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Body size affects measures of ovarian reserve in late reproductive age women

H. Irene Su, MD

Division of Reproductive Endocrinology and Infertility Department of Obstetrics and Gynecology
University of Pennsylvania School of Medicine 3701 Market Street, Suite 801 Philadelphia, PA
19104

Mary D. Sammel, ScD

Department of Biostatistics and Epidemiology Center for Clinical Epidemiology and Biostatistics
University of Pennsylvania School of Medicine Rm. 605 Blockley Hall 423 Guardian Drive
Philadelphia, PA, 19104

Ellen W. Freeman, PhD

Departments of Obstetrics and Gynecology and Psychiatry University of Pennsylvania School of
Medicine Mudd Professorship Suite 3701 Market Street, Suite 820 Philadelphia, PA, 19104

Hui Lin, MS

Center for Research on Reproduction and Women's Health University of Pennsylvania School of
Medicine Mudd Professorship Suite 3701 Market Street, Suite 820 Philadelphia, PA, 19104

Tracey DeBlasis, BA

Center for Research on Reproduction and Women's Health University of Pennsylvania School of
Medicine Mudd Professorship Suite 3701 Market Street, Suite 820 Philadelphia, PA, 19104

Clarisa R. Gracia, MD, MSCE

Division of Reproductive Endocrinology and Infertility Department of Obstetrics and Gynecology
University of Pennsylvania School of Medicine 3701 Market Street, Suite 801 Philadelphia, PA
19104

Abstract

Objective—To examine the association between obesity and serum and ultrasound measures of ovarian reserve in late reproductive age women.

Design—Cross-sectional study of 36 healthy women, ages 40–52. Women were recruited in a 1:1 ratio of normal weight (BMI<25) to obese women (BMI>30). Early follicular phase blood draw, anthropometric measurements and a transvaginal ultrasound were performed. Outcome measures were serum anti-Mullerian hormone, inhibin B, estradiol, follicle stimulating hormone and ultrasound ovarian volume and antral follicle count.

Results—Mean antral follicle count was 7.6 for normal weight and 6.3 for obese subjects ($p=0.35$). Proportions of normal weight (17%) versus obese subjects (22%) with antral follicle count less than 4 were similar. Ovarian volumes did not differ by body size. In adjusted models, anti-Mullerian hormone levels in obese women were 77% lower on average than in normal weight women ($p=0.02$). Inhibin B levels were 24% lower in obese women compared to normal weight women ($p=0.08$). Follicle stimulating hormone and estradiol were not associated with BMI.

Conclusions—While antral follicle count did not differ by body size, anti-Mullerian hormone was lower in obese compared to normal weight late reproductive age women. These data suggest that lower anti-Mullerian hormone levels in obese, late reproductive age women result from physiologic processes other than decreased ovarian reserve.

Keywords

Obesity; AMH; inhibin B; AFC; ovarian reserve; late reproductive age

Introduction

Reproductive aging is characterized by the depletion of a relatively fixed number of ovarian follicles over a woman's lifetime^{1, 2}. Clinically, reproductive aging results in infertility, increased risk of miscarriage, menopausal symptoms and ultimately menopause. Ovarian reserve represents the quantity and quality of the remaining ovarian follicle pool. While multiple serum and ultrasound measures have been found to reflect ovarian reserve and are used clinically to assess reproductive status, limited data are available as to whether body size affects these measures.

In natural ovarian aging, the following measures of ovarian reserve have been studied: anti-Mullerian hormone (AMH), inhibin B, follicle stimulating hormone (FSH), estradiol and antral follicle count (AFC). AMH, also known as mullerian-inhibiting substance, is a glycoprotein produced by granulosa cells surrounding ovarian follicles³. Because the majority of AMH is secreted by primary and preantral follicles, levels are gonadotropin-independent and are therefore a more consistent measure of ovarian reserve than other hormones such as FSH^{4, 5}. Low AMH has been associated with natural ovarian aging and infertility^{6–8}.

Inhibin B is a heterodimeric glycoprotein produced by small antral follicles in the ovary⁹. Inhibin B provides negative feedback to FSH secretion from the pituitary and is highest during the follicular phase⁹. In healthy women, inhibin B levels fall over reproductive life and the menopausal transition¹⁰.

AFC is an ultrasound marker of ovarian aging¹¹. With reproductive aging, the primordial follicle pool decreases. While the size of the primordial follicle pool is difficult to measure directly, it is correlated with the number of growing follicles^{12, 13}. These growing follicles can then be visualized via ultrasound. In the assisted reproductive technology literature, lower AFC has been associated with poor ovarian response^{14, 15}. Various cut points for low AFC, most often less than 4 or 6, have been described in predicting poor ovarian response¹⁵.

