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Subfertility reflects accelerated ovarian ageing

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BACKGROUND: The aim of the study was to explore the extent to which accelerated ovarian ageing may lead to subfertility early in reproductive life and eventually cause early menopause. METHODS: The population studied (*n* = 2393) never used oral contraceptives, hormone replacement therapy or an intrauterine device. Logistic regression analyses were performed using age at menopause as proxy for accelerated ovarian ageing. Measures of ovarian ageing and subfertility were menstrual cycle irregularity, ever consulted a physician for fertility problems, nulliparity, uniparity, miscarriage(s) and time interval >5 years between birth of first and second child. RESULTS: For every 5 years later menopause, the probability of reporting menstrual cycle irregularity was reduced by 26% (OR = 0.74, 95% CI: 0.63–0.86); the probability of ever consulting a physician for fertility problems was reduced by 18% (OR = 0.82, 95% CI: 0.71–0.95); the probability of staying nulliparous was reduced by 22% (OR = 0.78, 95% CI: 0.64–0.96); the probability of being uniparous was reduced by 22% (OR = 0.78, 95% CI: 0.66–0.91); the probability of having a miscarriage was reduced by 11% (OR = 0.89, 95% CI: 0.79–1.01); the probability of a large time interval between birth of first two children was reduced by 27% (OR = 0.73, 95% CI: 0.61–0.89). CONCLUSIONS: Fertility problems are frequently followed by early menopause. The findings support the view that both are an expression of accelerated ovarian ageing.

Key words: age at menopause/epidemiology/ovarian ageing/reproductive life/subfertility

Introduction

Ovaries, like other organs, age and finally lose their function. Menopause marks the definite end of the female reproductive life. Both age at natural menopause and fertility (a woman's capacity to reproduce) vary substantially between women. It has been suggested that the cumulative age distributions of reproductive events correspond with the cumulative distribution of age at menopause (te Velde *et al*., 1998). Furthermore, the distributions of age at last childbirth, which is considered the end of a female's fertile period, and of age at menopause are very similar (te Velde and Pearson, 2002). This implies that menopausal age and fertility may not be independent, but common underlying factors determine onset of fertility problems as well as age at menopause in individual women. In other words, fertility problems and age at menopause are likely to be expressions of the same underlying process, i.e. ovarian ageing. If early age at menopause is a marker for accelerated ovarian ageing, it may therefore be expected to be a retrospective indicator for fertility problems. The conventional way of analysing certain lifestyle factors and reproductive performance with age at menopause is to take age at menopause as the outcome measure. However, accepting menopausal age as retrospective proxy for ovarian ageing including age-related loss of fertility, it may be more appropriate to consider age at menopause as the determinant and indicators of subfertility as outcome measures. We set out to explore the extent to which ovarian ageing is expressed in fertility problems and in early age at menopause. Our hypothesis as well as the research question demand leaving the concept of regarding menopausal age as outcome. Consequently, although chronologically reverse, we studied menopausal age, as a proxy for ovarian ageing, as a determinant of indicators of subfertility. The population in which to address this research question is ideally non-contracepting. We were able to select a study population that consisted only of women who experienced natural menopause, never used oral contraceptives, never used an intra-uterine device and who reported whether or not they have tried to conceive.

Subjects and methods

Study population and age at natural menopause

The population for the present study was selected from the Prospect–EPIC (European Prospective Investigation into Cancer and Nutrition) project. The design and sampling

strategy of Prospect–EPIC has been described in detail elsewhere (Boker *et al*., 2002). Briefly, the Prospect cohort consisted of 17 357 women who were invited to join the study through an existing regional population-based programme of breast cancer screening. From 1993 to 1997, women aged 50–69 years living in the city of Utrecht and vicinity were enrolled in the study. All participants filled out detailed questionnaires on usual diet and on reproductive history, medical history and other risk factors for cancer. They underwent a brief medical examination and a blood sample was drawn.

