about 25 000 follicles is reached, and that a low threshold number triggers menopause. Since it is difficult, if not impossible, to verify these hypotheses *in vivo*, we have attempted to shed light on the dynamics by producing a more sophisticated model.

Materials and methods

The follicle data used in this study were obtained from several sources which were described in an earlier investigation of the age distribution (Faddy *et al.*, 1992). The total numbers per ovary were counted in sections of 110 pairs of whole organs obtained by oophorectomy from patients ranging from 0 to 51 years of age. The stage of the menstrual cycle was not recorded and, since most follicles at all ages are at either the primordial or preantral stage (which do not vary cyclically), this variable could be safely ignored. The distribution of menopausal age was obtained from Treloar (1981) in a classic longitudinal study of menstrual cycles in 393 healthy American women.

Results

These data were used to produce successively more realistic models of the relationship between the age-dependent disappearance of follicles and menopause. Analysis of the residuals of the original bi-exponential model (Faddy *et al.*, 1992) suggested that variation in the logarithmically transformed numbers of follicles is approximately normal. Taking 1000 follicles as the threshold number at menopause, the resulting distribution of menopausal age is also normal with a mean of 51.1 years and a standard deviation of 3.7 years; this distribution may be calculated from the relationship:

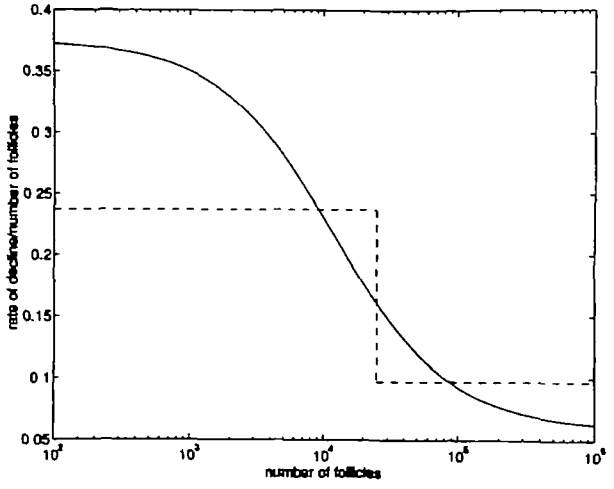

Figure 1 A comparison between two models accounting for the accelerated rate of disappearance of follicles in older ovaries: 'bi-exponential' model showing a step change in the rate when 25 000 follicles remain (– – –), and after fitting a model with a more gradual change (———). Note that the *x*-axis representing numbers of follicles runs in the opposite direction to age.

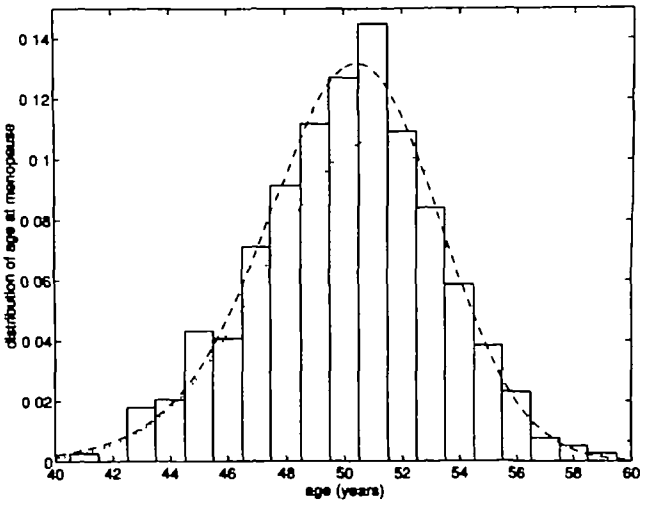

Figure 2. Distribution of ages at menopause in American women comparing observed data (bars) (Treloar, 1981) with fitted distributions: ·· ·· from 'bi-exponential' model, and – – – from the model with a more gradual change.

