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Changes with age in the level and duration of fertility in the menstrual cycle

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BACKGROUND: Most analyses of age-related changes in fertility cannot separate effects due to reduced frequency of sexual intercourse from effects directly related to ageing. Information on intercourse collected daily through each menstrual cycle provides the data for estimating day-specific probabilities of pregnancy for specific days relative to ovulation, and these estimates allow unconfounded analysis of ageing effects. METHODS: A total of 782 healthy couples using natural family planning methods contributed prospective data on 5860 menstrual cycles. Day of ovulation was based on basal body temperature measurements. Estimates of day-specific probabilities of pregnancy and the length of the fertile window were compared across age groups. RESULTS: Nearly all pregnancies occurred within a 6 day fertile window. There was no evidence for a shorter fertile window in older men or women. On average, the day-specific probabilities of pregnancy declined with age for women from the late 20s onward, with probabilities of pregnancy twice as high for women aged 19–26 years compared with women aged 35–39 years. Controlling for age of the woman, fertility was significantly reduced for men aged >35 years. CONCLUSIONS: Women's fertility begins to decline in the late 20s with substantial decreases by the late 30s. Fertility for men is less affected by age, but shows significant decline by the late 30s.

Key words: Bayesian/fertile interval/ovulation/menstrual cycle/pregnancy probabilities

Introduction

Although there is substantial interest in the association between male and female age and fertility, it has proven difficult to assess because of changes in sexual behaviour with age and the tendency for sexual partners to be of similar ages (Weinstein and Stark, 1994). The CECOS study of women having artificial insemination because of male factor infertility provided convincing evidence for a biological decline in female fertility (Fédération CECOS *et al*., 1982). In that study, inseminations were timed to coincide with ovulation, thus controlling for intercourse behaviour. No comparable data are available regarding male fertility. A decline in the conception rate with male age has been reported, which begins in men in their early 30s (Ford *et al*., 2000), but this finding may have been an artefact caused by defining age at the time of conception rather than the time of attempting pregnancy in a retrospective time-to-pregnancy analysis (Sallmen and Luukkonen, 2001). Descriptive birth rate data that adjust for female age and length of marriage or co-habitation show reduction in male fertility after the age of 40 years (Anderson, 1975), but length of marriage may not adequately control for sexual behaviour, and control for the effects of women's age may not have been adequate (Weinstein and Stark, 1994; Kidd *et al*., 2001).

Changes in semen characteristics with age have also been studied. The preponderance of the data suggest lower semen quality among men aged >50 years compared with men aged <30 years (Kidd *et al*., 2001), but there is limited evidence of declines with age in the 30s and 40s. Sperm motility is the parameter with the most evidence for an age-related decrease, even at relatively young ages. One study reports reduced post-thaw motility for men in their late 30s (Schwartz *et al*., 1983). However, the data are inconsistent (Kidd *et al*., 2001). For example, an analysis of 30 000 IVF cycles for women with tubal sterility found no 'important alteration of semen characteristic with age' (Guerin and deMouzon, 1997). Genetic defects in gametes increase with age for both males and females (Martin and Rademaker, 1987; Risch *et al*., 1987; Hassold *et al*., 1996). For females the rates clearly rise after age 35 years, but the evidence for men is less clear. In both the UK (British Andrology Society, 1999) and the USA (American Society for Reproductive Medicine, 1998) there are age limits of <40 years for semen donors in order to protect recipients from sperm that may be genetically defective.

The purpose of this study is to evaluate the effects of male and female age on natural fertility by carefully controlling for variation in sexual behaviour. We use data from a large multinational European prospective cohort study of couples practising natural family planning (Colombo and Masarotto, 2000). The probabilities of pregnancy associated with sexual

intercourse on specific days relative to ovulation are estimated and compared across age groups. In addition, we investigate differences among age groups in length of the fertile window during which intercourse can result in a clinically detectable conception.