In Figure 1 the selection procedure of our study population is outlined. Natural menopause was defined as proposed by the World Health Organization Scientific Group (1996) as ⩾12 consecutive months of amenorrhoea not due to surgery or other obvious causes. Women for whom age at natural menopause could not be determined were excluded. Natural menopause occurred in 6411 women of the initial sample. Women who were aged <58 years at time of recruitment were excluded (n = 2374) to avoid bias due to differential inclusion of women with an early age at menopause. Allowing women under this age into the study population would lead to a distortion of the age distribution of menopause. The number of women with a late age at menopause will be underestimated simply because women who were younger at the time of recruitment can only be included in the study population if they had an early menopause. At age 58 years, 97.8% of all women had reached menopause. Finally, we excluded women who had a unilateral oophorectomy pre-menopausally (n = 217) and women who ever used oral contraceptives (OC) or an intrauterine device (IUD) pre-menopausally (n = 1427). Our final study population thus consisted of 2393 women.

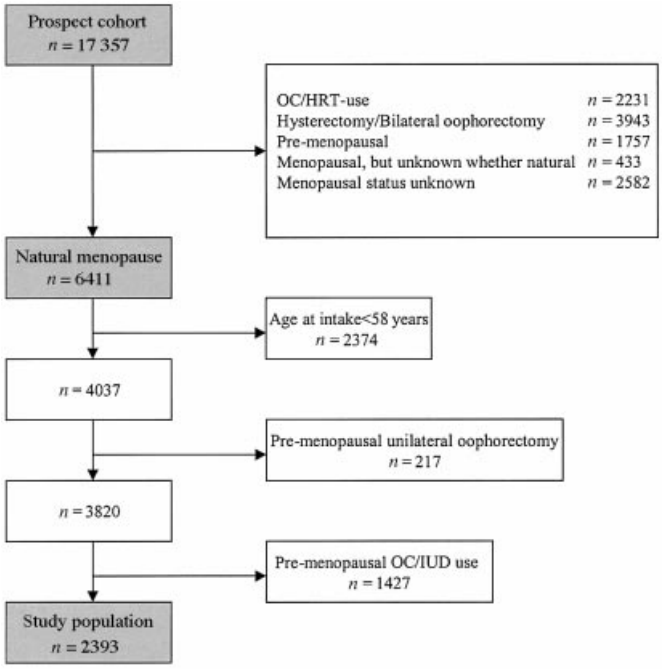

Figure 1. Flow chart representing the selection of the study population. OC = oral contraceptive; IUD = intrauterine device; HRT = hormone replacement therapy.

Table I. Characteristics of the 2393 women included in this study

Variable	Mean (SD) or %
Age at study recruitment (years)	63.8 (3.4)
Age at menopause (years)	49.9 (4.5)
Age at menarche (years)	13.8 (1.7)
Ever pregnant (%)	79.1
Any liveborn children (%)	78.3
Parity (if any children) (number)	3.0 (1.6)
Age at first childbirth (years)	26.3 (4.1)
Age at last childbirth (years)	32.3 (4.5)
Ever miscarriage (if ever pregnant) (%)	26.2
Ever had infertility consult (if ever tried to become pregnant) (%)	12.9
Diagnosed sub- or infertile (%)	2.9
Irregular menstrual cycle pattern (between ages 30 and 40 years) (%)	10.1
Smoking at time of menopause (%)	29.0
Marital status	
Married/living together (%)	67.0
Widow/divorced (%)	21.1
Unmarried (%)	11.9
Educational level	
Primary and intermediate education, general and vocational (%)	73.7
Secondary education, general and vocational (%)	19.5
Academic education (%)	6.8

Data analysis

Outcome variables

To define ovarian ageing and sub- or infertility, six reproductive correlates that are assumed to be a proxy for ovarian ageing or reduced fertility were chosen. These variables were: (i) having had an irregular menstrual cycle pattern between age 30 and 40 years, (ii) having consulted a physician for fertility problems, (iii) nulliparity, (iv) uniparity, (v) having had a miscarriage and (vi) a long time interval between birth of first and second child. The relationship between age at natural menopause and each of the above-mentioned variables was determined. For each analysis, an appropriate subpopulation was defined: (i) menstrual cycle irregularity, in all women reporting on menstrual cycle pattern (n = 2116). In all, 214 women reported irregular menstrual cycles. The information on menstrual cycle pattern concerned the period between age 30 and 40 years and irregularity of the menstrual cycle pattern was self-defined; (ii) subfertility, by studying women who ever consulted a medical doctor for fertility problems (n = 260) in all women who reported that they have tried to achieve pregnancy (n = 1996); (iii) nulliparity (n = 116), in women who reported that they have tried to achieve pregnancy (n = 1988); (iv) having only one child (n = 208), in all parous women (n = 1872); (v) at least one miscarriage (n = 495), in all women who were ever pregnant (n = 1888); (v) time interval >5 years between birth of first and second child (n = 112), in women with at least two live born children (n = 1274).

