Incessant Ovulation, Mucin 1 Immunity, and Risk for Ovarian Cancer

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Abstract

Background: **Risk for ovarian cancer correlates directly with ''ovulatory years or cycles'' estimated from time not pregnant, breast-feeding, or using oral contraceptives. Recently, we reported that several factors known to reduce ovarian cancer risk may operate by inducing antibodies against mucin 1 (MUC1), a glycoprotein overexpressed in ovarian cancer. Conversely, other events might increase risk by interfering with the development of protective immunity. In this study, we examined whether the total number of ovulatory cycles decreases the likelihood of anti-MUC1 antibodies and provides an immune basis for the association between ''incessant ovulation'' and ovarian cancer risk.**

Methods: **From 1998 to 2003, we enrolled 668 epithelial ovarian cancer cases and 721 controls residing in eastern Massachusetts or New Hampshire, collected information on menstrual and reproductive events, and obtained blood samples from controls to measure anti-MUC1 antibodies. Using logistic regression, we calculated odds ratios to evaluate the influence of reproductive factors, including the estimated lifetime number of ovulatory cycles on ovarian cancer risk and on the presence of MUC1 antibodies in controls.**

Results: **Overall, we observed that early age at first birth, cycle lengths ≥30 days, and oral contraceptive use increased the likelihood of having anti-MUC1 antibodies. Estimated ovulatory cycles were correlated positively with ovarian cancer risk and inversely with the presence of anti-MUC1 antibodies among controls ages 46 to 60 years.**

Conclusions: **These data suggest that suppression of MUC1-specific immunity should be considered as an additional explanation for the observation that ovarian cancer risk increases with the lifetime number of ovulatory cycles.** (Cancer Epidemiol Biomarkers Prev 2007;16(1):30–5)

Introduction

Risk for ovarian cancer is inversely related to number of pregnancies and lengths of breast-feeding and oral contraceptive use. These events, which interrupt ovulation, can be combined into a composite variable that estimates years of ovulation or, when menstrual cycle length is included, number of ovulatory cycles. Total ovulatory years or cycles directly correlate with ovarian cancer risk (1-4), providing a foundation for the popular ''incessant ovulation'' hypothesis for ovarian cancer (5). According to this hypothesis, monthly disruption and repair of the surface epithelium of the ovary with associated structural, genetic, or inflammatory damage increases risk of epithelial ovarian cancer.

Recently, we introduced a new paradigm (6) to explain ovarian cancer risk involving mucin 1 (MUC1), a member of the mucin family of glycoproteins that includes CA125. Many cancers, including ovarian, overexpress MUC1 (7-9) and can promote anti-MUC1 antibodies that correlate with a more favorable prognosis (10). By extension, anti-MUC1 immunity generated by events other than cancer might lower the risk of MUC1-positive cancers, including ovarian cancer. In our previous study, we identified events predicting anti-MUC1 antibodies in controls and then assessed the same events in relation to ovarian cancer risk. Events associated with anti-MUC1 antibodies included tubal sterilization, breast mastitis, and oral contraceptive use; these same events predicted lower risk for ovarian cancer. Thus, we proposed that several traditional and new risk factors for ovarian cancer might exert their influence through their ability to induce anti-MUC1 antibodies through immune recognition of MUC1 in the context of inflammatory or hormonal processes in tissues that normally express MUC1. In the current study, we examine whether hormonal factors, including menstrual and reproductive events, and oral contraceptive use have individual or cumulative effects on anti-MUC1 antibodies as an immune basis for the association between ''incessant ovulation'' and ovarian cancer risk.

Materials and Methods

Study Participants and Design. This population-based study was approved by the human subjects review committees at both Brigham and Women's Hospital and Dartmouth Medical School. Methods for this study have been previously described (6). Briefly, between July 1998 and July 2003, we identified 1,267 cases from tumor boards and statewide registries in eastern Massachusetts and all of New Hampshire. We excluded 119 cases who had died, 110 who moved from the study area, 24 who did not speak English or had no phone, and 46 found to have a nonovarian primary on review. Of the 968 eligible cases, physicians denied permission to contact 106 and 171 declined to participate, leaving 691 (71%) cases interviewed. Of these, 668 had epithelial ovarian cancer, including borderline malignancies. We identified controls through town books in Massachusetts and Drivers' License lists in New Hampshire and sampled them to match the age and residence distribution of previously accumulated cases. We sent 1,843 potential controls an invitation to participate. Of these, 318 had moved and could not be located or had died,

Received 8/11/06; revised 10/20/06; accepted 10/25/06.