Recently, we have reported that AMH and inhibin B were negatively associated with body size in a cohort of late reproductive aged women^{16, 17}. There are two possible explanations for the observed negative effect of body mass index (BMI) on AMH and inhibin B. First, lower levels of AMH and inhibin B in overweight and obese women may reflect a decrease in ovarian reserve. This first explanation is supported by the observation that obese women are less fertile (even in the presence of ovulatory menstrual cycles) and more prone to miscarriage than normal weight women^{18–20}. In addition, obese women exhibit decreased ovarian response to gonadotropin stimulation during superovulation^{21, 22}. Second, obesity may affect ovarian hormonal production, sequestration, or clearance^{23, 24}.

The overall goal of this study was to further examine the association between obesity and measures of ovarian reserve in late reproductive aged women. Our primary objective was to assess whether antral follicle count, an ultrasound marker of ovarian reserve, varies by body

size. We hypothesized that if obesity leads to decreased ovarian reserve, then antral follicle count would be lower in obese women than in normal weight women. Our secondary objective was to test whether other hormonal measures of ovarian reserve (AMH, inhibin B, FSH and estradiol) vary by body size in this sample of late reproductive aged women.

Materials and methods

Subjects

Thirty-six late reproductive age women were recruited from the Philadelphia area. Twenty-two subjects are participants of the Penn Ovarian Aging Study, an ongoing population-based cohort. These subjects were a separate sample than our prior reports on the associations between AMH, inhibin and obesity, and all data were collected specifically for this study. Eighteen women had normal BMI ($< 25 \text{ kg/m}^2$), and 18 women were in the obese group ($\geq 30 \text{ kg/m}^2$). Inclusion criteria included age ≥ 40 , menstrual cycles in the normal range (22 – 35 days), BMI $< 25 \text{ kg/m}^2$ or $\geq 30 \text{ kg/m}^2$, and intact uterus and ovaries. Exclusion criteria included hormonal therapy, contraception, and polycystic ovarian syndrome. The subjects were generally healthy. Several subjects were being treated for the following comorbidities: hypertension ($n = 5$), hypercholesterolemia ($n = 2$), hypothyroidism ($n = 2$), non-insulin dependent diabetes mellitus ($n = 1$), and history of breast cancer on tamoxifen ($n = 1$). The study was approved by the Institutional Review Board of the University of Pennsylvania. All subjects provided written consent.

Measures

Subjects underwent a non-fasting blood draw, anthropometric measurements and a transvaginal pelvic ultrasound within the first 4 days of a menstrual cycle. Height (without shoes) was measured to the nearest 0.5 cm with a vertical ruler. Body weight (in light clothing) was measured to the nearest 0.2 kg with a portable scale. Blood samples were centrifuged and frozen in aliquots at -80 degrees C. FSH, estradiol, inhibin B and AMH assays were conducted in the General Clinical Research Center of the University of Pennsylvania. For each sample, hormonal assays were performed in duplicate; means of the duplicates were used for analysis. Estradiol and FSH were measured by radioimmunoassay using Coat-A-Count commercial kits (Diagnostic Products, Los Angeles, CA). The intra- and interassay coefficients of variation were less than 5%. The lower limit of detection for estradiol was 20 pg/mL. The lower limit of detection for FSH was 0.06 mIU/mL. AMH was assayed using AMH ELISA kits (Diagnostic Systems, Webster, TX). The intra- and interassay coefficients of variation were less than 4% and 6%, respectively. The lower limit of detection was 0.05 ng/mL. Dimeric inhibin B was assayed using Inhibin B ELISA kits (Diagnostic Systems, Webster, TX). The intra- and interassay coefficients of variation were 3.5–4.6% and 6.3–7.6%, respectively. The lower limit of detection was 7 pg/mL. All values used in statistical analyses were actual values, even those below the kit threshold of detection.

Body mass index was calculated by weight (in kilograms) divided by the square of height (in meters). BMI was dichotomized into normal weight ($< 25 \text{ kg/m}^2$) or obese ($\geq 30 \text{ kg/m}^2$) groups as selected for the study.

Ovarian volume and antral follicle counts were determined by transvaginal pelvic ultrasonography within the first four days of a menstrual cycle. Pelvic ultrasounds were performed primarily by two trained gynecologists using a standard protocol. Both ovaries were measured in the maximum transverse, anterior-posterior and longitudinal diameters. The volume was estimated as $\pi/6 \times 3$ diameters. Abnormalities of morphology were collected. Antral follicles were defined as follicles between 2 and 10 millimeters in

diameter. Antral follicle count represents the total number of antral follicles from two ovaries.