Materials and methods

Description of study design and data

Data were drawn from a large multinational study of daily fecundability conducted in Europe (Colombo and Masarotto, 2000). The research protocol was reviewed and approved by the Institutional Review Boards of Fondazione Lanza (Padua, Italy) and Georgetown University (Washington, DC, USA). Briefly, the study enrolled 782 women (349 with at least one past pregnancy) recruited from seven centres (Milan, Verona, Lugano, Dusseldorf, Paris, London, Brussels) providing services on fertility awareness and natural family planning. Most participating couples were trying to avoid pregnancy. Women enrolled were between 18 and 40 years of age, had at least one menses after the most recent cessation of breastfeeding or delivery, and were not currently taking hormonal medication or drugs affecting fertility. In addition, neither partner could have a history of fertility problems and couples were required to not be in the habit of mixing unprotected and protected intercourse, e.g. by using condoms during fertile days. The participants kept daily records of basal body temperature (BBT) and recorded the days during which intercourse and menstrual bleeding occurred. Details of study methods, participants and pregnancy outcomes have been published (Colombo and Masarotto, 2000).

Briefly, ovulation days were estimated from the daily BBT data using published methods (Marshall, 1968; Colombo and Masarotto, 2000). Although the last day of hypothermia prior to the post-ovulatory rise in basal body temperature clearly does not correspond perfectly with the release of the oocyte, previous data suggest that BBT-based estimates of ovulation day have a high probability of being within ± 1 day of the true ovulation day (Dunson *et al*., 1999). More accurate measures require assays of daily urine specimens or ultrasound monitoring, both of which are prohibitively expensive in large studies.

Bayesian statistical analysis approach

A BBT-based estimate of ovulation day was available for 5860 menstrual cycles from 770 women. Of these, 2539 cycles from 647 women had at least 1 day with intercourse reported in the 10 day interval beginning 7 days prior to and ending 2 days after the estimated ovulation day. This interval was chosen as a conservative first guess for the fertile window. Our analysis is based on the 433 detected pregnancies that occurred in these 2539 cycles, along with the daily intercourse records and estimated ovulation days.

Since the specific intercourse act responsible for a pregnancy cannot be determined with certainty in a menstrual cycle having multiple days with intercourse in a window of potential fertility, we followed the established approach of using a statistical model to estimate the day-specific pregnancy probabilities (Barrett and Marshall, 1969; Wilcox *et al*., 1995, 1998; Dunson *et al*., 1999; Colombo and Masarotto, 2000). Most previous estimates of the day specific probabilities have been based on a published model (Schwartz *et al*., 1980), which assumes that batches of sperm introduced in the reproductive tract on different days mingle and then compete independently in attempting to fertilize the ovum. One of the primary drawbacks of the original Schwartz *et al*. model is that it implicitly assumes that all couples have the same probability of conceiving if they have intercourse at the same time relative to ovulation.

Extensions of the Schwartz *et al*. model have been developed for accommodating variability among couples in their fertility (Zhou *et al*., 1996; Dunson and Zhou, 2000; Dunson, 2001; Dunson *et al*., 2001a,b). In particular, the Bayesian approach of Dunson and Zhou (2000) accounts for known predictors of fertility (e.g. age) and unexplained heterogeneity among couples through a 'cycle viability' factor that has a multiplicative effect on the day-specific pregnancy probabilities. An alternative Bayesian approach developed by Dunson (2001) allows couples to vary with respect to both the probability of pregnancy following intercourse on the most fertile day of the cycle and the decrease in the pregnancy rate on less fertile days. The latter approach is more flexible in that it allows differences in the width of the fertile interval that are distinct from differences in the pregnancy rate on the most fertile day. As we are interested in assessing the impact of male and female age on the duration of the fertile interval and the day-specific pregnancy probabilities within the fertile window, our analysis will be based on the Dunson (2001) model. Differences among age groups are tested by estimating posterior probabilities (*PP*), with $PP \geqslant 0.95$ considered unlikely to be due to chance.