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doi:10.1158/1055-9965.EPI-06-0688

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197 (in Massachusetts) could not be recontacted because subjects declined through an ''opt out'' postcard, and 47 no longer had a working telephone. Of the remaining 1,281 who were contacted, 152 were ineligible because they had no ovaries or were not the correct age and 59 were incapacitated or did not speak English. Of the 1,267 eligible controls, 721 (67%) were interviewed.

After written informed consent, an in-person interview about demographic characteristics as well as medical, family, and reproductive history was conducted. Menstrual characteristics included age at menarche, regularity, and average length of the menstrual cycle during most of reproductive life (when not on oral contraceptives). Women were considered premenopausal if they had an intact uterus and reported their periods were still occurring naturally. Women were considered postmenopausal if they had an intact uterus and reported that their periods had stopped for at least a year or were occurring only because of hormones given for menopausal symptoms. Women who had had a hysterectomy (without oophorectomy) were considered premenopausal if they were <50 years of age and postmenopausal if they were >50 years of age. Women were also asked about birth control methods used and duration of use. Women who used progestin-only birth control pills as their only or longest birth control pill were classified with women who used other progesterone-only methods, such as progestin implants, whereas women who used (more common) combination pills were classified as oral contraceptive users.

ELISA Assay for Anti-MUC1 Antibodies. Heparinized blood specimens were collected from control participants agreeing to provide one (n = 705); separated into red cell, buffy coat, and plasma components; and stored at −80°C. Anti-MUC1 antibodies (total IgM, IgG, and IgA) were measured in the plasma (serially diluted from 1:20 to 1:160) of 705 controls using an ELISA assay according to a previously described protocol (10). Briefly, the level of anti-MUC1 antibody is estimated by reading the absorbance at 405 to 410 nm for each sample in the MUC1-coated plate compared with values in the antigen-negative plates to subtract nonspecific binding. Absorbance reactions at all dilutions are used for quality control of the assay. The assay was considered positive for anti-MUC1 antibodies based on the absorbance at 1:20 dilution with an absorbance reading of ≥0.6. Anti-MUC1 antibodies were not assessed in cases because antibody levels may reflect changes in response to the cancer.

Statistical Methods. Number of ovulatory years was calculated by subtracting the age at menarche from their current age (if premenopausal) or age at last period (if postmenopausal), which would yield the maximum number of ovulatory years, and reducing this by time spent pregnant, breast-feeding, or using oral contraceptives. For time spent pregnant, we assigned 0.15 year to abortion, miscarriages, and ectopic pregnancies, 0.65 year to preterm birth or stillborn, and 0.92 year to a term birth. The latter number represents ~8 weeks longer than a 40-week term pregnancy based on evidence that ovulation does not return for this period in women who do not breast-feed after a pregnancy (11). For women who breast-fed, we assigned the amount of time breast-feeding up to 1 year for each pregnancy based on data indicating few women remain amenorrheic after 1 year (12). Women who failed to answer the question about age at menarche (n = 6) were assigned to the most common age at menarche in this population (13 years). Women who were missing age at menopause (n = 4) were assigned age 50 for this variable. The number of menstrual (and presumed ovulatory) cycles was then estimated by multiplying the number of ovulatory years by 365 and then dividing by the number of days women reported as their average menstrual cycle length. Women who reported their periods were never regular were excluded from this analysis (n = 114). Because age restricts the number of possible ovulatory cycles and reflects birth cohort, separating the effect of ovulatory cycles from age or temporal trends in childbearing is problematic. To address this issue, we created a relative variable for ovulatory cycles based on tertile cut points in three age groups (≤45 years, 46-60 years, and >60 years). Among women ages ≤45 years, ≤196 ovulatory cycles are defined as low, 197 to 298 as medium, and >298 as high. Among women ages 46 to 60 years, these categories are ≤375, 376 to 436, and >436, and among women ages >60 years, these categories are ≤399, 400 to 457, and >457. Consequently, we assessed the risk associated with a low, medium, or high number of ovulatory cycles for women of a certain age rather than evaluating the risk associated with an absolute number of ovulatory cycles for women across all ages. For comparability, we examined the individual reproductive variables contributing to the composite ovulatory cycle variable for each age group as well as all combined.

