Cohabitation, infection and breast cancer risk.

Leo J Kinlen[1*], Clare Gilham[2], Roberta Ray[3], David B Thomas[3] and Julian Peto[2]

1. Cancer Research UK Cancer Epidemiology Unit, Nuffield Department of Population Medicine, University of Oxford, Richard Doll Building, Roosevelt Drive, OX3 7LF, UK.

2. Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

3. Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA 98104, USA.

*Correspondence to Prof L J Kinlen:

leo.kinlen@gtc.ox.ac.uk

+44 (0)1865 289600

Key words: breast cancer, age at marriage, age at first birth, cohabitation, infection

Abbreviations:

AFB	age at first birth
AFM	age at first marriage
AFSR	age at first sexual relationship
CI	confidence interval

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ijc.33319

CSNSC WHO Collaborative Study of Neoplasia and Steroid Contraceptives

IMFB interval from marriage to first birth

OC oral contraceptive

OR odds ratio

SES socioeconomic status

SCS Seven Country Study

Novelty statement

Reanalysis of a large international case-control study suggests that age at marriage (a surrogate for age at beginning cohabitation) is strongly associated with breast cancer risk in parous and in nulliparous women. The effect of age at first birth that has been accepted for 50 years appears to be due largely and perhaps entirely to confounding. This surprising conclusion should be tested in other data. An underlying infective mechanism might be targeted to prevent breast cancer.

Abstract

For 50 years the effect of age at first birth (AFB) has been thought to explain the strong association between breast cancer risk and age at first marriage (AFM), which was first reported in 1926. The independent effects of AFM, AFB and number of sexual partners adjusted for parity and other risk factors were estimated in reanalysis of a large international case-control study conducted in 1979-82 (2,274 breast cancers, 18,209 controls) by unconditional logistic regression. Respective AFB and AFM breast cancer odds ratios (ORs) for ≥31 years relative to ≤18 years were 3.01 (95%CI 2.44-3.71; p(trend)<0.0001) and 3.24 (95%CI 2.62-4.01; p(trend)<0.0001) in univariate analyses. Among married parous women these ORs fell to 1.38 (95%CI 0.98-1.95; p(trend)<0.03) for AFB and 1.70 (95%CI 1.17-2.46; p(trend) < 0.002) for AFM when fitted together in multivariate analysis including other risk factors. A similar adjusted OR for AFM ≥31 years relative to ≤18 years was seen among married nulliparous women (OR 1.71, 95%CI 0.98-2.98; p(trend)<0.001). AFM (a surrogate for age at starting prolonged cohabitation) is thus strongly associated with breast cancer risk. This suggests an effect of close contact. Identifying the (probably infective) mechanism might lead to effective prevention of breast cancer. The independent effect of AFB is smaller and could be due to residual confounding.

Introduction

Early case-control studies by Lane-Claypon[1] and others[2, 3] reported a marked association of breast cancer risk with late age at first marriage (AFM), which at that time was a good surrogate for age at beginning cohabitation. This was investigated in 1970 in the Seven Country Study (SCS)[4] by MacMahon and colleagues, who concluded that the apparent effect of AFM was entirely due to its strong correlation with age at first birth (AFB). This was despite a positive trend with increasing AFM among married nulliparous women for which they offered no explanation. More recently some effect of AFM was also noted in parous women in data from one of the SCS centres[5, 6]. These observations led us to analyse the effects of AFM and AFB in a large international study of similar size to the SCS.

Methods

The WHO Collaborative Study of Neoplasia and Steroid Contraceptives (CSNSC) was carried out in 1979-82 and included 2,760 cases of breast cancer and 18,381 controls aged <65 years. The methods have been described previously[7, 8]. The 12 participating centres were in ten countries: Australia, Chile, China, Colombia, East Germany, Israel, Kenya, Mexico, the Philippines and Thailand (three centres). Stata 15.1 was used to calculate odds ratios (ORs) for invasive breast cancer by unconditional logistic regression. Table 1 shows univariate ORs for each variable adjusted for age (five-year age groups) and centre (n=12). Subsequent analyses were adjusted for age, centre, oral contraceptive (OC) use and socioeconomic status (SES), and were restricted to the 98.7% (2,724/2,760) of cases and 99.1% (18,209/18,381) of controls with complete data on AFM (excluding 5 cases and 27 controls reporting first intercourse >2 years after marriage), AFB, parity (number of live births), duration of breastfeeding, age at first sexual relationship (AFSR) and number of sexual partners. The effects of AFM and AFSR in nulliparous women are shown in table 2. In married parous women AFM and years from first intercourse to marriage were fitted jointly (table 3). Table 4 shows multivariate regression results among married parous women fitting AFM, AFB, parity, duration of breast feeding and number of partners (model 1) and the effect of omitting each variable (models 2 to 6). For each fitted variable the significance of the difference in trend among married parous women between women aged 50-64 v. below age 50 years and between more v. less developed countries (table 1S) was assessed by including an interaction term. For trend analyses each categorical variable was refitted as continuous retaining other variables as categorical. Table 5 shows estimates of the increase in OR per year for AFM, AFB and interval from marriage to first birth (IMFB) in married parous women when these variables were fitted individually and in pairs. All significance levels are 2-sided.

Results

Table 1 shows univariate ORs adjusted only for age and centre for each variable. The trend in OR is negative for parity, duration of breastfeeding and number of sexual partners and positive for AFB, AFM, AFSR and higher SES ($p<0.0005$ for all these trends). The OR is significantly elevated for current OC use of ≥5 years' duration (heterogeneity $p=0.0002$). Few women had 5 or more alcoholic drinks per week, and alcohol consumption, which showed no effect on risk (p(trend)=0.9), was not considered further. Subsequent analyses were adjusted by including age, centre, OC use and SES in all models categorized as in table 1. The analyses in table 2, which are restricted to nulliparous women and adjusted for variables not related to childbirth, show a highly significant trend with increasing AFM in married nulliparous women (p(trend)<0.001) and a weak and non-significant trend with increasing AFSR (p(trend)=0.13) which is further reduced when these variables are fitted jointly (p(trend)=0.6).