Probability of number of follicles at age t ≥ threshold = Probability of age at menopause ≥ t

However, there was poor concordance between this result and the independent data of Treloar (1981) for menopausal age, with the distribution failing to account for the quite noticeable negative skew of the data: Figure 2, dotted curve, χ^2 goodness of fit = 44.67 on 14 degrees of freedom.

The bi-exponential model of Faddy *et al.* (1992) may be described in terms of the rate of decline in follicle numbers as a changing proportion of the numbers of follicles (N) remaining, where the proportionality constants increase with age as the numbers fall, according to:

$$\text{Rate of decline/number of follicles} = \begin{cases} 0.097, \text{ if } N \geq 25\,000 \\ 0.237, \text{ if } N < 25\,000 \end{cases}$$

It is biologically more realistic to assume that this increase is more gradual, rather than abrupt (as above). A gradual change can be represented by the model:

Rate of decline/number of follicles = $a + b/(c + N)$,

with the mean number of follicles then declining according to the differential equation:

$dN(t)/dt = -N(a + b/(c + N))$

Here N represents the number of follicles present and a, b and c are parameters which were estimated by least squares from the data on declining follicle numbers, resulting in the values a: 0.0595, b: 3716, c: 11 780, and 701 200 for the initial number of follicles. This is illustrated in Figure 1, where the increase in the fitted rate of follicle decline is more gradual as the number of follicles decreases. The fit of this model to the data is comparable to the bi-exponential model [cf. Gougeon *et al.* (1994)], with a residual sum of squares of 91.83 on 106 degrees of freedom. However, the resulting distribution of menopausal age, based on a threshold level of 1000 follicles, is now much closer to Treloar's (1981) data: χ^2 goodness of fit = 22.02 on 12 degrees of freedom The associated P-value for this statistic is 0.04, which indicates that there are still some discrepancies between the observations and the fitted model.

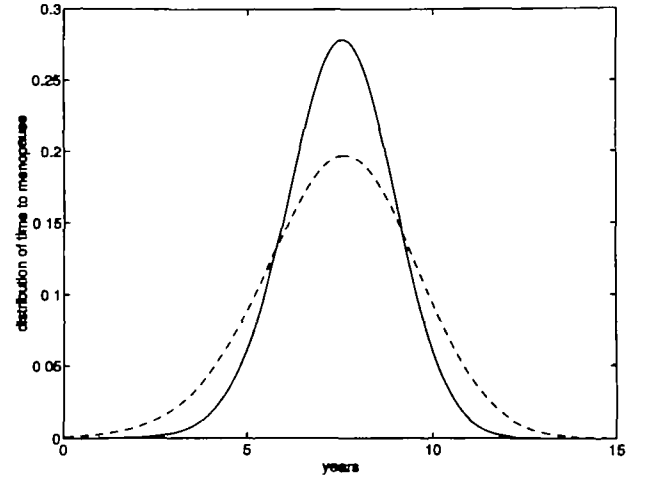

Figure 3. Years to menopause predicted from a stochastic threshold model, when 10 000 follicles remain. The probability distributions represented are when this number of follicles is known exactly (———) and when estimated with a 50% standard error (– – –)

These discrepancies are due to the fitted distribution having insufficient dispersion. More variation is achieved by a stochastic threshold level. This too is more biologically realistic than a fixed level of 1000, and is in accord with expected variation in the population. A normally distributed log-threshold, with an estimated mean of 6.99 and standard deviation of 0.50, produces a more acceptable distribution of menopausal age compared with Treloar's data (Figure 2: dashed curve, χ^2 goodness of fit = 4.46 on 12 degrees of freedom). The fitted distribution follows more faithfully the skewness observed in the data, where the original bi-exponential model failed. According to this model, the median threshold for triggering menopause should be revised to 1100 follicles.