We calculated odds ratios (OR) and 95% confidence intervals (95% CI) using unconditional logistic regression analyses to estimate the association between positivity for anti-MUC1 antibodies and hormonal characteristics, including the estimated total number of ovulatory cycles in controls. We used similar techniques to assess the association between factors predicting positivity for anti-MUC1 antibody and ovarian cancer risk. We adjusted all analyses for age (continuous) and study center (Massachusetts or New Hampshire). In addition, we evaluated all predictors of anti-MUC1 antibodies identified previously, including bone fracture/osteoporosis, mastitis, pelvic surgeries, intrauterine device use, no genital talc use, oral contraceptive use, and current smoking as potential confounders, by including them individually in the model (6). Only pelvic surgeries (tubal ligation, cesarean section, and hysterectomy) influenced the estimate of the association between ovulatory cycles and occurrence of anti-MUC1 antibodies appreciably. To evaluate the trend in associations, a median value was assigned to each category and a Wald test was used to test the significance of the trend (two sided, $P < 0.05$). In addition, we assessed the correlation between estimated lifetime number of ovulatory cycles and absorbance of anti-MUC1 antibodies using Spearman rank correlations.

Results

Although there was no association between number of children and likelihood of anti-MUC1 antibodies, there was a significant trend of increasing presence of antibodies with an earlier age at first birth and a decreased likelihood of antibodies for women who had their last child after age 35 years (Table 1). Lifetime duration of breast-feeding did not influence occurrence of anti-MUC1 antibodies (Table 1) nor did number of breast-feeding episodes or duration of breast-feeding with each pregnancy (data not shown). Women with menstrual cycles lasting 30 or more days were more likely to have anti-MUC1 antibodies compared with women with menstrual cycles lasting 28 days (OR, 1.49; 95% CI, 1.01-2.19), although there was no clear general trend between cycle length and antibodies. Cycle regularity, age at menarche, and age at menopause were not associated with likelihood of anti-MUC1 antibodies.

Women who had used oral contraceptives had a greater likelihood of antibodies, and the association was most apparent in premenopausal women in whom there was a 2-fold increase in the likelihood of antibodies associated with ever use (OR, 2.28; 95% CI, 1.18-4.42). Although the category was very small, women who used progestin-only methods of contraception had a lower (but not significantly so) likelihood of having antibodies.

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Table 1. Reproductive events and likelihood of anti-MUC1 antibodies

