Title: An age-dependent ovulatory strategy explains the evolution of dizygotic twinning in humans

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Abstract

Dizygotic twinning, the simultaneous birth of siblings when multiple ova are released, is an evolutionary paradox. Twin bearing mothers often have elevated fitness; but despite twinning being heritable, twin births only occur at low frequencies in human populations. We resolve this paradox by showing that twinning and non-twinning are not competing strategies, instead dizygotic twinning is the outcome of an adaptive conditional ovulatory strategy of switching from single to double ovulation with increasing age. This conditional strategy when coupled with the well-known decline in fertility as women age, maximizes reproductive success and explains the increase and subsequent decrease in twinning rate with maternal age that is observed across human populations. We show that the most successful ovulatory strategy would be to always double ovulate as an insurance against early fetal loss, but to never bear twins. This finding supports the hypothesis that twinning is a byproduct of selection for double ovulation rather than twinning.

Main

The stable existence of alternative phenotypes in a single population is of interest because it suggests a balance of evolutionary forces at play. Elucidating those forces, particularly when they apply to humans, is an important focus of evolutionary biology [1]. For example, the tendency to produce dizygotic twins, where the ovulation of two ova gives rise to the birth of siblings, is heritable [2] and varies within and among populations, its rate rising and then falling with increasing maternal age [3]. Here we show that a conditional strategy of switching from single to double ovulation with increasing age explains why twinning rate rises and falls as females age, and how switching from single to double ovulation with increasing age maximizes individual reproductive success, thus explaining why dizygotic twinning persists in humans.

The evolutionary forces that account for twinning and the age-dependent change in its rate are poorly understood. While the birthing of twins has fitness costs for both mothers and offspring, mothers that produce twins often have greater fitness than mothers who have never produced twins [4-8]. We focus on how selection operates on the reproductive trait that makes dizygotic twins possible, double ovulation. The ova insurance hypothesis [9] posits that twinning is a maladaptive consequence of the ovulation of multiple ova, which serves as an adaptive counter to poor prenatal offspring survival. Because prenatal mortality is known to dramatically increase with maternal age [10-12], we hypothesized that the age-dependent pattern of dizygotic twinning observed in humans might be due to an adaptive ovulatory strategy of switching from single ovulation to double ovulation with increasing age. This hypothesis makes two predictions. First, an increasing rate of double ovulation coupled with a decreasing live birth rate should explain the observed pattern of age-dependent twinning in humans. Second, the expected lifetime reproductive success of women using this conditional strategy should exceed that of women who always single or always double ovulate. We verified the first prediction by extending a simple mathematical model that derives the twinning rate given the probabilities of double ovulation and live birth. We asked whether a declining probability of live birth coupled with an increasing probability of double ovulation with increasing maternal age could explain the pattern of age-dependent twinning observed in human populations. To verify the second prediction, we used postnatal and maternal survival rates for twins and singletons from a natural fertility population and age-dependent prenatal survival rates at different stages of pregnancy to compare the expected lifetime reproductive success of women who always single or double ovulate with that of women who switch from single to double ovulating with increasing age.

Results and Discussion

With respect to the first prediction, only two parameters, the rate of double ovulation and the live birth rate, are needed to predict the twinning rate. For a woman at age t, the twinning rate (T_t) at birth is given by the formula $T_t = F_t p_t / (1 + F_t (1-p_t))$, where F_t is the double ovulation rate and p_t is the probability of survival from fertilization to birth (see SI for derivation) [13]. If the observed change in twinning rate with age (T_t) is due to a conditional ovulatory strategy that compensates for declining fertility, then a double ovulation rate (F_t) that increases with age and a live birth rate (p_t) that decreases with age should produce age dependent twinning rates (T_t) that closely match those observed in human populations. We used a cumulative normal function for F_t so that the probability of double ovulation increases with age depending on the mean and standard deviation of a normal distribution in age of switching from single to double ovulation. We chose this function because if forms the basis of the environmental threshold model, the most commonly used quantitative genetic model for conditional strategies [14] (Fig. S1). Based on studies of a natural fertility population [10], we used a declining exponential function for the probability of live birth (p_t), where p_t declines at a constant rate with female age from a value of 0.55 at age 18 (SI). We then derived age-dependent twinning rates (T_t) using combinations of values for the mean and standard deviation of the cumulative normal distribution for age of switching from single to double ovulation (F_t) and the annual rate of decline in probability of live birth (p_t). We used *Microsoft Excel's* iterative model fitting function *Solver* to compare the expected twinning rates (T_t) with those observed in nine data sets from human populations unbiased by fertility treatments [3,15-19]. *Solver* identified the combinations of values for the three parameters (mean and standard deviation of the cumulative normal distribution for age of switching from single to double ovulation, and the annual rate of decline in probability of live birth) that maximized R^2, we were able to determine what values of these parameters could best explain the pattern of age-dependent twinning observed in each population.