Confounders

Because of the potential for confounding, all analyses are adjusted for smoking and socio-economic status. Women were defined as smokers if they reported smoking pre-menopausally until time of menopause. Educational level was used to adjust for socio-economic status. In The Netherlands, the majority of women of this generation were not educated to their capacity nor

gainfully employed, but usually stayed home to take care of household and children. In the case of married women, educational attainment of the husband is considered a better indicator for socio-economic status of the household to which a woman belongs (Shinberg, 1999). So, for married women adjustments were made for the educational level of their spouse.

Statistical methods

Numbers and percentages of all reproductive correlates according to categories of menopausal age were computed. Six separate logistic regression analyses were performed producing an odds ratio (OR) and corresponding 95% confidence interval (CI) for each association between a subfertility measure and age at menopause. All analyses were performed using SPSS 10.1 for Windows. Age at menopause was collapsed into a variable with a 5-year difference in menopausal age per unit. This new variable was used as a continuous variable in the analyses. Therefore results can be interpreted as follows: the OR represent the risk for having the outcome variable, i.e. reduced reproductive potential, per unit change in menopausal age, e.g. a 5 year difference in age at menopause.

Results

General characteristics of the study population are presented in Table I. Mean age at menopause was 50 years (SD 4.5); 29% of the women reported being a current smoker when reaching menopause. Table II shows the distribution of the reproductive events by age at menopause. In Table III results for the relationship between menopausal age and risk of indicators of ovarian ageing and reduced fertility are shown. The risk of having irregular menstrual cycles between the ages of 30 and 40 years was decreased by 26% per 5 years menopause commenced later (OR = 0.74; 95% CI: 0.63–0.86). Compared with women with a given menopausal age, women with a 5 year later occurrence of menopause experienced an 18% decreased risk of having consulted a doctor for infertility problems (OR = 0.82; 95% CI: 0.71–0.95). Per five years later menopause, women had a 22% lower risk of staying nulliparous (OR = 0.78; 95% CI: 0.64–0.96). In the group of parous women, the probability of having only one child was reduced by 22% per 5 years later menopause (OR = 0.78; 95% CI: 0.66–0.91). The risk of ever having a miscarriage was related to an earlier age at menopause, without, however, reaching statistical significance (OR = 0.89; 95% CI: 0.79–1.01). In women with at least two children, women with a given age at menopause had a 27% reduced risk of having a time interval of >5 years between their first and second child, compared to women with age at menopause 5 years earlier (OR = 0.73; 95% CI: 0.61–0.89). Additional adjustment for breastfeeding the first child for >6 or <6 months did not affect the results. The association was not modified by age at birth of first child.

Discussion

The results unambiguously show that an earlier age at menopause is associated with a higher frequency of all investigated reproductive characteristics that reflect subfertility.