	All				<45 y		46-60 y		>60 y	
	MUC1 antibodies		Adjusted* OR (95% CI)	*P* trend*	Adjusted* OR (95% CI)	*P* trend*	Adjusted* OR (95% CI)	*P* trend*	Adjusted* OR (95% CI)	*P* trend*
	Negative, *n* (%)	Positive, *n* (%)								
No. liveborn										
0	83 (67)	40 (33)	1.00	0.19	1.00	0.71	1.00	0.03	1.00	0.87
1	61 (64)	34 (36)	1.24 (0.70-2.20)		1.12 (0.48-2.62)		3.23 (1.09-9.52)		0.31 (0.07-1.51)	
2	150 (68)	72 (32)	1.09 (0.67-1.77)		0.80 (0.38-1.70)		3.07 (1.19-7.91)		0.35 (0.10-1.24)	
≥3	173 (65)	92 (35)	1.41 (0.86-2.31)		0.92 (0.39-2.14)		3.69 (1.39-9.79)		0.61 (0.22-1.67)	
Any	384 (66)	198 (34)	1.22 (0.79-1.89)		0.91 (0.47-1.76)		3.30 (1.33-8.16)		0.53 (0.20-1.43)	
Age at first birth (among parous women), y										
≤21	63 (57)	48 (43)	1.81 (1.08-3.05)	0.04	3.15 (1.11-8.94)	0.04	1.00 (0.47-2.11)	0.94	4.77 (1.18-19.36)	0.05
22-25	118 (68)	55 (32)	1.16 (0.71-1.89)		1.59 (0.61-4.18)		0.83 (0.41-1.68)		2.20 (0.58-8.33)	
26-29	96 (68)	45 (32)	1.09 (0.66-1.81)		1.02 (0.43-2.42)		0.78 (0.36-1.70)		2.76 (0.69-11.03)	
>29	107 (68)	50 (32)	1.00		1.00		1.00		1.00	
Age at last birth (among parous women), y										
≤25	45 (56)	35 (44)	1.00	0.17	1.00	0.07	1.00	0.72	1.00	0.75
26-30	123 (68)	58 (32)	0.61 (0.35-1.05)		0.68 (0.21-2.17)		0.56 (0.26-1.20)		0.57 (0.18-1.80)	
31-35	130 (66)	67 (34)	0.67 (0.39-1.14)		0.52 (0.17-1.66)		0.97 (0.45-2.07)		0.51 (0.17-1.55)	
>35	86 (69)	38 (31)	0.59 (0.33-1.07)		0.32 (0.09-1.21)		0.67 (0.29-1.56)		0.70 (0.22-2.24)	
Lifetime duration of breast-feeding (among parous women), mo										
0	156 (65)	83 (35)	1.00	0.58	1.00	0.37	1.00	0.74	1.00	0.09
≤2	51 (65)	27 (35)	0.93 (0.54-1.61)		0.61 (0.21-1.74)		1.16 (0.46-2.95)		0.96 (0.39-2.37)	
3-11	83 (68)	39 (32)	0.82 (0.51-1.31)		0.38 (0.13-1.08)		1.06 (0.54-2.06)		0.85 (0.32-2.27)	
≥12	94 (66)	49 (34)	0.87 (0.55-1.37)		1.06 (0.46-2.46)		0.92 (0.46-1.83)		0.37 (0.12-1.17)	
Any	228 (66)	115 (34)	0.86 (0.60-1.24)		0.72 (0.34-1.53)		1.01 (0.59-1.75)		0.71 (0.37-1.37)	
Menstrual cycle pattern										
Regular	435 (66)	224 (34)	1.00	NA	1.00		1.00	NA	1.00	
Irregular	32 (70)	14 (30)	0.84 (0.44-1.62)		0.60 (0.19-1.90)		0.65 (0.17-2.40)		1.22 (0.43-3.48)	
Menstrual cycle length (among women with a regular pattern), d										
≤27	48 (64)	27 (36)	1.23 (0.73-2.07)	0.32	1.00 (0.43-2.34)	0.50	1.59 (0.67-3.80)	0.82	1.08 (0.37-3.14)	0.31
28	251 (69)	112 (31)	1.00		1.00		1.00		1.00	
29	36 (62)	20 (38)	1.33 (0.73-2.42)		2.49 (0.80-7.80)		1.48 (0.58-3.81)		0.63 (0.19-2.08)	
≥30	100 (61)	65 (39)	1.49 (1.01-2.19)		1.23 (0.61-2.48)		1.45 (0.80-2.62)		1.96 (0.92-4.21)	
Age at menarche, y										
≤11	86 (65)	46 (35)	1.18 (0.74-1.89)	0.62	1.28 (0.57-2.85)	0.34	1.41 (0.67-2.99)	0.52	0.72 (0.28-1.86)	0.51
12	134 (64)	75 (36)	1.22 (0.80-1.84)		1.61 (0.80-3.23)		1.18 (0.61-2.29)		0.87 (0.37-2.02)	
13	137 (69)	63 (31)	1.00		1.00		1.00		1.00	
≥14	110 (67)	54 (33)	1.13 (0.72-1.76)		1.02 (0.44-2.36)		1.93 (0.95-3.93)		0.56 (0.24-1.28)	
Age at menopause (among postmenopausal women), y										
≤47	65 (67)	32 (33)	1.19 (0.65-2.16)	0.59	NA		1.22 (0.49-305)	0.77	0.97 (0.41-2.34)	0.92
48-49	42 (70)	18 (30)	1.05 (0.52-2.11)				1.06 (0.36-3.14)		1.31 (0.50-3.43)	
50-51	89 (70)	39 (30)	1.07 (0.61-1.88)				1.73 (0.74-4.06)		0.80 (0.37-1.69)	
≥52	78 (71)	32 (29)	1.00				1.00		1.00	
Oral contraceptive use, mo †										
≤3	174 (71)	70 (29)	1.00	0.23	1.00	0.85	1.00	0.08	1.00	0.59
3-18	76 (66)	40 (34)	1.22 (0.76-1.98)		2.51 (0.97-6.50)		1.17 (0.53-2.56)		0.77 (0.30-1.98)	
19-48	86 (66)	45 (34)	1.16 (0.72-1.86)		1.59 (0.67-3.77)		0.98 (0.45-2.11)		1.90 (0.63-5.77)	
49-90	59 (60)	40 (40)	1.49 (0.89-2.48)		1.74 (0.70-4.31)		1.30 (0.57-2.93)		4.23 (1.16-15.49)	
>90	72 (63)	43 (37)	1.33 (0.82-2.17)		1.31 (0.55-3.12)		2.02 (0.90-4.52)		0.81 (0.27-2.42)	
Any	293 (64)	168 (36)	1.28 (0.90-1.83)		1.42 (0.68-2.93)		1.28 (0.70-2.35)		1.25 (0.67-2.36)	
Progestin shots, implants, or pills										
Never	425 (66)	221 (34)	1.00	NA	1.00		1.00	NA	1.00	NA
Ever	42 (72)	17 (28)	0.81 (0.45-1.47)		0.60 (0.17-2.08)		0.81 (0.32-2.02)		0.99 (0.34-2.84)	