The independent contributions of AFM and time from first sexual relationship to marriage in married parous women are shown in table 3. The trend with AFM remains significant after adjustment for all variables (p(trend)=0.002), but time from first intercourse to marriage has no independent effect (p(trend)=0.8). AFSR was therefore omitted in subsequent analyses. The independent effects of AFM, AFB, parity, duration of breast feeding and number of sexual partners were investigated in married parous women by fitting all five variables (table 4 model 1) and each subset of four variables (table 4 models 2 to 6). Effect estimates for each variable among unmarried parous women (29 cases, [illegible] controls) were consistent with the estimates unadjusted for AFM among married parous women (table 4 model 2) but were very imprecise due to small numbers and are uninformative on the joint effects of AFM and AFB (data not shown). In the fully adjusted analysis restricted to married parous women (table 4 model 1) the quantitative effect of each variable is lower than in the univariate analyses in table 1. Fully adjusted significance levels for trend in married parous women are 0.001 for AFM, 0.03 for AFB, <0.0001 for parity, 0.02 for number of partners and 0.04 for duration of breast feeding. These trends are quantitatively similar in more developed v. less developed countries and below age 50 v. 50-64 years (table 1S: p>0.3 for all differences between trends). Correlations between these variables and IMFB In married parous controls are shown in table 2S. There was a strong correlation between AFM and AFB (correlation = 0.84) but a negative and much weaker correlation between AFM and IMFB (correlation = -0.07). AFB equals AFM plus IMFB, so the independent effects of these variables were estimated by fitting each pair (table 5). The OR for IMFB showed little or no effect when fitted alone (p(trend) > 0.9) or jointly with AFM (p(trend) > 0.3) but decreased sharply with increasing IMFB (3.9% per year, p(trend) = 0.003) when fitted together with AFB.

Discussion

The effect of AFM

Table 2 shows the marked effect of AFM on breast cancer risk in married nulliparous women (table 2: p(trend)<0.001) unconfounded by AFB and parity, and a weak trend with AFSR (p(trend) > 0.1) that disappears (p(trend) = 0.6) when AFM and AFSR are fitted together. Table 3 shows similar results in married parous women after adjusting for other variables including AFB and parity. There is a consistent and significant trend with increasing AFM (last row, table 3; p(trend) = 0.002), and no evidence that time from first intercourse to marriage has any independent effect (p(trend)=0.8). AFSR was therefore ignored in subsequent analyses. Table 4 shows results in married parous women for AFM, AFB, parity, duration of breast feeding and number of partners in the fully adjusted multiple regression (model 1). The fully adjusted ORs for AFM (p(trend) = 0.001) are almost identical to those shown in table 3, the only difference between the analyses being exclusion of AFSR from the model. Adjusted ORs for AFB also increase (p(trend) = 0.03) but are lower than for AFM at each age. The reduction in risk with increasing numbers of sexual partners is statistically significant (p(trend) = 0.002) but weak (OR relative to 1 partner = 0.85 for 2 partners, 95%CI 0.73-0.99;

0.78 for >2 partners, 95%CI 0.66-0.93). The estimated effects of breast feeding and high parity are similar to estimates based on pooled analysis of the worldwide data[9].

Table 4 also shows the effect of omitting each of these factors from the regression (models 2 to 6). OR estimates for number of partners are almost identical in all models. Parity and duration of breast feeding are highly correlated (table 2S: correlation = 0.64), and the estimated effect of each is inflated when the other is omitted (models 4 and 5) but unaltered by excluding other factors. Excluding parity in the multiple regression substantially inflates the trend in risk for AFB but not for AFM (model 1 v. model 4), consistent with the evidence from nulliparous women that the effect of AFM is independent of childbirth. ORs for AFM are increased by excluding AFB (model 1 v. model 3) but are virtually unaltered by excluding other factors, and ORs for AFB are increased by excluding AFM (model 1 v. model 2). We conclude that age at marriage (i.e. beginning prolonged cohabitation) is a cause or correlate of an important risk factor in both nulliparous and parous women, and that the estimated effect of AFB is substantially inflated if AFM is not adjusted for.

AFM and AFB are strongly correlated (table 2S: correlation = 0.84) because AFB equals AFM plus IMFB. The estimates of the effects of AFM and AFB when fitted together may therefore be distorted by residual confounding[12]. However, AFM and IMFB are likely to be reported reliably and are virtually uncorrelated (correlation = -0.07), and their estimated effects are similar whether fitted separately (table 5: increase in OR per year 5.2%, 95%CI 3.9%-6.4% for AFM; 0.1%, 95%CI -2.4%-2.6% for IMFB) or together (increase in OR 5.5% per year, 95%CI 4.2%-6.9% for AFM; 1.3% per year, 95%CI -1.2%-3.9% for IMFB). These results confirm the strong independent effect of AFM and the weaker (and possibly negligible) independent effect of IMFB and hence of AFB. IMFB and hence AFB would be associated with increased risk if less fertile women were at higher risk and took longer to conceive, but no association between delay in conception and subsequent breast cancer risk was seen in British women trying to conceive[10]. AFM equals AFB minus IMFB, so if AFM is the relevant risk factor and neither AFB nor IMFB has any independent effect the joint analysis of AFB and IMFB excluding AFM would be expected to show the strong positive trend with AFB and strong negative trend with IMFB seen in the right-hand column of table 5. Evidence from larger studies is needed to estimate the independent effect of AFB (and ages at subsequent births[11]) more precisely, but the strong negative trend with IMFB when fitted jointly with AFB confirms that AFM is an important risk factor.