Since the ages of the male and female partners are highly correlated, we cannot simply include both male and female age in the model without facing problems with co-linearity. To avoid such problems, we instead included the age of the woman (categorized according to the intervals 19–26, 27–29, 30–34 and 35–39 years) and the difference in years of age between the male and female partners. There were 481, 923, 807 and 328 cycles and 103, 154, 140 and 36 pregnancies in the respective age categories. Most (76%) of the men were older than their partners, and the average difference in age between the men and the women was 2.4 years (SD = 3.5, interquartile range = 0.3, 4.4). Our hypothesis was that the effect of male age would be more pronounced among the older men, with minimal differences in fertility between 20 and 30 year old men. To assess this hypothesis, we estimated *PP* of an association between the age difference and the level and duration of fertility separately for men in different age categories.

To complete a Bayesian specification of our model, we chose mildly informative prior distributions for the baseline fertility parameters, based on a published analysis (Wilcox *et al*., 1998), and non-informative prior distributions for the age-effect parameters. We then used a Markov chain Monte Carlo algorithm (Gilks *et al*., 1996) to obtain posterior summaries of each of the parameters.

Results

There was no evidence that the duration of the fertile interval was dependent on male or female age. The estimated interval during which intercourse had a $\geqslant$5% chance of resulting in a pregnancy consisted of the 6 days ending on the identified ovulation day, for women in each age group adjusting for their male partner's age. For women in the first three age categories (19–26, 27–29 and 30–34 years), the estimated fertile interval was the same regardless of their male partner's age. For women in the 35–39 year category having male partners that were 5 years older, the estimated interval was 1 day shorter (beginning 5 days prior to the identified ovulation day). However, this difference was not statistically significant.

Since it has been hypothesized that heterogeneity among couples in fecundity increases with age, we considered a model that allowed the magnitude of heterogeneity among couples in their fertility to vary depending on the age category. The

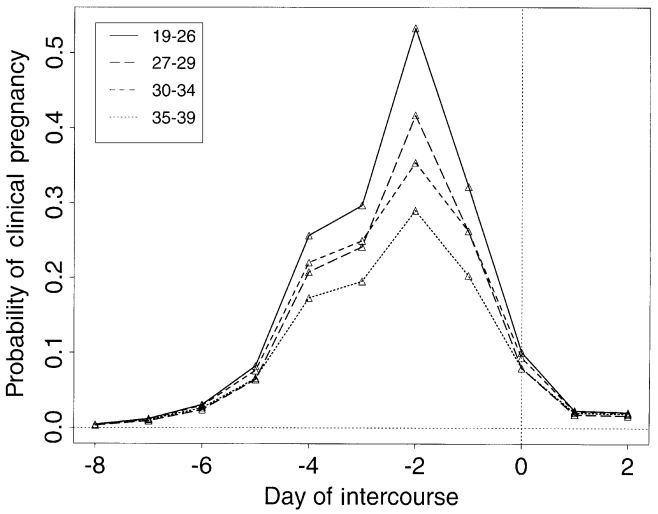

Figure 1. Probability of clinical pregnancy following intercourse on a given day relative to ovulation for women of average fertility aged 19–26, 27–29, 30–34 and 35–39 years (European Study of Daily Fecundability, 433 pregnancies), adjusted for male partner's age.

resulting posterior means for the heterogeneity parameters were similar across age categories, with no evidence of an increasing or decreasing trend with age. Therefore, we simplified our analysis by focusing on a model with a single heterogeneity parameter.