Our results show that 90% or more of the variation in twinning rates in these populations is explained by a shift from single ovulation to double ovulation with increasing age (Fig. 1; Table 1), supporting the hypothesis that age-dependent twinning results from a conditional ovulatory strategy. An increase in double ovulation rates, F_t, from near zero at menarche to over 50% prior to menopause (Fig. 1e), coupled with a constant decline in live birth rate, p_t, to less than 10% as women approach menopause (Fig. 1f), readily explains the pattern of age-dependent twinning (Fig. 1a-d). Because the decline in prenatal survival ensures that double ovulations increasingly result in only singleton births or reproductive failure, the increased rate of double ovulation does not translate into ever-increasing rates of twin births because as prenatal survival rates decline it become increasingly unlikely that both offspring survive to birth. Hence, patterns of age-dependent twinning are consistent with a conditional strategy that combines an age-dependent switch to double ovulation with the well-established age-dependent decline in prenatal viability [10-12].

Our results also suggest that differences in age-dependent twinning rates amongst populations can be explained by differences in the mean and standard deviation in age of switching from single to double ovulation and the decline in probability of live birth per conception with age. For example, the age-dependent twinning rates in a Nigerian population (Fig. 1a), which has the steepest increase and subsequent decline in twinning rate with age, requires an average age of switching to double ovulation of 33 years and an annual decline in probability of live birth of 7% per year, compared with a switching age of 46 years for a Japanese population, which has the lowest twinning rates (Fig. 1e; Table 1), and an annual decline in probability of live birth of 11% (Fig. 1e, Table 1).

We used two complementary approaches, simulations and probabilistic modeling (see SI, Figs. S3-S5, Tables S1 and S3) to evaluate the prediction that women playing a conditional strategy of switching from single to double ovulation will have greater lifetime reproductive success than women who always single or always double ovulate. Both approaches allowed us to estimate the lifetime reproductive success of women switching from single to double ovulation at different ages. By setting the age of switching to less than what we functionally defined as menarche (age 18, based on the average age of first reproductive attempt in a natural fertility population[5]) we could estimate the fitness of a strategy of always double ovulating. When the switching age was set to greater than what we functionally defined as menopause (age 40, based on the mean age of last birth in a natural fertility population[5]) we could estimate the fitness of always single ovulating. Setting the switching age to ages between 18 and 40, allowed us to estimate the fitness of conditional ovulatory strategies that switched from single to double ovulation at different ages. We compared the success of the different strategies using the average number of offspring surviving to age 15. The modeling allowed us to calculate the expected number of zygotes per ovulatory cycle surviving to age 15, and the time between successive cycles for different-aged single and double ovulating women (SI). From these data we estimated age specific reproductive rates for women playing different ovulatory strategies. We treated the survival of individual conceptions resulting from double ovulation as independent events, assumed that each ovum was fertilized, and that women resumed ovulation only after complete brood loss or weaning (SI).