Origin of the assumption that the effect of AFM is due to confounding by AFB

The association of breast cancer with late AFM was first reported by Lane-Claypon in 1926[1], but in 1970 McMahon and colleagues[4] concluded from their analysis of the SCS that any effect of AFM is entirely accounted for by the effect of AFB. They wrote:

"The pooled data for all centres do suggest lower risks for nulliparous women married under the age of 25 years than for those married later. However, relative to the trend in risks associated with age at first birth, that with age at marriage is weak. In addition, the deficit of cases observed among nulliparous women first married under the age of 20 years is confined to 2 centres. If these are excluded the trend disappears. We have no explanation for the appearance of this feature in these two centres. In view of the relatively small change in risk associated with it and its limitation to 2 of the 7 centres, we conclude that early marriage is not associated with reduction in risk of cancer of the breast, unless it is associated with early confinement"[4].

Despite the weakness of this rationale the role of AFM seems never to have been reconsidered. In a study investigating whether late AFB reflects difficulty in conceiving, and hence some hormonal aspect affecting breast cancer risk, about 80% of the difference in AFB between cases and controls was accounted for by differences in their ages at starting regular sexual activity[10]. An effect of cohabitation was not considered, however. AFM was even used as a surrogate for AFB in a recent reanalysis of two early studies[12]. Our data suggest the opposite conclusion: that some effect of cohabitation substantially influences breast cancer risk, and that most of the unadjusted effect of AFB is due to its correlations with AFM, parity and other factors (AFB ≥31 years v. ≤18 years: unadjusted OR 3.01 in table 1, fully adjusted OR 1.38 in table 4 model 1).

In the original SCS report the effect of AFM in parous women was not analysed[4], but evidence of an effect in parous women in the SCS data from Wales[5] prompted one of us (LK) to ask Dimitrios Trichopoulos, one of the original SCS investigators, to examine the joint effects of AFM and AFB in parous women in the SCS. He died before this analysis was completed and we no longer have access to the SCS data, but the results of the preliminary analysis of the independent effects of AFB and AFM, fitted jointly but unadjusted for parity and the other factors in table 4, are similar to the CSNSC results analysed in the same format (table 3S). Both studies show a marked trend with increasing AFM ($p<0.001$) and a weaker although still significant trend with increasing AFB ($p<0.001$). A long-standing hypothesis was thus discarded inappropriately, as the study generally thought to have shown that AFM has no independent effect on breast cancer risk in fact suggests a marked effect. The protective effect of high parity may have made a protective effect of young AFB seem plausible, perhaps contributing to the failure by cancer epidemiologists over the last half century to examine the joint effects of AFB and AFM in other studies; but in fact there are no strong grounds for expecting AFB to affect breast cancer risk merely because high parity is protective. The risk of ovarian cancer also declines with increasing parity but is unaffected by age at first birth [13]. AFM is strongly related to breast cancer risk in the only three datasets in which we could estimate its effects independent of or adjusted for AFB (nulliparous and parous women in the CSNSC and the overall results of the SCS). Our results imply that the marked effect of AFB unadjusted for AFM seen in these data and in every other study was greatly inflated by failure to adjust for AFM. Pending joint analysis of AFM and AFB in other studies we conclude that there is no published evidence that AFB is an important risk factor for breast cancer. The majority of unmarried women were virgins in the CNSNC but cohabitation is now common, so age at beginning prolonged cohabitation rather than AFM must be adjusted for in more recent studies.

The effects of early and later cohabitation on breast cancer risk

Nulliparous women (table 2) were reanalysed with the unmarried, 82% of whom were virgins, as the reference group. The risk is lower in those who marry before age 25 (OR 0.82 95%CI 0.63-1.06) but higher in those who marry after age 25 (OR 1.50, 95%CI 1.12-2.03), suggesting that early marriage is protective and late marriage increases breast cancer risk compared with unmarried women. However, beginning cohabitation could be slightly protective even after age

30 if women who will remain single are atypical in other ways that reduce their risk, perhaps having a pattern of interpersonal contacts different from women who will marry. Conversely, it is also possible that women who remain single are at greater risk for other reasons and cohabitation increases the risk slightly when it begins before age 19 and more when it begins later. The lower risk in women with more than one partner suggests a protective rather than a carcinogenic effect, but pending discovery of the underlying mechanism(s) this may be difficult to resolve.