The estimated pregnancy probabilities following intercourse on a given day relative to ovulation for average women aged 19–26, 27–29, 30–34 and 35–39 years (with their partners of the same age) are shown in Figure 1. The day-specific pregnancy probabilities decreased with age, with the pattern similar across age categories. To simplify assessment of differences between groups, we focused on a reduced model that incorporated age effects through a multiplicative cycle viability factor. Based on the simplified model, which produced estimates consistent with Figure 1, women aged 27–29 years were predicted to have lower pregnancy rates on average than women aged 19–26 years given equivalent timing of intercourse ($PP = 0.99$). Women in the 27–29 and 30–34 year age categories had statistically indistinguishable rates, and there was evidence of a decline between the 30–34 and 35–39 year age groups ($PP = 0.95$).

We found that, adjusting for woman's age, 35–39 year old men had significantly reduced day-specific pregnancy probabilities relative to younger men ($PP > 0.99$). Figure 2 shows the estimated day-specific pregnancy probabilities for typical women in different age categories having partners the same age or 5 years older. For a 35 year old woman with a partner of the same age, the probability of pregnancy following intercourse on only the most fertile day was 0.29. This estimated probability decreased to 0.18 for 35 year old women with 40 year old partners.

Controlling for both male and female age, there was considerable heterogeneity among couples in the day-specific probabilities, with no evidence that the level of heterogeneity varies with age. Figure 3 shows the estimated day-specific probabilities of pregnancy for couples in the 5th, 25th, 50th, 75th and 95th percentiles of the distribution for fertility among couples with the woman aged 27–29 and the man the same age. All couples had a low probability of conceiving a clinical pregnancy following intercourse $\geqslant$6 days prior to ovulation or $\geqslant$1 days after ovulation. For an average couple (i.e. a couple in the 50th percentile of the distribution for fertility), the daily probabilities followed a similar pattern to that seen in the North Carolina Early Pregnancy Study (Wilcox *et al*., 1998). In particular, the probabilities increased towards a peak of 0.37 occurring 2 days prior to ovulation and decreased thereafter. The peak probability varied from 0.05 for a couple in the 5th percentile of the population distribution to 0.83 for a couple in the 95th percentile.

Discussion

The length of the fertile window may decline with male age if sperm from older men do not remain viable as long in the female reproductive tract. Current estimates of the fertile window suggest that it is rarely longer than 6 days, with the day of ovulation being the final day. However, estimates from the literature have a high degree of uncertainty, and pertain to average couples, not age-specific groups.

Data appropriate for estimation of the day-specific pregnancy probabilities have been collected in a British study of couples using natural family planning in the 1960s (Barrett and Marshall, 1969), in a North Carolina study of early pregnancy conducted in the 1980s (Wilcox *et al*., 1995), and most recently in our multinational European study of daily fecundability (Colombo and Masarotto, 2000). The data from the earlier studies have been analysed to estimate the fertile days of the menstrual cycle and the day-specific probabilities of conception (Royston, 1982; Weinberg *et al*., 1994; Royston and Ferreira, 1999; Dunson and Weinberg, 2000; Dunson *et al*., 2001a). However, a detailed assessment of the effect of male and female age on the fertile window and daily probabilities of pregnancy has not been possible, given the limited number of participants and conceptions in earlier studies. Our data set is larger than prior studies in terms of numbers of couples, clinical pregnancies, and menstrual cycles with daily records of intercourse and menstrual bleeding. Moreover, many couples had intercourse on only one day in the fertile window since many were using fertility awareness methods to avoid conception. These factors allow more precise estimation of the day-specific probabilities of pregnancy. In addition, a recently developed statistical model provides the methodology for these analyses (Dunson, 2001).