These approaches required data on offspring survival rates between conception and birth, maternal survival rates at the birthing of twins and singletons, singleton and twin survival rates between birth and weaning and to reproductive age (assumed to be 15 yrs), and the time intervals between successive ovulatory cycles. Such data are unavailable from any single population, so we used multiple sources. Natural fertility populations in Bangladesh [10] and Gambia [5] provided data for live birth rates per zygote at age 18 (0.55/conception), and maternal childbirth survival rates and postnatal offspring survival rates for twin and singleton births (Table S1). We assumed a decline in live birth rate of 11% which was the average decline rate estimated from our analyses of observed twinning rates (Table 1). Data for determining prenatal survival rates at different times between conception and birth for different aged women were based on an analysis of the fates of over 1.2 million pregnancies in Denmark [20] (Table S2, Fig.S2). Estimates of the time intervals between successive ovulatory cycles were taken from the literature.[21]

Both simulations and modeling indicated that fitness was maximized when women switched from single to double ovulation at approximately age 25 (Fig 2a, Fig 3a). This result supports the hypothesis that the age-dependent conditional ovulatory strategy we have identified as best explaining the observed age-dependent twinning rates is adaptive [1]. The modeling results show why fitness is maximizes by switching from single to double ovulation with increasing age. While at all ages the expected per capita number of offspring surviving to 15 per ovulatory cycle was greater for a strategy of double ovulating than single ovulating (Fig 3b), this was especially so in older women (91% greater at age 40 versus 26% greater at age 18). However, the lower probability of livebirth per ovulatory cycle shortens the time between successive ovulations in single ovulators, while in double ovulators the increased probability of at least one livebirth and subsequent lactation, and lengthens the time between ovulations (Fig 3c). So, although the per capita offspring per cycle was greater for double ovulations, this was more than compensated by the increased number of ovulations of young single ovulators. Young double ovulators were also more likely to birth twins whose poorer postnatal survival depressed fitness and whose birthing incurred greater maternal risks (Fig. S6). Hence, double ovulating when young has fitness costs in the investment of time in low-fitness high-risk pregnancies that produce twins, while double ovulating when older has fitness benefits because twins are rarely produced. These differences in the age-dependent reproductive effects of double and single ovulating results in reproductive rates in young single ovulators that exceed those of young double ovulators, but in older women the reverse is true (Fig. 3d). This age-dependent trade-off in reproductive rate fulfills an important requirement for maintenance of a conditional strategy, and in the case of conditional double ovulation, produces stabilizing selection on an optimal switching age between menarche and menopause [22].

Our modeling indicates that the optimal switching age is sensitive to differences in prenatal survival probabilities of embryos resulting from single versus double ovulation. For example, our results are based on the assumption that prenatal probabilities of survival of embryos resulting from single versus double ovulation are the same and independent (SI). While this assumption is more likely to be valid early in pregnancy, later in development the survival of individual twin fetuses are likely to be less than singletons [23,24] and when one of the twin fetuses is lost, the probability of the second fetus being lost increases [25], as do other adverse perinatal outcomes [26]. As a result, the true optimum switching age is likely later than our analyses in Figures 2 and 3 indicate, and more in line with the switching ages observed in human populations (Fig. 1).

Finally, we asked whether twinning is a maladaptive byproduct of an otherwise adaptive conditional ovulatory strategy [9]. This hypothesis predicts that if women who double ovulate throughout their lives could avoid birthing twins, for example via reabsorption of one fetus, then their lifetime reproductive success should exceed that of women who switch from single to double ovulation with increasing age, and therefore occasionally produce twins. We repeated the simulations, but now women who double ovulated were only allowed to only give birth to singletons even if they were expected to carry twins to term. Hence, they received the fertility benefits of double ovulation without incurring the costs of twin births. In these simulations, lifetime reproductive success was maximized at switching ages less than or equal to the age of menarche (Fig. 2b). Since these women double ovulated during each ovulatory cycle, this result supports the byproduct hypothesis [9].