Possible explanations of the effect of cohabitation

The evidence that earlier age at beginning cohabitation is associated with reduced breast cancer risk seems strong and consistent, but we can only speculate on the explanation. That cohabitation, an extreme example of interpersonal contact, may influence breast cancer risk is more plausible now than 50 years ago. Interpersonal contacts are central in the biology of infections some of which, while mainly immunising, have malignancy as a rare response. Several such carcinogenic infections are now known[14], although the cause of the significant excesses of childhood leukaemia following the new contacts promoted by sudden large influxes into rural areas, where susceptible individuals are likely to be more prevalent[15], remains undiscovered. A recent review concluded that oncogenic viruses are the major plausible hypothesis for a direct cause of human breast cancer[16] while another concluded that they are unlikely to play any significant role[17]. Earlier exposure to an oncogenic infection might reduce its long-term effect through a mechanism analogous to the reduced risk of paralysis following early poliovirus infection, but such a pattern has not been shown for any infective cause of human malignancy. Highly infectious sexually transmitted agents are unlikely to be involved. The risk of cervical cancer, which is caused almost entirely by sexually transmitted human papillomaviruses, is strongly associated with early age at first intercourse and increases steeply with increasing numbers of sexual partners. In contrast, early age at first intercourse has no detectable effect on breast cancer risk, and the OR is slightly lower for 2 sexual partners than for 1 but shows little further reduction for 3 or more partners (OR for >2 v. 2 partners 0.91, 95% CI 0.75-1.11, p = 0.4). This suggests a protective effect requiring prolonged contact with a partner. Recent findings suggest that more complex infective mechanisms, including effects of the microbiome on the immune system, may also affect tumorigenesis[18, 19]. The epidemiology of breast cancer in relation to menarche, childbirth and menopause as well as OC use and hormone replacement therapy is plausibly accounted for by hormonal effects. An influence on differentiating mammary cells via circulating hormone levels was proposed to explain the strong protective effect of high parity[20], and Pike and colleagues[21] suggested that changes in breast cancer incidence associated with menarche, birth and menopause are consistent with concomitant changes in breast stem cell division rates. Both gut and mammary microbiota may influence breast cancer risk by hormonal mechanisms[22]. A dose-related protective immunological response to sperm[23, 24] has been suggested, but an effect of the microbiome, perhaps through anticancer immune responses[19], may be more plausible. Correlates of prolonged cohabitation include convergence of gut microbiota between couples and increased diversity of gut microbiota compared with those who live alone[25]. Microbiota at other sites, including the breast, could also be relevant.

We hope that our results will encourage others with relevant data to examine the effects on breast cancer risk of age at beginning cohabitation, number of long-term relationships, parity and ages at first and subsequent births. Confirmatory evidence that age at beginning prolonged cohabitation influences risk and accounts for much of the effect of age at childbirth would be important whatever the mechanism. If infection were involved transmission must occur within families and through other social contacts as well as between couples. The resulting variation in risk in the general population could be greater than the variation associated with age at beginning cohabitation, and might even account for a substantial proportion of the non-genetic familial risk. However, contacts before menarche when the breast has not developed or in childhood while the immune system is still evolving could be less important than later exposures. If an underlying infective process were identified it might be targeted to reduce breast cancer incidence.

Acknowledgements

[illegible] is very grateful to Valerie Beral for providing facilities in her Unit.

Statement of authorship

LK conceived the idea for the analysis and wrote the first draft of the report. CG conducted the statistical analyses. RR prepared the data and performed initial statistical analyses. DT provided the data and suggested further analyses. JP advised on the statistical analyses and revised the report. LK had full access to all the data and had final responsibility for the decision to submit for publication. All authors contributed to revision of the manuscript and approved the final version.

Conflict of interest

None of the authors declares any conflict of interest.

Data availability statement

The data that support the findings of this study are available from RR upon reasonable request.

Funding

No funding was received for this work.

References

1. Lane-Claypon JE. A further report on cancer of the breast with special reference to its associated antecedent conditions. Reports on Public Health and Medical Subjects no. 32. London: HMSO; 1926.
2. Wainwright JM. A Comparison of Conditions Associated with Breast Cancer in Great Britain and America. American Journal of Cancer. 1931;15:2610-45

3. Stocks P. Social status in relation to carcinoma of the breast. Schweiz Z Pathol Bakteriol. 1955;18(4):706-17

4. MacMahon B, Cole P, Lin TM, Lowe CR, Mirra AP, Ravnihar B, et al. Age at first birth and breast cancer risk. Bull World Health Organ. 1970;43(2):209-21

5. Kinlen LJ. Breast cancer and ages at first marriage and first birth: a new hypothesis. Eur J Cancer Prev. 2014 Jan;23(1):53-7

6. Lowe CR, MacMahon B. Breast cancer and reproductive history of women in South Wales. Lancet. 1970 Jan 24;1(7639):153-6

7. WHO. Invasive cervical cancer and combined oral contraceptives. WHO collaborative study of neoplasia and steroid contraceptives. Br Med J (Clin Res Ed). 1985 Mar 30;290(6473):961-5

8. WHO. Breast cancer and combined oral contraceptives: results from a multinational study. The WHO Collaborative Study of Neoplasia and Steroid Contraceptives. Br J Cancer. 1990 Jan;61(1):110-9

9. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. Lancet. 2002 Jul 20;360(9328):187-95

10. Vessey MP, McPherson K, Roberts MM, Neil A, Jones L. Fertility in relation to the risk of breast cancer. Br J Cancer. 1985 Oct;52(4):625-8

11. Wohlfahrt J, Melbye M. Age at any birth is associated with breast cancer risk. Epidemiology. 2001 Jan;12(1):68-73

12. Press DJ, Pharoah P. Risk factors for breast cancer: a reanalysis of two case-control studies from 1926 and 1931. Epidemiology. 2010 Jul;21(4):566-72

13. Gaitskell K, Green J, Pirie K, Barnes I, Hermon C, Reeves GK, et al. Histological subtypes of ovarian cancer associated with parity and breastfeeding in the prospective Million Women Study. Int J Cancer. 2018 Jan 15;142(2):281-9