Age, race, smoking status and alcohol use were collected through a structured questionnaire by self-report at the time of ultrasound as potential confounders of the association between body size and measures of ovarian reserve.

Power calculation

Estimates of statistical power to detect a difference in antral follicle count between obese and normal weight women were conducted. Low AFC was defined as follicle count less than 4. The prevalence of low AFC in normal weight subjects was assumed to be 50%. Type I error was set to 5%. In a 1:1 ratio of obese to normal weight subjects, a total of 30 subjects were required to have 80% power to detect a relative risk of 2 when comparing the proportion of obese subjects to the proportion of normal weight subjects with AFC less than 4. A post hoc power calculation determined that this study had 86% power to detect the observed difference, which corresponds to an effect size of 0.88 sd units or greater between mean antral follicle counts in the two groups.

Statistical methods

Baseline characteristics were compared between the normal weight and obese groups. Student's t test (for normally distributed data) or Wilcoxon rank-sum test (for non-normally distributed variables) were used to compare continuous variables. Categorical variables were characterized by proportions and compared by chi-square. Graphic displays of outcome variables were explored to determine data distributions. Hormone measures were transformed to natural log values to minimize the impact of their skewed distributions. Mean values for hormones are reported as geometric means. Two-sample t-tests were performed to compare mean log transformed hormonal values between the two weight groups. Ovarian volume and antral follicle counts were compared between the two groups using the t-test or chi-square as appropriate for the data. Correlations between hormone values and ultrasound measurements were also computed. Finally, multivariable linear regression models were constructed to examine the independent associations of AMH, inhibin B, and AFC with BMI, adjusting for other potential confounders. A two-tailed p value of <0.05 was considered statistically significant.

Results

Demographic characteristics are shown in Table 1. The mean age ($\pm$ SD) was 45 ± 2.0 years. The mean BMI (95% CI) was 22.4 (21.6–23.2) in normal weight subjects and 37.6 (34.2–40.8) in obese subjects. All variables except age significantly differed between the two groups.

Mean antral follicle count, the primary outcome, was similar between the two groups (Table 2). Using a cut-point of 4 for low AFC, the proportion of normal weight subjects (17%) and the proportion of obese subjects with low AFC (22%) were similar ($p = 0.80$). The relative risk of low AFC in obese compared to normal weight women was 1.33 (95% CI 0.35–5.13). Results were similar using cut-points of 3 or 5 for low AFC. The proportion of women with ovarian cysts was high (44%), but comparable between the groups. Ovarian volume was not significantly higher in the obese group when compared to normal weight ($p=0.06$).

Hormone measures of ovarian reserve by BMI group are depicted in Table 2. AMH and inhibin B were significantly lower in obese women. FSH and estradiol were similar between the two groups.

We then examined the adjusted associations between clinically and statistically significant study variables with AMH and inhibin B in linear regression models. BMI ($p=0.02$) and age ($p = 0.003$) remained independent predictors of AMH in a multivariable model also adjusted for smoking, alcohol and race (Table 3). The adjusted geometric mean AMH levels were lower in obese subjects compared to normal weight subjects, 0.06 ng/mL (95% CI 0.03–0.13) and 0.28 ng/mL (95% CI 0.12–0.67), respectively. Smoking ($p = 0.26$), alcohol ($p = 0.43$) and race ($p = 0.71$) were not significantly associated with AMH in the model.

BMI ($p = 0.08$) was not independently associated with inhibin B in a multivariable model that included age, smoking, alcohol and race (Table 3). The adjusted geometric mean inhibin B levels appeared lower in obese women compared to normal weight subjects, 9.5 ng/mL (95% CI 8.0–11.3) and 12.5 ng/mL (95% CI 10.1–15.4), respectively, but this finding did not reach statistical significance. Age ($p = 0.2$), smoking ($p = 0.82$), alcohol ($p = 0.89$) and race ($p = 0.39$) were not significantly associated with inhibin B. In both AMH and inhibin B models, we also tested the interaction between AFC and BMI and found no statistical significance. Finally, AMH and inhibin B models excluding the subject on tamoxifen showed no change in the association between each hormone and BMI.

Discussion

The overall goal of this study was to investigate whether measures of ovarian reserve differed between obese and normal weight women in late reproductive age. Specifically, we conducted a comprehensive evaluation of measures of ovarian reserve, including both serum and ultrasound measures. We hypothesized that all measures of ovarian reserve would be affected by body size. We found mean AMH levels were significantly lower in obese women compared to normal weight women, but other hormone measures and ultrasound measures did not differ by body size^{16, 17}. To our knowledge, this study is the first to examine antral follicle count and body size in late reproductive age women. These findings do not support the notion that ovarian reserve is impaired in obese women.