Our results potentially shed light on why mothers who have produced twins often have greater fitness than mothers who never do so [4,5,7,8]. Follicle development and ovulation are controlled by the hypothalamic-pituitary axis which is able to integrate an adaptive response to cues associated with maternal condition and age [27]. Mothers who produce twins often do so at higher parities (independent of maternal age) [3], and often possess physical characteristics, such as height and body mass indices, that potentially increase both the probability of double ovulation, lifetime reproductive success and prenatal survival [5,28]. In this study, we have only attempted to explain the evolution of age-dependent double ovulation. However, if double ovulation and prenatal survival are conditional on these factors, in addition to maternal age, then it would not be surprising if women who have produced twins often have greater fitness than those who have not.

Our findings indicate that the ultimate cause (*sensu* Mayr [29]) of the pattern of age-dependent twinning in human populations is natural selection favoring double ovulation with increasing age in response to declining fertility. These results suggest that in human populations in which increasing numbers of women delay first reproduction [30], a substantial fraction of singleton births will result from double ovulation with the subsequent prenatal loss of one of the siblings. For example, if the probability of double ovulation and probability of live birth per ova for women at age 27 are 0.05 and 0.2 (Figure 1e, f), respectively, approximately 8% of all singletons will have been produced following double ovulation. In contrast, at age 37, if the probability of double ovulation and probability of live birth per ova are 0.4 and 0.05 (Fig. 1e, f), respectively, the fraction of singletons produced by double ovulation rises to approximately 56%. Alongside this, when reproduction is delayed, we also expect a population level increase in the incidence of twin births, a phenomenon already seen in developed countries[31].

Materials and Methods

Estimating expected age-dependent twinning rates from the decline in of live birth rate and increase in double ovulation rate

Using the formula that calculates twinning rate as a function of the rates of live birth per zygote and double ovulation, we used the *Solver* add-in in *Microsoft Excel* to determine the rates of age-dependent decline in live birth per zygote and age-dependent increase in double ovulation that provided the best fit to the observed age-dependent twinning rates (maximized the R_2 with observed twinning rates) in large samples from populations with African, European and Asian ancestries (Table 1). The models were fitted subject to several constraints. We fixed the probability of live birth at age 18 (*dra*) at 0.55 based on the best estimate of which we are aware and coming from a natural fertility population in Bangladesh [10]. The probability of live birth per zygote declines with maternal age [10,12] and we assumed an exponential decline, where the annual decline was constrained to be less than 0.99, and greater than 0.55 (no model approached either boundary; see SI). Based on quantitative genetic models for conditional strategies[22,32] (SI), we assumed that the increase in probability of double ovulation per ovulatory cycle with maternal age was best described by an increasing cumulative normal function, with mean (*spm*) and standard deviation (*spSD*) constrained in our *Solver* searches to be greater than two years.

Simulations

The simulation model, which was written in the language R [33], estimates lifetime reproduction in a cohort of (nominally 1000) women by simulating ovulatory cycles from first reproductive attempt until reproduction stops due to death or menopause (Table S1, Figs. S3 & S4). Dependent on inputs governing the probability of prenatal loss, these cycles can result in early loss, abortion, late fetal loss (miscarriage) or live birth (Table S2). The failure of a pregnancy to proceed to term delays the commencement of cycling following the event by a variable amount depending on when the pregnancy ends (Table S1). The simulation allows women to either single or double ovulate based on her age at ovulation relative to a double ovulation switch point that determines the age at which they switch from single to double ovulation (*spm* in Table S1). We were able to simulate different conditional ovulatory strategies by setting the ovulation switch point to values between age of menarche (age 18) and age of menopause (age 40). Women could also be constrained to double or single ovulate throughout their reproductive lives by setting the switch to ages less than 18 or greater than 40, respectively. Depending on the number of ova released and the probabilities of surviving the prenatal period (considered to be independent where there are two zygotes), either none, one or twin offspring are born. Subject to inputs governing the probabilities of still birth, background adult death rate, and maternal death in childbirth, successful live births occur (Table S1). Contingent on inputs controlling postnatal mortality, children survive for periods of one month, one year, two years (weaning) or to 15 years (Table S1). The duration of offspring survival influences the duration of time that the female stops cycling (Table S1). Survival to 15 years is considered a successful reproductive event for the mother and per capita number of offspring surviving to 15 years was our measure of maternal fitness. Women are continually subject to the probability of death including when pregnant and weaning (Table S1). When women died during childbirth, the children they were birthing also died. The simulation allows for up to 20 parities; however a woman's reproductive longevity is curtailed by declining ova quality (Table S2 and Fig. S2d). Women older than the user-defined age at menopause, at the time that they are due to begin cycling again after a pregnancy attempt or child bearing (Table S1), do not continue cycling, but women that are not pregnant and younger than this age can cycle beyond that age.