14. Kinlen L. Infections and immune factors in cancer: the role of epidemiology. Oncogene. 2004 Aug 23;23(38):6341-8
15. Kinlen LJ. An examination, with a meta-analysis, of studies of childhood leukaemia in relation to population mixing. Br J Cancer. 2012 Sep 25;107(7):1163-8
16. Lawson JS, Salmons B, Glenn WK. Oncogenic Viruses and Breast Cancer: Mouse Mammary Tumor Virus (MMTV), Bovine Leukemia Virus (BLV), Human Papilloma Virus (HPV), and Epstein-Barr Virus (EBV). Front Oncol. 2018;8:1
17. Gannon OM, Antonsson A, Bennett IC, Saunders NA. Viral infections and breast cancer - A current perspective. Cancer Lett. 2018 Apr 28;420:182-9
18. Salmon H, Remark R, Gnjatic S, Merad M. Host tissue determinants of tumour immunity. Nat Rev Cancer. 2019 Apr;19(4):215-27
19. Zitvogel L, Ayyoub M, Routy B, Kroemer G. Microbiome and Anticancer Immunosurveillance. Cell. 2016 Apr 7;165(2):276-87
20. Russo J, Moral R, Balogh GA, Mailo D, Russo IH. The protective role of pregnancy in breast cancer. Breast Cancer Res. 2005;7(3):131-42
21. Pike MC, Krailo MD, Henderson BE, Casagrande JT, Hoel DG. 'Hormonal' risk factors, 'breast tissue age' and the age-incidence of breast cancer. Nature. 1983 Jun 30;303(5920):767-70
22. Fernandez MF, Reina-Perez I, Astorga JM, Rodriguez-Carrillo A, Plaza-Diaz J, Fontana L. Breast Cancer and Its Relationship with the Microbiota. Int J Environ Res Public Health. 2018 Aug 14;15(8):1747
23. Rossing MA, Stanford JL, Weiss NS, Daling JR. Indices of exposure to fetal and sperm antigens in relation to the occurrence of breast cancer. Epidemiology. 1996 May;7(3):309-11
24. Li X, Hemminki K. Cancer risks in women who had children with different partners from the Swedish Family-Cancer Database. Eur J Cancer Prev. 2002 Oct;11(5):433-8
25. Dill-McFarland KA, Tang ZZ, Kemis JH, Kerby RL, Chen G, Palloni A, et al. Close social relationships correlate with human gut microbiota composition. Sci Rep. 2019 Jan 24;9(1):703

Table 1. WHO Collaborative Study of Neoplasia and Steroid Contraceptives. Numbers of cases and controls, and univariate odds ratios (OR) adjusted for age and centre for each other variable.

	Cases (n=2,760) n (%)	Controls (n=18,381) n(%)	OR (95% CI)	p-trend
Centre				
Siriraj Hospital, Thailand	266 (9.6)	3552 (19.3)		
Chiang Mai, Thailand	262 (9.5)	3025 (16.5)		
Chulalongkorn, Thailand	259 (9.4)	3036 (16.5)		
Israel	671 (24.3)	1645 (9.0)		
Mexico	143 (5.2)	1729 (9.4)		
GDR	504 (18.3)	1215 (6.6)		
Philippines	162 (5.9)	977 (5.3)		
Chile	142 (5.1)	946 (5.2)		
Australia	81 (2.9)	702 (3.8)		
China	189 (6.9)	621 (3.4)		
Kenya	46 (1.7)	714 (3.9)		
Colombia	35 (1.3)	219 (1.2)		
Age group at diagnosis/interview				
15-24	20 (0.7)	1950 (10.6)		
25-29	100 (3.6)	1697 (9.2)		
30-34	252 (9.1)	2126 (11.6)		
35-39	390 (14.1)	2939 (16.0)		
40-44	599 (21.7)	3001 (16.3)		
45-49	791 (28.7)	3336 (18.2)		
50-54	516 (18.7)	2495 (13.6)		
55-64	92 (3.3)	837 (4.6)		
Socioeconomic status[1]				<0.0001
professional	461 (16.7)	1746 (9.5)	1.00 (ref)	
skilled worker	1490 (54.0)	8241 (44.8)	0.70 (0.62-0.79)	
unskilled worker	744 (27.0)	7375 (40.1)	0.57 (0.49-0.66)	
student/unemployed/no data	65 (2.4)	1019 (5.5)	0.74 (0.55-0.99)	
Oral Contraceptive (OC) use				0.0002[2]
never	1709 (61.9)	11443 (62.3)	1.00 (ref)	
current OC use, <5 yrs duration	73 (2.6)	809 (4.4)	1.18 (0.91-1.54)	
current OC use, ≥5 yrs duration	183 (6.6)	673 (3.7)	1.54 (1.27-1.86)	
past OC use, <5yrs duration	133 (4.8)	972 (5.3)	1.15 (0.94-1.40)	
past OC use, ≥5yrs duration	101 (3.7)	446 (2.4)	1.08 (0.85-1.37)	
Incomplete OC history[3]	561 (20.3)	4038 (22.0)	0.94 (0.84-1.04)	
Alcoholic drinks per week[4]				0.9
None	1373 (49.8)	9002 (49.1)	1.00 (ref)	

<1	912 (33.1)	7434 (40.6)	0.96 (0.87-1.07)	
1-4	300 (10.9)	1106 (6.0)	0.94 (0.79-1.11)	
≥5	171 (6.2)	780 (4.3)	1.09 (0.89-1.34)	

Table 1 continued.