Table II. Reproductive characteristics of the study population

Age at menopause (years)	Irregular menstrual cycles		Consult for sub- or infertility		Nulliparity		Uniparity		Ever miscarriage		Interval between first and second child >5 years	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
<40	12 (5.6)	20 (1.1)	9 (3.5)	32 (1.8)	6 (5.2)	36 (1.9)	7 (3.4)	29 (1.7)	9 (1.8)	27 (1.9)	5 (4.5)	11 (0.9)
40–44	26 (12.1)	146 (7.7)	29 (11.2)	125 (7.2)	10 (8.6)	143 (7.6)	32 (15.4)	111 (6.7)	41 (8.3)	103 (7.4)	11 (9.8)	71 (6.1)
45–49	50 (23.4)	477 (25.1)	77 (29.6)	448 (25.8)	38 (32.8)	480 (25.6)	54 (26.0)	426 (25.6)	138 (27.9)	351 (25.2)	29 (25.9)	297 (25.6)
50–54	99 (46.3)	1019 (53.6)	113 (43.5)	913 (52.6)	53 (45.7)	973 (52.0)	87 (41.8)	886 (53.2)	253 (51.1)	724 (52.0)	58 (51.8)	618 (53.2)
⩾55	27 (12.6)	240 (12.6)	32 (12.3)	218 (12.6)	9 (7.8)	240 (12.8)	28 (13.5)	212 (12.7)	54 (10.9)	188 (13.5)	9 (8.0)	165 (14.2)
Total	214 (100)	1902 (100)	260 (100)	1736 (100)	116 (100)	1872 (100)	208 (100)	1664 (100)	495 (100)	1393 (100)	112 (100)	1162 (100)

Values in parentheses are percenpboard1tages.

Table III. Relationship between increased age at menopause and risk of indicators of ovarian ageing and reduced fertility

Outcome	No. of women in subpopulation (no. of cases)	Adjusted OR (95% CI)[a]	*P*
Irregular cycles between age 30–40	2116 (214)	0.74 (0.63–0.86)	< 0.001
Ever consult for sub- or infertility	1996 (260)	0.82 (0.71–0.95)	0.007
Nulliparity	1988 (116)	0.78 (0.64–0.96)	0.02
Uniparity	1872 (208)	0.78 (0.66–0.91)	0.002
Ever a miscarriage	1888 (495)	0.89 (0.79–1.01)	NS (0.06)
Interval of >5 years between first and second child	1274 (112)	0.73 (0.61–0.89)	0.001

[a]Adjusted for smoking and educational level.
OR = odds ratio; CI = confidence interval; NS = non-significant.

To appreciate the findings, some aspects of the study need to be discussed. An irregular menstrual cycle pattern has previously been described as a strong predictor for reduced fertility (Kolstad *et al*., 1999). Unfortunately, it appears that no unequivocal description is available to define an irregular menstrual cycle pattern. Comparisons of results between studies should therefore be made with care.

This study is the first to report data on age at menopause in relation to reduced fertility as defined by having consulted a physician for fertility problems. The group of women who sought medical advice for fertility problems is possibly heterogeneous as for underlying cause resulting in their sub- or infertility. However, this misclassification of sub- or infertile women occurred independently of menopausal age and is thus non-differential. Non-differential misclassification leads to an underestimation of the true association.

To assess the relationship between menopausal age and nulliparity, a major advantage of our study is that the group of nulliparous women excluded women who by choice remained nulliparous and thus consisted only of women who reported that they tried to conceive. Still, non-differential misclassification could have been introduced due to a male factor resulting in nulliparity of a couple. In general, this leads to underestimation of the effect.

Two previous studies described the relationship between miscarriages and subfertility. Both concluded that women with a history of subfertility, defined as failure to conceive within 1 year and difficulty achieving conception and seeking a physician's help respectively, have increased rates of (subclinical) early pregnancy loss, relative to women without such a history (Hakim *et al*., 1995; Gray and Wu, 2000).

Time to pregnancy is widely used to estimate the degree of subfertility (Baird *et al*., 1986; Greenhall and Vessey, 1990). The outcome variable 'time interval between birth of first and second child' is a substitute for time to pregnancy as we were unable to directly determine time to pregnancy. A consequence is that subfertility in this analysis is of relatively minor magnitude, because all women in this studied subpopulation were able to conceive at least twice. However, since we were able to exclude all oral contraceptive users and all intrauterine device users, the interval between first and second child comprises for a major part unintentional waiting time.

In previous studies, several of these indicators of reproductive potential were shown to be related to age at menopause (Stanford *et al*., 1987; Whelan *et al*., 1990; Parazzini *et al*., 1992; Cramer *et al*., 1995a; Bromberger *et al*., 1997; Hardy and Kuh, 1999; Gold *et al*., 2001). These studies imply a causative relationship between the factor and menopausal age. We hypothesize that both are related to (a) common factor(s), causing accelerated ovarian ageing.