This model carries implications for attempts to forecast age of menopause from knowledge of the number of follicles present at a given age Figure 3 shows the estimated distribution of years to menopause for women with 10 000 follicles remaining, calculated from the fitted model but starting with

this number of follicles. The solid curve describes the distribution if this number of follicles were known exactly, while the dashed one gives the distribution if the numbers were estimated with a 50% standard error. The shapes of these distributions are unchanged if the number of follicles remaining is larger, but their modes increase approximately according to $0.077\times\sqrt{}$(number of follicles remaining), while the number of follicles remaining $\leq$ 100 000.

Discussion

The early onset of senescence is one of the most arresting characteristics of the human ovary. There can be little doubt that primary ovarian ageing is responsible since, after the menopause, secretion of oestrogen and inhibin fall, plasma levels of FSH and luteinizing hormone (LH) rise and exogenous gonadotrophins can no longer elicit follicle maturation or ovulation (Gow *et al.*, 1994). Histological examination too leaves little doubt that only a tiny residue of the original follicle store remains in perimenopausal women, and this too disappears shortly after the menopause (Richardson *et al.*, 1987). Ovarian failure probably makes a contribution to the cessation of cycles in animals too (Gosden *et al.*, 1983), but the evidence is more clear cut in humans than in any other species. The cell biology underlying the timing mechanism is unknown, but at least the dynamics of follicle utilization are becoming clearer.

At one time it was assumed that follicle numbers in the human ovary decline as a simple exponential function of age, as in animals. Recently, however, the rate of attrition was claimed to be greater after 37.5 years of age until the menopause, which is advanced by some 20 years in consequence (Faddy *et al.*, 1992). Step changes of physiological activity are rare and, though the menarche and menopause are notable exceptions, it would be surprising if an abrupt change in follicle dynamics takes place when approximately 25 000 follicles still remain. While this question is relatively intractable to the empirical approach, mathematical models provide opportunities to investigate whether a gradual change to a higher rate of follicle utilization can provide an improved fit to the data or harmonize better with the age distribution for menopausal data. Our revised model demonstrates good concordance with menopause.

We made the assumption that follicles decline at a rate expressed as a simple function of the numbers remaining. This hypothesis is physiologically plausible if, as we suspect, paracrine factors play a crucial role in regulating the ovarian economy. The gradual transition from a low to high rate of follicle disappearance in the new model broadly conforms to the distribution of menopausal age in American women (Treloar, 1981). However, it failed to match the dispersion when the threshold number of follicles remained fixed at 1000. Since members of a follicle cohort probably vary in their threshold response to gonadotrophins (Brown, 1978), it would not be surprising if there were also variation at different ages, with those remaining after the menopause no longer fully responsive (Rannevik *et al.*, 1986). In that event it would seem unlikely that a fixed threshold number triggered the menopause and we should expect to find some random variation. A stochastic threshold generated a degree of variation that gave better correspondence to observed menopausal ages. The model produced a negatively skewed distribution matching that of menopausal ages, which is the more persuasive for being predicted by results obtained independently of Treloar (1981).

These results not only harmonize follicle numbers and menopausal ages, but help to lay a theoretical framework for predicting the age of menopause from follicle numbers. Currently, there is no reliable non-invasive test for monitoring the total numbers of follicles in the ovary, nor has any evidence been produced yet to show whether ovarian biopsies can be used to estimate the size of the reserve (Gosden and Faddy, 1995). However, if a technology were developed, menopause might be predicted with a fair degree of precision from estimates of the number of follicles present. Our model predicts that when 10 000 follicles remain the great majority of women will reach the menopause within the next 5–10 year period. Those with 100 000 follicles will reach the same stage in 21.5–26.5 years. The age spread is, of course, broader when follicle counts are imprecise, but even if the estimates carried an error of $\pm$ 50% the difference is not that great. In other words, even a crude estimate of the follicle reserve might provide a useful forecast of the number of menstrual years remaining. A woman could then plan her pregnancies with fewer fears of an unexpectedly early menopause.

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