The simulation assumes that women are not sperm limited and make no socially driven decisions to delay or reduce the number of children they attempt to have, so successful pregnancy was only a function of embryo survival to live birth. The simulation makes no provision for siblings, grandparents or males assisting in the care of offspring. The estimates of the demographic parameters used in the simulation come from multiple sources. The probability of live birth at age 18 comes from a natural fertility population in Bangladesh[10]. The decline in probability of live birth with increasing age was based on the decline in live birth probability needed to explain the observed twinning rate averaged over all the populations summarised in Table 1. The probabilities associated with different prenatal fates of zygotes were based on the ratio of spontaneous abortions, stillbirths (late pregnancy losses) to live births in a study of over one million pregnancies in Danish women of different ages (Table S2) [20], conditional on our estimates of probability of live birth per zygote in women of different ages (Table 1). Postnatal survival rates were based on those taken from a Gambian natural fertility population [5]. We assumed prenatal survival probabilities to be the same for twins and singletons and independent for zygotes produced by double ovulation (i.e. if the probability of survival per zygote is p, then in double ovulations, the probability of both zygotes surviving is p^2, one surviving is $2p(1-p)$ and neither surviving is $(1-p)^2$). Other than parameters such as age of switching from single to double ovulation and whether double ovulation could result in twin births which defined the strategy, all ovulatory strategies had the same set of parameters of the simulated biological background and so represented a comparison only of the differences that arise from the changes in strategy.

In replicate simulations (Figs. S3 & S4 for further details), we estimated fitness by tallying the number of offspring surviving to 15 produced by cohorts of 1000 women playing different ovulatory strategies, each defined by the age at which women switched from single to double ovulation. Simulations designated as 'Twins not produced' were identical to previously described, except that whenever two offspring survived to birth, twin births were replaced with a singleton birth. In each simulation we followed women from their first ovulation, through each ovulatory cycle to either death or menopause and tabulated the number of offspring surviving to age 15.

Probabilistic model comparing fitness of single vs double ovulation strategies

We have defined the fitness of a woman at age *x* as $\sum_{k=x}^{M} C(k)$ where $C(k)$ = number of children conceived when the woman is age *k* that survive to age 15, and *M* is the age of menopause. Our model estimates the expected value of this expression as

$$F(x) = \sum_{k=x}^{M} N(k)S(k \mid x)$$

where $N(k)$ is the average number of offspring conceived to a woman age *k* that survive to age 15, and $S(k \mid x)$ is the probability that a woman alive at age *x* has not died before age *k*. We determine $N(k)$ by

$$N(k) = \frac{12V(k)}{T(k)}$$

where $V(k)$ is the expected number of offspring per ovulation that survive to age 15 when a woman is age *k*, and $T(k)$ is the expected time between ovulations (in months) when a woman is age *k*. $S(k \mid x)$ is determined by the recurrence relation

$$S(x \mid x) = 1$$

$$S(x+1 \mid x) = R(x) \cdot asr$$

$$S(x+2 \mid x) = S(x+1, x) \cdot R(x+1) \cdot asr$$

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$$S(k \mid x) = S(k-1, x) \cdot R(k-1) \cdot asr$$

where $R(t)$ is the probability a woman age *t* does not die in childbirth and *asr* is the annual survival rate of the adult population. (See SI for details regarding the constructions of the functions *V*, *T*, and *R* for single and double ovulators.)