Abbreviation: NA, not available.
*Adjusted for age (continuous) and study center (Massachusetts or New Hampshire).
†Does not include progestin-only formulations.

We observed a strong positive association between the relative lifetime number of ovulatory cycles and ovarian cancer risk across all age groups. Conversely, we observed an inverse association between the relative number of ovulatory cycles and likelihood of anti-MUC1 antibodies of borderline significance that was strongest for women of middle age (46-60 years; Table 2). Overall, the estimated lifetime number of ovulatory cycles was weakly ($r = -0.13$) but significantly ($P = 0.0008$) correlated with anti-MUC1 antibodies.

As expected, we observed a strong association between lifetime number of ovulatory cycles and risk for all epithelial types of ovarian cancer combined (P for trend < 0.0001; Table 3). The association varied by the histologic type of ovarian cancer, being present in women with serous invasive, endometrioid, clear cell, and other/undifferentiated types of ovarian cancer but absent in those with serous borderline type. For mucinous ovarian cancer, a positive association with ovulatory cycles is suggested but not statistically significant, most likely due to the small number of cases in this histologic category. Point estimates for the OR associated with ovulatory cycles were especially strong for endometrioid types with a 4-fold increase in risk for women in the highest category of ovulatory cycles compared with women in the lowest category.

Discussion

Recently, we proposed that reduced risk of ovarian cancer associated with tubal ligation, oral contraceptive use, and mastitis might be explained in part by their ability to promote

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Table 2. Estimated relative lifetime number of ovulatory cycles and ovarian cancer risk or likelihood of anti-MUC1 antibodies