	Cases (n=2760)	Controls (n=18,381)	OR (95% CI)	p-trend
Number of lifetime sexual partners[5]				0.0004
Virgins[7]	223 (8.2)	2318 (12.7)	1.70 (1.44-2.00)	
1	1819 (66.6)	12002 (65.8)	1.00 (ref)	
2	308 (11.3)	2254 (12.4)	0.84 (0.73-0.96)	
3	155 (5.7)	737 (4.0)	0.92 (0.75-1.12)	
4	81 (3.0)	341 (1.9)	0.93 (0.71-1.22)	
≥5	144 (5.3)	600 (3.3)	0.89 (0.72-1.10)	
Age at first sexual relationship[8]				<0.0001[6]
≤18	841 (30.5)	6856 (37.3)	1.00 (ref)	
19-21	747 (27.1)	4586 (25.0)	1.31 (1.17-1.46)	
22-24	411 (14.9)	2344 (12.8)	1.59 (1.39-1.83)	
25-27	265 (9.6)	1244 (6.8)	2.19 (1.86-2.58)	
28-30	147 (5.3)	630 (3.4)	2.45 (1.99-3.01)	
≥31	71 (2.6)	263 (1.4)	3.26 (2.60-4.09)	
Virgins[7]	223 (8.1)	2318 (12.6)	2.55 (2.13-3.04)	
Age at first marriage[9]				<0.0001[10]
≤18	490 (17.8)	5221 (28.4)	1.00 (ref)	
19-21	760 (31.3)	4746 (31.9)	1.40 (1.24-1.59)	
22-24	546 (22.5)	2736 (18.4)	1.70 (1.48-1.95)	
25-27	306 (12.6)	1419 (9.5)	2.04 (1.73-2.40)	
28-30	187 (7.7)	678 (4.6)	2.79 (2.29-3.40)	
≥31	162 (6.7)	467 (3.1)	3.24 (2.62-4.01)	
Unmarried	308 (11.2)	3100 (16.9)	2.39 (2.03-2.82)	
Parity (number of live births)				<0.0001
None	488 (17.7)	3909 (21.3)	1.00 (ref)	
1-2	1121 (40.6)	5637 (30.7)	0.79 (0.69-0.89)	
3-4	794 (28.8)	4573 (24.9)	0.59 (0.52-0.68)	
5-6	215 (7.8)	2359 (12.8)	0.35 (0.29-0.42)	
7-8	91 (3.3)	1124 (6.1)	0.31 (0.25-0.40)	
≥9	51 (1.9)	779 (4.2)	0.24 (0.18-0.33)	
Age at first live birth in parous women				<0.0001
≤18	269 (11.8)	3076 (21.3)	1.00 (ref)	
19-21	592 (26.1)	4627 (32.0)	1.25 (1.06-1.46)	
22-24	565 (24.9)	3259 (22.5)	1.54 (1.31-1.81)	
25-27	413 (18.2)	1884 (13.0)	1.96 (1.65-2.33)	
28-30	222 (9.8)	923 (6.4)	2.40 (1.96-2.94)	
≥31	211 (9.3)	703 (4.9)	3.01 (2.44-3.71)	
Breastfeeding duration in parous women[11]				<0.0001

<1 year	1286 (56.6)	5679 (39.3)	1.00 (ref)	
1-3 years	689 (30.3)	4944 (34.2)	0.77 (0.69-0.87)	
≥4 years	297 (13.1)	3846 (26.6)	0.47 (0.41-0.56)	

Footnotes to table 1:

[1] Occupation of woman, or of husband if higher SES

[2] P-value for heterogeneity

[3] Includes 419 cases and 2892 controls with time of last OC use NK, 29 cases and 301 controls with duration of OC use NK, 111 cases and 827 controls with both last use and duration NK, and 2 cases and 18 controls with any OC use NK

[4] Alcohol consumption not known for 4 cases and 59 controls

[5] Number of sexual partners not known for 30 cases and 129 controls

[6] Trend test excluding virgins

[7] Includes 2 controls who were married and reported no sexual relationships

[8] Age at first sexual relationship not known for 55 cases and 140 controls

[9] Marriage status or age at marriage not known for 1 case and 14 controls

[10] Trend test excluding unmarried women

[11] Duration of breastfeeding not known for 3 controls

Table 2. Nulliparous women: effects of age at first sexual relationship and age at marriage analysed separately (univariate – all women) and jointly (multivariate, restricted to married women). Odds ratios (ORs) adjusted for age group, centre, socioeconomic status and oral contraceptive use.

Age at first sexual relationship							**Age at first marriage**				
	Cases		Controls		Univariate	Multivariate[1]		Cases	Controls	Univariate	Multivariate[1]
	married	total	married	total	OR (95% CI)	OR (95% CI)				OR (95% CI)	OR (95% CI)
≤18	59	72	302	500	0.97 (0.67-1.39)	1.00 (ref)	≤18	25	185	1.00 (ref)	1.00 (ref)
19-21	43	56	281	425	0.76 (0.52-1.12)	0.76 (0.42-1.35)	19-21	33	271	0.87 (0.48-1.58)	1.07 (0.52-2.21)
22-24	20	29	168	235	0.69 (0.44-1.10)	0.80 (0.36-1.79)	22-24	29	221	0.74 (0.40-1.36)	0.91 (0.40-2.11)
25-27	28	34	130	185	0.94 (0.61-1.45)	0.99 (0.40-2.45)	25-27	33	155	1.18 (0.64-2.17)	1.26 (0.48-3.32)
28-30	20	26	82	108	1.17 (0.72-1.89)	0.54 (0.20-1.49)	28-30	32	101	1.71 (0.92-3.18)	3.01 (1.10-8.23)
≥31	38	47	100	132	1.26 (0.85-1.86)	0.79 (0.31-2.04)	≥31	56	132	1.71 (0.98-2.98)	2.20 (0.85-5.69)
	p trend excluding virgins				p=0.13	p=0.57	p trend excluding unmarried			p=0.0009	p=0.017
Virgins	0	223	2	2318	1.00 (ref)		[2] Unmarried	279	2840	1.14 (0.71-1.83)	

[1]The multivariate analysis was restricted to 203 case and 1063 control married nulliparous women who reported ever having a sexual relationship.

[2] The OR for unmarried nulliparous non-virgins with virgins as the reference group is 0.83 (95%CI 0.57-1.22).

Table 3. Married parous women: age at marriage and years from first intercourse to marriage. Numbers of cases and controls (upper part) and odds ratios (ORs) adjusted for parity, age at first birth, duration of breast feeding, number of sexual partners, socioeconomic status, oral contraceptive use, age group and centre.