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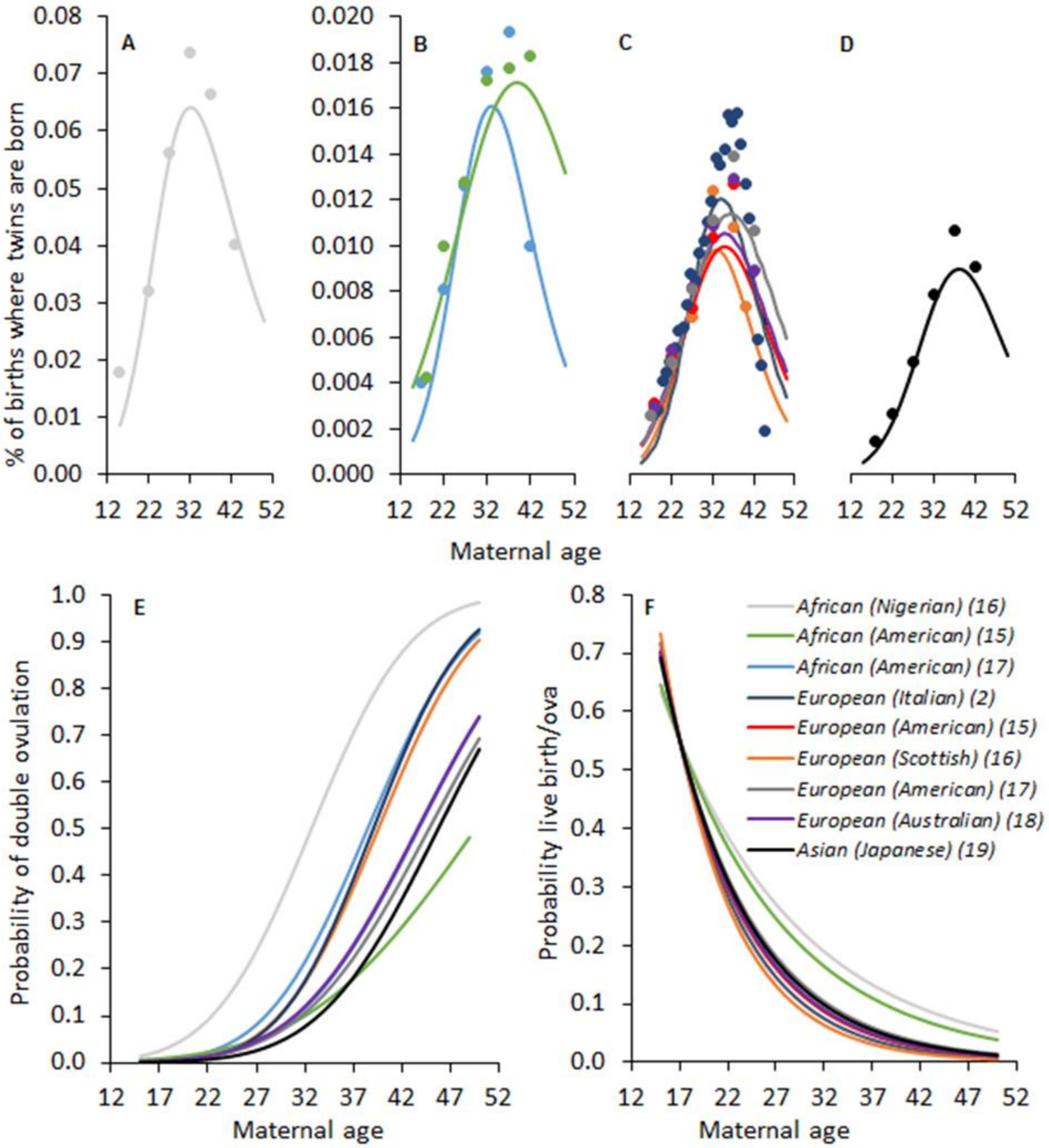

Fig. 1. Declining prenatal survival and increasing double ovulation rates explain age-dependent twinning rates. **a-d,** Observed twinning rates (points) as a function of maternal age and those expected (lines) given **e,** increasing probability of double ovulation, and **f,** decreasing probability of live birth per zygote, both as a function of maternal age. Curves in **e** and **f** were chosen for their ability to minimize the squared deviations between the observed and expected twinning rates in **a-d** (See Table 1).