Lifetime no. ovulatory cycles*	Association with ovarian cancer				Association with anti-MUC1 antibodies			
	Cases, *n* (%)	Controls, *n* (%)	OR† (95% CI)	*P* trend	Negative, *n* (%)	Positive, *n* (%)	OR† (95% CI)	*P* trend
≤45‡								
Low	44 (39)	68 (61)	1.00	0.001	38 (56)	30 (44)	1.00	0.99
Medium	44 (39)	69 (61)	1.15 (0.65-2.05)		42 (64)	24 (36)	0.91 (0.41-2.01)	
High	91 (56)	71 (44)	2.97 (1.53-5.75)		41 (60)	27 (40)	0.99 (0.38-2.64)	
46-60								
Low	62 (39)	95 (61)	1.00	0.001	57 (61)	37 (40)	1.00	0.04
Medium	83 (46)	96 (54)	1.38 (0.89-2.15)		65 (71)	27 (29)	0.62 (0.33-1.18)	
High	115 (55)	94 (45)	2.10 (1.35-3.29)		68 (74)	24 (26)	0.49 (0.25-0.97)	
>60								
Low	39 (39)	60 (61)	1.00	0.06	41 (68)	19 (32)	1.00	0.76
Medium	55 (48)	59 (59)	1.41 (0.81-2.46)		39 (67)	19 (33)	1.12 (0.51-2.47)	
High	68 (52)	62 (62)	1.70 (0.99-2.93)		44 (72)	17 (28)	0.88 (0.40-1.96)	
All ages								
Low	145 (39)	223 (61)	1.00	<0.0001	136 (61)	86 (38)	1.00	0.06
Medium	182 (45)	224 (55)	1.25 (0.94-1.67)		146 (67)	70 (32)	0.76 (0.51-1.13)	
High	274 (55)	227 (45)	1.88 (1.42-2.47)		153 (69)	68 (30)	0.68 (0.45-1.03)	

*There are 114 women missing estimated lifetime number of ovulatory cycles because they reported that their menstrual cycles were never regular.
†Adjusted for reference age (continuous), study center (Massachusetts or New Hampshire), cesarean section, tubal ligation, and hysterectomy.
‡The ≤45 group was not adjusted for hysterectomy because only one woman was MUC1 positive with a hysterectomy, so estimate was unstable.

anti-MUC1 antibodies through immune recognition of MUC1 in the context of inflammatory or hormonal processes in tissues of the genital tract and breast ducts that normally express MUC1 (6). In the current study, we examined in greater detail key reproductive events that are important in the epidemiology of ovarian cancer. An early age at first birth, average cycle lengths ≥30 days, and oral contraceptive use (especially in premenopausal women) were associated with a significantly greater likelihood of having anti-MUC1 antibodies. Although the individual variables used to calculate the lifetime number of ovulatory cycles were not significantly associated with anti-MUC1 antibodies, we generally observed that variables that reduced the number of ovulatory cycles, such as number of pregnancies, longer cycle length, late age at menarche, oral contraceptive use, and early age at menopause, were positively associated with anti-MUC1 antibodies. After creating a variable to estimate lifetime number of ovulatory cycles, we found that an increasing number of ovulatory cycles, which predict greater risk for ovarian cancer, correlated with a lower likelihood of anti-MUC1 antibodies, particularly among women ages 46 to 60 years. This association may be less apparent in younger women (≤45 years) because they have not completed all reproductive events contributing to ovulatory cycles, whereas the association may be attenuated in older women (>60 years) because it has been longer since they were exposed to ovulatory cycles. In addition, older women may be experiencing other events, such as bone fracture or osteoporosis, which may be altering antibody levels more profoundly (6). Our discussion will explore the possibility that ''incessant ovulation'' might be associated with higher risk for ovarian and perhaps other cancers in women by, in some way, interfering with immune surveillance.

In 1971, Fathalla (5) proposed that repeated damage and repair of the ovarian epithelium from ''incessant ovulation'' increased ovarian cancer risk and suggested that this explained why events that interrupt ovulation, such as pregnancy, breast-feeding, and oral contraceptive use, reduce ovarian cancer risk. Subsequently, several investigators estimated ovulatory years or cycles and showed that these variables did, indeed, correlate directly with ovarian cancer risk (1-4). Ovulation may lead to the formation of inclusion cysts whereby hormonally responsive ovarian epithelium becomes entrapped in the hormonally active ovarian stroma (13). Alternatively, with ovulation stromal cells are recruited to