Years from first intercourse to marriage	**Age at first marriage** ≤18	19-21	22-24	25-27	28-30	≥31	Total	
	case/control	case/control	case/control	case/control	case/control	case/control	case/control	
≤1	427/4715	548/3765	351/1997	217/1031	113/493	74/260	1730/12261	
2-4	33/247	160/592	102/305	16/69	9/19	3/14	323/1246	
5-7	1/11	10/79	50/170	23/72	6/20	4/6	94/358	
≥8	0/2	1/11	8/32	16/80	23/40	24/53	72/218	
Total	461/4975	719/4447	511/2504	272/1252	151/572	105/333	2219/14083	
	OR (95%CI)	OR (95%CI)	OR(95%CI)	OR(95%CI)	OR(95%CI)	OR(95%CI)	p for trend within row	OR adjusted for age at marriage (95%CI)
≤1	1.00 (ref)	1.24 (1.04-1.48)	1.37 (1.09-1.71)	1.53 (1.16-2.01)	1.56 (1.10-2.20)	1.75 (1.14-2.67)	0.0045	1.00 (ref)
2-4	1.24 (0.82-1.87)	1.24 (0.96-1.60)	1.26 (0.92-1.72)	0.87 (0.48-1.60)	1.87 (0.79-4.44)	0.70 (0.19-2.64)	0.59	0.96 (0.80-1.14)
5-7	1.16 (0.13-10.01)	1.12 (0.55-2.29)	1.28 (0.87-1.90)	1.13 (0.66-1.94)	1.22 (0.46-3.24)	2.62 (0.67-10.23)	0.34	0.90 (0.68-1.19)
≥8		0.80	2.09	0.89	2.78	1.67	0.37	1.03

		(0.10-6.60)	(0.90-4.87)	(0.49-1.61)	(1.55-5.01)	(0.95-2.94)		(0.73-1.45)
p for trend within column	0.56	0.63	0.88	0.46	0.13	0.37		Overall trend p=0.77
OR adjusted for time since first intercourse	1.00 (ref)	1.24 (1.04-1.46)	1.35 (1.09-1.67)	1.37 (1.05-1.78)	1.64 (1.19-2.27)	1.68 (1.13-2.49)	Overall trend p=0.0023	

Table 4. Effects among married parous women of age at marriage, age at first live birth, parity, duration of breast feeding, number of sexual partners and age at first sexual intercourse. Multivariate analyses adjusted for age group, centre, socioeconomic status and oral contraceptive use.

	Cases	Controls	Model 1: fitting all variables OR (95% CI)	Model 2: excluding age at marriage OR (95% CI)	Model 3: excluding age at first live birth OR (95% CI)	Model 4: excluding parity OR (95% CI)	Model 5: excluding duration of breast feeding OR (95% CI)	Model 6: excluding number of sexual partners OR (95% CI)
Age at marriage								
≤18	461	4,975	1.00 (ref)		1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
19-21	719	4,447	1.23 (1.04-1.45)		1.24 (1.08-1.42)	1.24 (1.05-1.46)	1.23 (1.04-1.46)	1.23 (1.04-1.46)
22-24	511	2,504	1.33 (1.08-1.64)		1.45 (1.25-1.68)	1.34 (1.08-1.65)	1.34 (1.08-1.65)	1.33 (1.08-1.64)
25-27	272	1,252	1.36 (1.06-1.76)		1.62 (1.35-1.94)	1.37 (1.06-1.77)	1.38 (1.07-1.78)	1.36 (1.05-1.75)
28-30	151	572	1.64 (1.20-2.25)		2.08 (1.66-2.60)	1.65 (1.21-2.26)	1.67 (1.22-2.28)	1.64 (1.20-2.24)
≥31	105	333	1.70 (1.17-2.46)		2.24 (1.72-2.91)	1.72 (1.19-2.50)	1.72 (1.18-2.49)	1.67 (1.15-2.41)
Age at first live birth								
≤18	263	2,943	1.00 (ref)	1.00 (ref)		1.00 (ref)	1.00 (ref)	1.00 (ref)
19-21	578	4,514	0.97 (0.81-1.18)	1.09 (0.93-1.29)		1.00 (0.83-1.21)	0.98 (0.81-1.19)	0.98 (0.81-1.19)
22-24	560	3,200	1.03 (0.82-1.29)	1.27 (1.07-1.51)		1.09 (0.87-1.36)	1.04 (0.83-1.30)	1.05 (0.84-1.31)
25-27	403	1,851	1.17 (0.91-1.52)	1.52 (1.26-1.83)		1.27 (0.98-1.64)	1.19 (0.92-1.54)	1.20 (0.92-1.55)
28-30	214	902	1.25 (0.92-1.70)	1.75 (1.41-2.19)		1.38 (1.02-1.86)	1.27 (0.94-1.73)	1.28 (0.94-1.73)
≥31	201	673	1.38 (0.98-1.95)	2.12 (1.68-2.67)		1.54 (1.10-2.16)	1.41 (1.00-1.99)	1.42 (1.01-2.00)