We find that the fertile interval lasts ~6 days and ends on the day of ovulation, in agreement with both the North Carolina and British cohorts (Dunson *et al*., 1999). The similarities to the results from the North Carolina study, which used a highly accurate surrogate for ovulation day based on urinary hormone metabolites (Baird *et al*., 1991), suggest that bias caused by measurement error in our BBT-based marker of ovulation may be low. We had speculated that as men age, sperm survival after insemination would be reduced, thus reducing the length of the fertile window. The estimated fertile interval was indeed 1 day shorter for 40 year old men with 35 year old partners

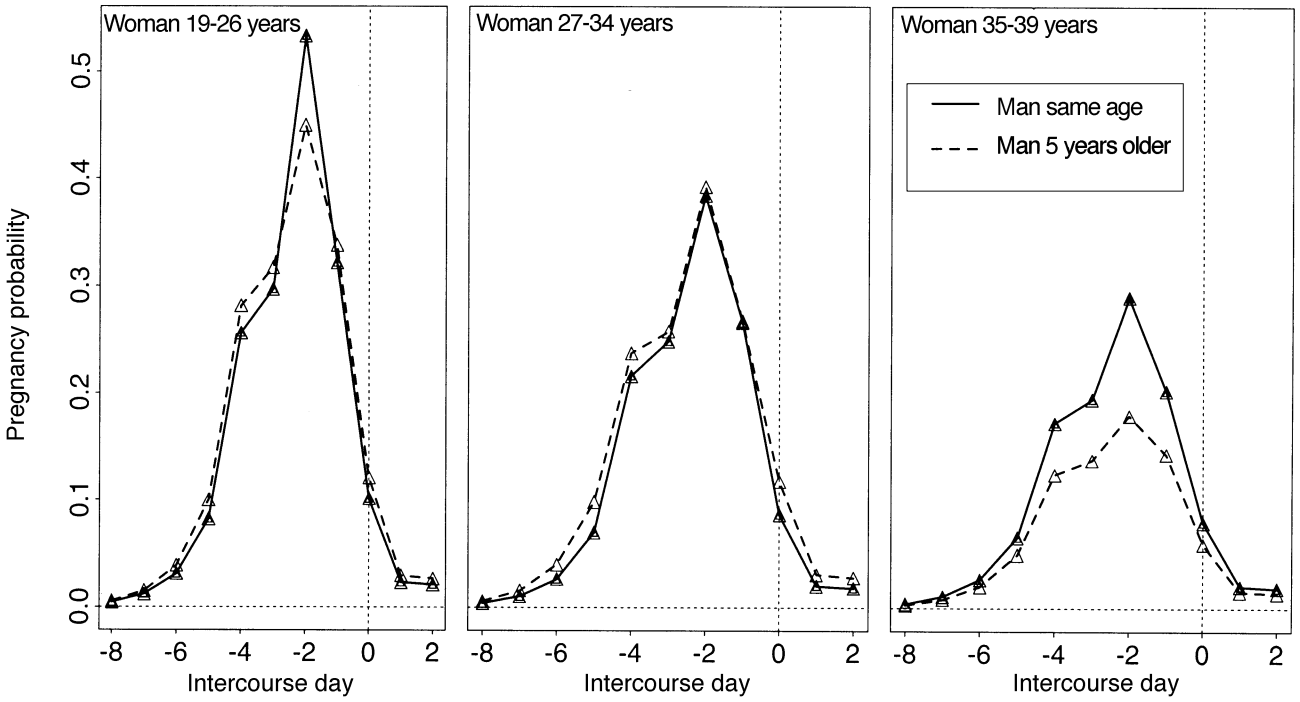

Figure 2. Probability of clinical pregnancy following intercourse on a given day relative to ovulation for women of average fertility aged 19–26, 27–29, 30–34 and 35–39 years having partners of the same age or 5 years older.