Table 3. Relative lifetime number of ovulatory cycles and ovarian cancer risk

	Lifetime number of ovulatory cycles*			*P* trend
	Low, *n* (%)	Medium, *n* (%)	High, *n* (%)	
Controls	223 (33)	224 (33)	227 (34)	
All cases	145 (24)	182 (30)	274 (46)	<0.0001
OR† (95% CI)	1.25 (0.94-1.67)	1.87 (1.42-2.47)	1.88 (1.42-2.47)	
Serous borderline cases	34 (42)	24 (30)	23 (28)	0.54
OR† (95% CI)	1.00	0.80 (0.45-1.42)	0.84 (0.47-1.52)	
Serous invasive cases	60 (24)	65 (26)	121 (49)	0.0005
OR† (95% CI)	1.00	1.01 (0.67-1.51)	1.84 (1.27-2.65)	
Mucinous cases	12 (22)	24 (44)	19 (35)	0.10
OR† (95% CI)	1.00	2.19 (1.06-4.52)	1.93 (0.89-4.19)	
Endometrioid cases	18 (18)	22 (22)	58 (59)	<0.0001
OR† (95% CI)	1.00	1.33 (0.69-2.56)	3.92 (2.19-7.04)	
Clear cell cases	16 (20)	28 (35)	36 (45)	0.002
OR† (95% CI)	1.00	1.91 (0.99-3.66)	2.71 (1.42-5.17)	
Other cases	5 (12)	19 (46)	17 (42)	0.04
OR† (95% CI)	1.00	3.66 (1.34-10.01)	3.24 (1.17-8.99)	

*There are 114 women missing estimated lifetime number of ovulatory cycles because they reported that their menstrual cycles were never regular.
†Adjusted for reference age (continuous), study center (Massachusetts or New Hampshire), cesarean section, tubal ligation, and hysterectomy.

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be steroid producers and a greater number of cells with this potential may persist in a women with many past ovulations (14). Damage and repair of the ovarian epithelium might also lead to the accumulation of mutations in the tumor suppressor gene *p53* as a molecular basis for the theory (15). A broader theory suggests that ovulation is just one of a several inflammatory events that might stimulate ovarian epithelial proliferation through growth-promoting cytokines or reactive oxygen species causing DNA damage (16).

Although there is little doubt that ''incessant ovulation'' correlates with increased ovarian cancer risk, the biological mechanisms proposed to explain this are not entirely satisfactory. About surface epithelial damage, there is not complete agreement that the surface epithelium is the cell of origin for ovarian cancer (17). An attempt to replicate histopathologic data supporting p53 mutations as a consequence of ovulation did not confirm the model (18), and meta-analyses about the association between anti-inflammatory drugs on ovarian cancer risk are conflicting, undermining a possible role for inflammatory damage (19, 20). More importantly, all of the above theories, which largely focus on local effects, would not explain why incessant ovulation might also increase the risk for cancers of the endometrium (21) or breast (22) as may be the case.

We propose that an immunologic basis connecting ''incessant ovulation'' with ovarian cancer risk could explain the role of ovulatory cycles in relation to breast and endometrial cancers, which also overexpress MUC1. We previously showed that acute, relatively short-term, and diverse events, such as pelvic surgery and breast mastitis, involve injury or inflammation of tissues that normally express MUC1 and may cause its release into the circulation leading to its presentation to the immune system. Although a single event may prime anti-MUC1 immunity, the cumulative effect of several of these events may boost this response leading to the establishment of protective anti-MUC1 immunologic memory and lower ovarian cancer risk. Our current investigation showed that incessant ovulation is associated with a lower likelihood of anti-MUC1 antibodies and increased risk for ovarian cancer among women ages 46 to 60 years. This effect could derive either from an absence of those reproductive events, which promote the formation of anti-MUC1 antibodies, or to the presence of repetitive and chronic changes associated with ovulation that dampen MUC1-specific immunity. Although both are possible, we will explore the latter explanation in more detail.

Although not normally expressed by ovarian surface epithelium, MUC1 is expressed in epithelia of the vagina, endocervix, endometrium, fallopian tubes, and ducts of the mammary glands, where its function may include protection of epithelial cells from dehydration, proteolysis, and microbial challenge (23). It is not known whether MUC1 expression changes in breast or fallopian tube epithelium during a menstrual cycle, but expression of MUC1 in the endometrium is highest during the luteal phase of the menstrual cycle when high levels of estrogen and progesterone are present (24, 25). The glycosylation pattern of MUC1 may also vary during a natural cycle resulting in decreased accessibility to the immunogenic protein core of MUC1 during the luteal phase (26). Although the specific effects of pregnancy and breast-feeding on MUC1 expression in the endometrium are unknown, it is known that pregnancy leads to a thin cuboidal epithelial layer of endometrium beneath the implantation site during most of gestation (27), whereas combination oral contraceptives and breast-feeding lead to inactive endometrial glands (28, 29). In emphasizing a connection between endometrial expression of MUC1, ovulatory cycles, and ovarian cancer risk, we think it notable that the histologic types of ovarian cancer most strongly associated with ovulatory cycles are endometrioid, serous, and clear cell types, which are also observed in endometrial cancer and are correlated with MUC1 expression. Using immunohistochemistry, Feng et al. (9) observed that MUC1 was expressed on at least 80% serous, clear cell, and endometrioid ovarian cancer cells but only in 44% of mucinous ovarian cancer cells.