Table 4 continued

	Cases	Controls	Model 1: All variables OR (95% CI)	Model 2: excluding age at marriage OR (95% CI)	Model 3: excluding age at first live birth OR (95% CI)	Model 4: excluding parity OR (95% CI)	Model 5: excluding duration of breast feeding OR (95% CI)	Model 6: excluding number of sexual partners OR (95% CI)
Parity								
1-2	1,080	5,377	1.00 (ref)	1.00 (ref)	1.00 (ref)		1.00 (ref)	1.00 (ref)
3-4	784	4,507	0.94 (0.83-1.06)	0.95 (0.84-1.07)	0.91 (0.81-1.03)		0.91 (0.81-1.03)	0.95 (0.84-1.07)
5-6	215	2,326	0.68 (0.56-0.83)	0.68 (0.56-0.83)	0.66 (0.54-0.80)		0.63 (0.53-0.75)	0.69 (0.57-0.83)
7-8	89	1,109	0.64 (0.49-0.85)	0.64 (0.49-0.84)	0.62 (0.48-0.81)		0.59 (0.45-0.75)	0.65 (0.50-0.86)
≥9	51	764	0.56 (0.40-0.79)	0.56 (0.40-0.78)	0.54 (0.38-0.76)		0.50 (0.36-0.69)	0.57 (0.41-0.80)
Duration of breast feeding								
<1 year	1,252	5,470	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)		1.00 (ref)
1-3 years	673	4,826	0.93 (0.82-1.05)	0.92 (0.82-1.05)	0.92 (0.81-1.04)	0.90 (0.79-1.01)		0.93 (0.82-1.05)
≥4 years	294	3,787	0.81 (0.67-0.99)	0.80 (0.66-0.97)	0.81 (0.66-0.98)	0.65 (0.55-0.78)		0.81 (0.67-0.99)
Number of sexual partners								
1	1,660	10,817	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	
2	269	1,986	0.85 (0.73-0.99)	0.85 (0.73-0.98)	0.85 (0.73-0.99)	0.86 (0.74-1.01)	0.85 (0.73-0.99)	
≥3	290	1,280	0.78 (0.66-0.93)	0.78 (0.66-0.93)	0.77 (0.65-0.92)	0.79 (0.66-0.93)	0.78 (0.65-0.92)	

[1]*Excluding 15 with missing marriage status, 32 who had first sex >2 year after first marriage, 3 with unknown duration of breastfeeding and 158 with unknown number of sexual partners*

Table 5. Married parous women: Odds ratios (ORs) for age at first marriage (AFM), age at first birth (AFB) and interval from first marriage to first birth (IMFB) adjusted for age group, centre, socioeconomic status, oral contraceptive use, parity, duration of breast feeding and number of sexual partners.

			Univariate analyses	Fitting AFM and AFB Correlation 0.84	Fitting AFM and IMFB Correlation -0.07	Fitting AFB and IMFB Correlation 0.36
	Cases	Controls	OR (95%CI)	OR (95%CI)	OR (95%CI)	OR (95%CI)
AFM ≤18	461	4,975	1.00 (ref)	1.00 (ref)	1.00 (ref)	
19-21	719	4,447	1.24 (1.08-1.42)	1.23 (1.04-1.45)	1.25 (1.09-1.43)	
22-24	511	2,504	1.45 (1.25-1.68)	1.33 (1.08-1.64)	1.47 (1.26-1.71)	
25-27	272	1,252	1.62 (1.35-1.94)	1.36 (1.06-1.76)	1.66 (1.38-1.99)	
28-30	151	572	2.08 (1.66-2.60)	1.64 (1.20-2.25)	2.14 (1.71-2.69)	
≥31	105	333	2.24 (1.72-2.91)	1.70 (1.17-2.46)	2.36 (1.80-3.09)	
Increase per year			1.052 (1.039-1.064) p(trend)<0.0001	1.033 (1.012-1.053) p(trend)=0.0015	1.055 (1.042-1.069) p(trend)<0.0001	
AFB ≤18	263	2,943	1.00 (ref)	1.00 (ref)		1.00 (ref)
19-21	578	4,514	1.09 (0.93-1.29)	0.97 (0.81-1.18)		1.12 (0.95-1.33)
22-24	560	3,200	1.27 (1.07-1.51)	1.03 (0.82-1.29)		1.35 (1.13-1.61)
25-27	403	1,851	1.52 (1.26-1.83)	1.17 (0.91-1.52)		1.65 (1.36-2.00)
28-30	214	902	1.75 (1.41-2.19)	1.25 (0.92-1.70)		1.94 (1.54-2.43)
≥31	201	673	2.12 (1.68-2.67)	1.38 (0.98-1.95)		2.39 (1.87-3.05)
Increase per year			1.048 (1.036-1.061) p(trend)<0.0001	1.022 (1.002-1.042) p(trend)=0.035		1.057 (1.043-1.070) p(trend)<0.0001
IMFB ≤-3	39	204	1.01 (0.68-1.48)		0.74 (0.50-1.10)	1.03 (0.70-1.51)
-2	12	92	0.65 (0.34-1.23)		0.57 (0.30-1.08)	0.64 (0.34-1.21)
-1	32	146	0.91 (0.59-1.38)		0.92 (0.60-1.41)	0.95 (0.62-1.46)
0	330	1,989	1.00 (ref)		1.00 (ref)	1.00 (ref)
1	995	6,611	0.98 (0.85-1.14)		0.99 (0.86-1.15)	0.94 (0.81-1.09)

2	385	2,650	0.87 (0.73-1.03)		0.89 (0.75-1.06)	0.80 (0.67-0.95)
3	175	1,048	0.91 (0.74-1.13)		0.94 (0.76-1.16)	0.80 (0.64-0.99)
4	85	499	0.87 (0.66-1.14)		0.91 (0.69-1.20)	0.73 (0.55-0.96)
5-9	130	714	0.95 (0.75-1.20)		1.01 (0.79-1.28)	0.72 (0.56-0.92)
≥10	36	130	1.14 (0.76-1.73)		1.32 (0.87-2.01)	0.71 (0.46-1.10)
Increase per year[1]			1.001 (0.976-1.026) p(trend)=0.94		1.013 (0.988-1.039) p(trend)=0.32	0.961 (0.936-0.987) p(trend)=0.0033

[1] *Women married after first birth are excluded in trend analyses of years from marriage to first birth*