Inconsistent results have been reported on menstrual cycle pattern and age at menopause (Stanford *et al*., 1987; Whelan *et al*., 1990; Bromberger *et al*., 1997). In our data, early menopause was related to a higher risk for an irregular cycle pattern. The lack of agreement can be due to differences in defining irregular menstrual cycle pattern. Our data on menstrual cycle pattern concern the age period of 30–40 years, while earlier studies based their results on the menstrual cycle pattern at an earlier age. It is however unlikely that the results are distorted by this, because the median age of inception of peri-menopause is at age 47–48 years and lasts for 4–5 years (McKinlay *et al*., 1992; Den Tonkelaar *et al*., 1998; Hardy and Kuh, 1999). For the majority of women, the menstrual cycle pattern between ages 30 and 40 years represents their normal pre-menopausal pattern. This is endorsed by the fact that excluding women with menopause before 45 years from the analysis did not change the results.

Most studies agree on parity delaying menopause. The proposed mechanism for this is that during pregnancy and lactation, oocytes are 'saved' and menstrual cycles continue longer (Stanford *et al*., 1987; Whelan *et al*., 1990; Cramer *et al*., 1995b). We suggest two alternative explanations. Firstly, following our hypothesis, women subjected to accelerated ovarian ageing are likely to have fewer children. Accelerated ovarian ageing is also reflected in an earlier age at menopause and consequently low parity is correlated with earlier age at menopause and high parity with later age at menopause. The second explanation is methodological. Relating two age-dependent factors may induce bias: women experiencing menopause at higher age by definition had more chance of having more children. In this study, we bypassed this problem because we examined the risk of having only one child in parous women.

Fertility declines with increasing age in all women (Krey *et al*., 2001). Therefore, the relationship between age at menopause and the time interval between birth of first and second child was tested for effect modification by age at first child birth, but was found to be absent. Analogous with the reasoning described for parity, a positive relationship is expected between age at menopause and the time interval, even in absence of a true association. Again, this is because both are age-dependent. To illustrate, women with a later age at

menopause have by definition had more chance to have a large time interval between birth of the children. Our results show an opposite relationship, a higher age at menopause decreases the risk of a large time interval, emphasizing the strength of this relationship.

The use of age at menopause in our analyses as a 'determinant' may be confusing because it is a parameter that can only chronologically be assessed after the 'outcome'. Indeed it would have been better to use a measure of ovarian ageing that preceded the fertility problems. Unfortunately, such a measure is not available. In the cohort we included women that had all reached menopause. Consequently, an ultimate measure for ovarian ageing, age at menopause, could be used. If age at menopause may indeed be used as a measure for accelerated ovarian ageing, and if the aim is to explore the role of accelerated ovarian ageing in (causing) fertility problems, then it makes sense to consider it a determinant in a relationship in which fertility problems are the outcome.

The question remains what determines ovarian ageing. The most consistently reported environmental factor to influence age at menopause is smoking. Smoking is proposed to decrease the size of the follicle pool (Westhoff *et al*., 2000), to interfere with the pituitary–ovarian axis, and to have an effect on the metabolism of sex hormones (Midgette and Baron, 1990). Also, it has been found that chemicals in cigarette smoke up-regulate a pro-apoptosis gene resulting in enhanced follicular damage and premature ovarian failure in mice (Matikainen *et al*., 2001). All mechanisms are plausible to accelerate ovarian ageing. Smoking and other factors that might influence the oocyte pool explain only a small part of the large variation in menopausal age (Van Noord *et al*., 1997). Other arguments for the possible underlying mechanisms of accelerated ovarian ageing such as the importance of the fetal period, selection and growth of oocytes/follicles from the follicle pool, possible endocrine pathways and genetic determinants have recently been reviewed (te Velde and Pearson, 2002). Twin studies (Snieder *et al*., 1998; Treloar *et al*., 1998) and a sibling study (De Bruin *et al*., 2001) have shown age at menopause to be heritable to a large extent. Genes, or genes in interaction with environmental factors, are therefore good candidates to have a major impact on ovarian ageing. Identifying these genes and their function will help us understand female reproductive ageing.

In conclusion, the results of this study show that fertility problems are frequently followed by early menopause. The findings support the view that both are an expression of accelerated ovarian ageing.

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