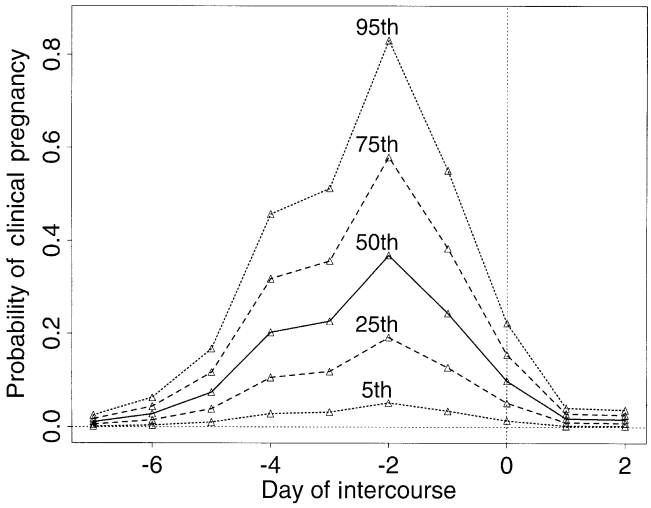

Figure 3. Probability of clinical pregnancy following intercourse on a given day relative to ovulation (day 0) for couples in the 5th, 25th, 50th, 75th and 95th percentiles of the population distribution of fertility among couples aged 27–29 years.

compared with 35 year old men with 35 year old partners. However, this difference was not statistically significant.

As expected, advancing female age was strongly associated with reduced fertility. The day-specific probabilities of pregnancy were observed to decline in women in their late 20s, slightly earlier than reported in the CECOS study of women with artificial insemination (Fédération CECOS, 1982). Nearly a 50% drop occurred between women in their early 20s and women in their late 30s. These estimates do not include the increased occurrence of spontaneous abortion that is evident in older women, but do include early, preclinical loss, which is not distinguishable from non-conception in these data.

Perhaps the most interesting result from our study is the observed decrease in fertility with male age, beginning in the late 30s. Studies of couples using assisted reproduction services show decreases in pregnancy rates with age starting as early as the 30s, but most are not statistically significant (Kidd *et al*., 2001). The limited data for non-clinical populations suggest reductions in fertility beginning in the 40s (Anderson, 1975). Our analysis is much more convincing than prior studies because it accounts for female age and variability in sexual behaviour.

There are several possible biological mechanisms for this decrease. Achieving a clinical pregnancy depends upon testicular production of sperm that can mature properly, survive insemination and passage through the female reproductive tract and remain viable until the oocyte is available, undergo capacitation and the acrosome reaction, penetrate the zona pellucida, fertilize, and provide sufficiently normal genetic material for early development. Genetic defects in the form of sperm chromosomal abnormalities increase in frequency with male age, and the increase can be seen as early as the 30s (Risch *et al*., 1987). This could adversely affect sperm function and early embryonic development. Sperm motility may decline at these early ages (Schwartz *et al*., 1983), but unless the male has quite low sperm counts, this alone is unlikely to directly affect fertility. Other semen characteristics are less predictably affected at such early ages (Schwartz *et al*., 1983; Kidd *et al*., 2001). Average FSH levels appear to increase in the 30s (Zumoff *et al*., 1982), suggesting that age-related changes in the gonadal–pituitary axis (Veldhuis, 1999) may begin during midlife. In addition, the testes and prostate show morphological

changes that might adversely affect both sperm production and the biochemical properties of the semen (Hermann *et al*., 2000). Though few age-related differences in semen biochemistry have been reported (Schirren *et al*., 1977), newly identified substances, such as fertilization-promoting peptide, that may be critical for sperm function (Fraser and Adeoya-Osiguwa, 2001) have not been investigated. Whatever the mechanism, the reduction in fertility with male age in these healthy couples suggests that male gamete 'overproduction' does not fully buffer against reproductive failure.

This study also documents the enormous heterogeneity in fertility among healthy couples that is not accounted for by age. The interquartile range in the probability of pregnancy on the peak day of the fertile window extends from a 20% probability of pregnancy to a 60% probability of pregnancy. Epidemiological studies have identified some of the factors associated with this variability in fertility, including prenatal exposures, sexually transmitted disease history, smoking, and occupational exposures (Bonde, 1999; Baird and Strassmann, 2000), but much of this heterogeneity remains unexplained.

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