''Incessant ovulation'' is just one of several risk factors related to breast, endometrial, or ovarian cancer that might be mediated by MUC1 immunity. Tubal ligation, intrauterine device use, bone fracture, and current smoking, which we have shown may increase anti-MUC1 antibodies, are risk factors for two or more of these female cancers (6, 30-34). Notably, we found in this study that an early age at first birth, which protects against ovarian, endometrial, and especially breast cancer, was strongly associated with a greater likelihood of anti-MUC1 antibodies.

In the discussion above, we have equated the presence of anti-MUC1 antibodies with protection against ovarian and possibly other reproductive cancers, but it should be appreciated that this association does not imply a direct clearing effect of cancer cells by the antibody. Although the antibody most certainly plays a role, we consider the presence of anti-MUC1 antibodies to be also a marker for the presence of other immune mechanisms, such as MUC1-specific T cells. Furthermore, the conditions leading to anti-MUC1 immunity may also promote immune responses to other epithelial cell antigens, adding to the overall complexity and strength of protective immunity.

Although our data suggest that anti-MUC1 antibodies may explain in part the well-established association between lifetime number of ovulatory cycles and ovarian cancer risk, prospective studies are needed to establish an association between MUC1 and ovarian cancer, including measurement of MUC1 in women before the development of ovarian cancer.

Besides its retrospective nature, other potential weaknesses of our study include the imprecision in estimating lifetime ovulations, confounding factors, and selection bias. Over the years, investigators have estimated lifetime ovulations with increasing level of detail by adding breast-feeding, converting ovulatory years to cycles (assuming a 28-day cycle for all), and factoring in menstrual irregularity. We used an individual woman's average cycle length and further modified the algorithm based on assumptions about when cycles return after pregnancies accompanied or unaccompanied by breast-feeding. Despite the inherent imprecision in estimating lifetime ovulations, it is noteworthy that, even for studies with the crudest estimates, ovulatory years or cycles continue to be one of the strongest risk factors for ovarian cancer.

Age is perhaps the most important confounder because we previously found anti-MUC1 antibodies decreased with age (6) and age also directly correlates with ovulatory cycles. Temporal trends, such as lower family size or greater birth control pill use, may also contribute to older women having had more ovulatory cycles than younger women. To address this issue in the analyses presented here, we have considered the relative number of ovulatory cycles (low, medium, and high) for young women (≤45 years), middle-aged women (46-60 years), and older women (>60 years) separately. About selection bias, differences between study participants and the general population must be considered in any case-control study; however, these differences are unlikely to lead to bias in this study because participation is probably not associated with reproductive characteristics.

In conclusion, we have suggested that immune response may be an additional mediator of the relationship between ''incessant ovulation'' and ovarian cancer by lowering protective antibodies against the tumor antigen MUC1. This model accommodates other (protective) risk factors for ovarian cancer, including pelvic surgery, such as tubal ligation, and emerging risk factors, such as breast mastitis and intrauterine device use. Importantly, this model offers an explanation for

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why incessant ovulation and certain other risk factors are common to breast and endometrial cancer, which also overexpress MUC1. A variety of prospective studies will be necessary to define the scope of MUC1 immunity in ovarian, breast, and endometrial cancer. If confirmed, the results of these studies could have profound implications in developing preventive strategies for female reproductive cancers through vaccines to stimulate MUC1 immunity.

Acknowledgments

We thank Allison Vitonis for her programming expertise as well as the participants of this study and their physicians who made this research possible.

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Cancer Epidemiol Biomarkers Prev 2007;16:30-35.

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