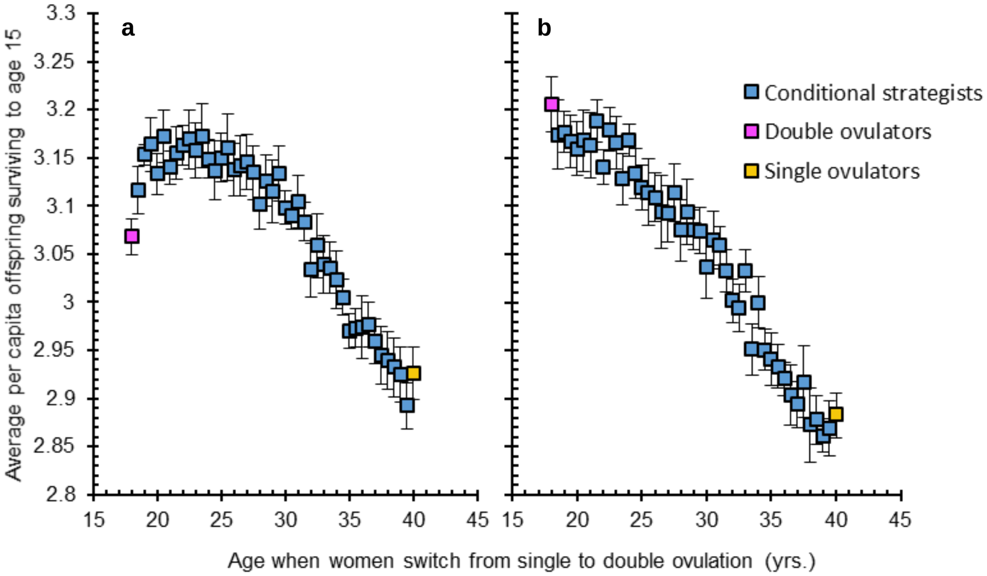

Fig. 2. Average per capita numbers of offspring surviving to 15 years (±95% CI, n=10) from simulations of the reproductive lives of 1000 women. When age of switch is 18 women always double ovulate throughout their reproductive lives; when it is 40 (age of menopause), they always single ovulate throughout their reproductive lives. When the switch age is between 18 and 40, women are playing different conditional strategies defined by the switch age. **a,** Women that double ovulated could give birth to twins. **b,** Women that double ovulated carried only one offspring to term.

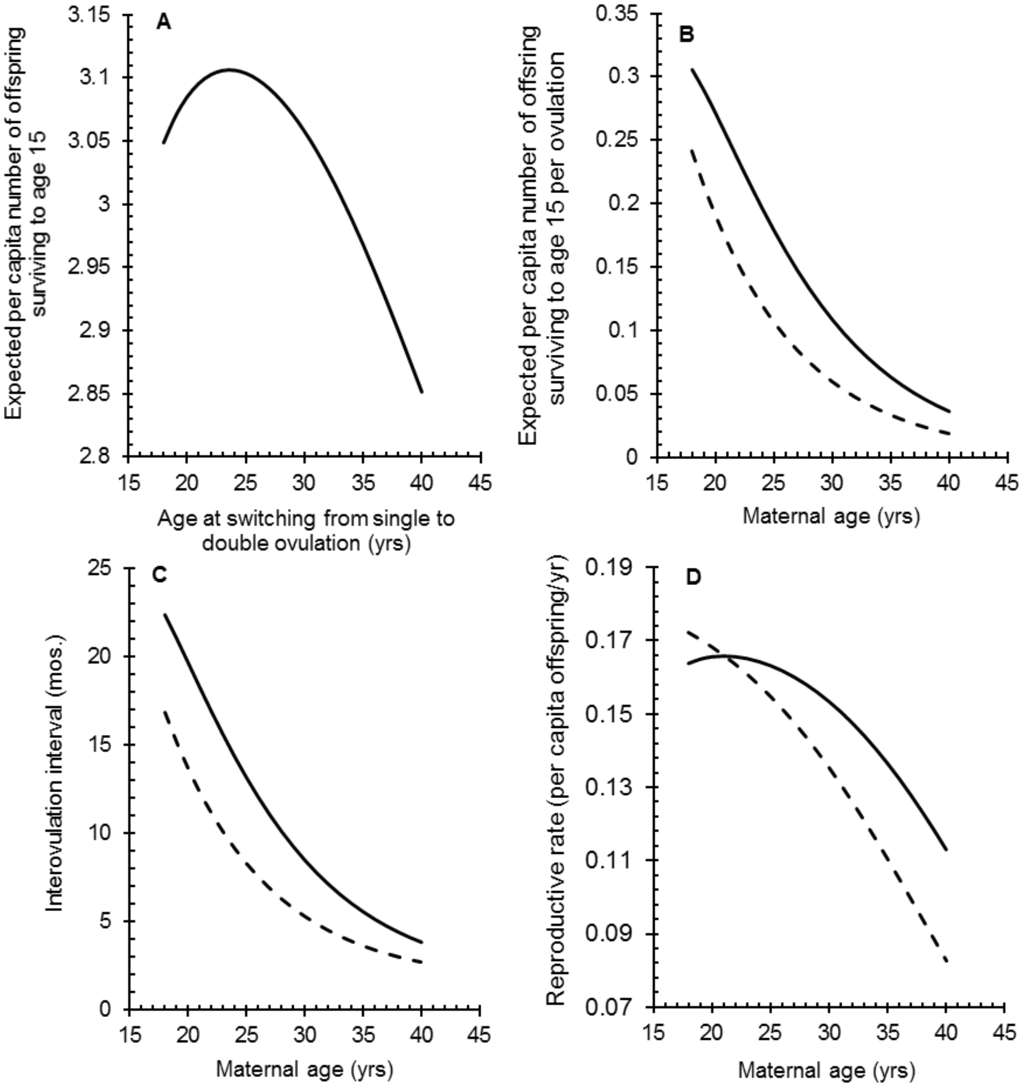

Fig. 3. Results of mathematical modeling of the reproductive characteristics of different ovulatory strategies. **a**, Expected lifetime reproductive success for women switching from single to double ovulation at different ages. **b**, Per capita number of offspring surviving to age 15 per ovulation for double (solid line) and single ovulators (dashed line) at different ages. **c**, Time between successive ovulations for double (solid line) and single ovulators (dashed line) at different ages. **d**, Expected annual reproductive rates for double (solid line) and single ovulators (dashed line) at different ages.

Table 1. Estimates of the parameters that explain age-dependent twinning rates in nine human populations

Population	n	*drb*	*dra*	*spm*	*spSD*	R_2	study
African (Nigerian)	18,400	0.93	0.55	32.67	8.02	0.93	*16*
African (American)	22,100	0.92	0.55	49.62	13.76	0.96	*15*
African (American)	3,679	0.89	0.55	38.47	8.22	0.91	*17*
European (American)	21,809	0.89	0.55	44.80	10.27	0.97	*17*
European (Scottish)	49,000	0.87	0.55	39.56	7.98	0.90	*16*
European (American)	5,672,228	0.88	0.55	43.70	9.84	0.91	*15*
European (Italian)	1,817,736	0.88	0.55	39.05	7.53	0.99	*3*
European (Australian)	45,956	0.89	0.55	43.61	9.89	0.96	*18*
Asian (Japanese)	72,180	0.89	0.55	45.74	9.64	0.99	*19*
Mean ±	858,120.89	0.89	0.55	41.91	9.46	0.95	
SE	633136.269	0.007		1.664	0.636	0.012	

drb is the annual decline in prenatal survival rate per zygote with increasing maternal age; *dra* is the live birth rate per zygote at maternal age 18 yrs.; *spm* and *spSD* are the mean and standard deviation, respectively, of the cumulative normal function for the double ovulation rate with increasing maternal age; R_2 is fraction of variation in age-dependent twinning explained by these parameters (see Fig. 1).