Although AFC was slightly lower and ovarian volume appeared clinically larger in obese subjects compared to normal weight subjects, the differences were not statistically significant. Given small sample size, the mean ovarian volumes could be influenced by the presence of outliers, as evidenced by wide confidence intervals. Similar proportions of the groups had ovarian cysts, and therefore, presence of cysts did not confound the association. Because most ovarian cysts were measured in two dimensions, we were unable to subtract ovarian cyst volume from total ovarian volume for more detailed analysis. However, because several volumes were affected by large cysts, subtracting the cyst volumes would have likely resulted in more similar ovarian volumes between the two weight groups.

Our examination of AFC with other hormone measures of ovarian reserve showed that AFC had a significant, positive association with AMH. However, AFC was not associated with inhibin B. This finding suggests that AFC and AMH both reflect the pool of early, gonadotropin-independent follicles. In contrast, inhibin B is secreted from gonadotropin-dependent, recruited follicles and may be a less consistent measure of the ovarian reserve pool.

Taken together, these results indicate that decreased ovarian reserve is not the direct cause of lower AMH in obese, regularly cycling, late-reproductive-aged women. In published data, serum and ovarian measures of decreased ovarian reserve do not show consistent changes with body size^{17, 26–28}. One explanation for this lack of consistency is that these measures are not ideal surrogates of ovarian reserve. Alternatively, body size may alter hormone production at the level of the ovary, increase sequestration or elimination of serum

hormones^{23–25}. For example, the observation that adiponectin modulates ovarian steroidogenesis in conjunction with insulin and gonadotropins provides indirect evidence that obesity alters ovarian hormonal synthesis²⁵.

Several limitations to our study should be considered. As observed in the general population, race and BMI were highly associated with one another in this study, limiting the ability to separate the effect of the two variables on the ovarian reserve measures. It is not clear if this affects the validity of our results. Data on racial differences in ovarian reserve measures are mixed. While the Study of Women's Health Across the Nation has reported higher FSH in African American women compared to Caucasian women, we have not observed this difference in the Penn Ovarian Aging cohort^{16, 17, 29}. Therefore, in the present study, we did not recruit subjects or stratify statistical analyses based on race. Antral follicles, 2 to 10 millimeters in size, can be difficult to visualize with increased body size. Had this occurred, it would have biased the results toward finding a difference in AFC between the two BMI groups. In addition, using surrogate markers to measure ovarian aging in late reproductive age women is another potential limitation. Ideal surrogate markers are closely linked with the clinical outcome of interest. In younger patients, these surrogate hormonal and ultrasound measures of ovarian reserve have been validated using assisted reproductive technology outcomes³⁰. However, in late reproductive age women, the association of these measures with the gold standard measurement of time to menopause has not been established. Another limitation is the cross-sectional design, where risk factors (BMI) and outcomes of interest are examined at one time point. Therefore, causality, or the sequence of events, cannot be determined. Nonetheless, it is biologically plausible that body fat contributes to different levels of AMH.

Study subjects had several comorbidities representative of women in this age group. By including these subjects, our findings are potentially more generalizable. One subject had a history of breast cancer and was on tamoxifen. Because of the potential effect of tamoxifen on hormonal measures of ovarian reserve, analyses were rerun excluding this subject³¹. No significant departures from our reported results were observed.

Finally, while this pilot study had small numbers, we were sufficiently powered for our outcome of interest. Because we sampled subjects by BMI, difference in demographics, such as smoking and race, arose by chance. However, the association of BMI with the AMH persisted when we adjusted for these potential confounders, an indication that our findings are robust. Unfortunately, while mean inhibin B levels appeared lower in obese subjects than in normal weight subjects after adjustment, the association was no longer statistically significant in this limited sample.

In conclusion, we present the first report on the relationship between body size and ovarian antral follicle count. While certain hormone measures such as AMH are decreased in obese women, ultrasound measures of ovarian reserve do not differ by body size. Therefore, it does not appear that obese women have decreased ovarian reserve compared to normal weight women. These findings may be a function of different hormone metabolism, sequestration, or clearance in obese women. Further studies of the mechanisms underlying these observations are warranted. Importantly, given the increased use of these hormone measures in clinical practice, clinicians should be aware that levels may be altered in obese women. Serum measures of ovarian reserve, especially Inhibin B and AMH, should be interpreted with caution in these women.

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Table 1

Demographic characteristics, n = 36

	Normal weight (BMI < 25) n = 18	Obese (BMI ≥ 30) ^b n = 18	p-value
Age (mean, range) ^a	45.0 (40–52)	45.1 (41–46)	0.88
BMI (mean, 95% CI) ^{a,b}	22.4 (21.6–23.2)	37.6 (34.2–40.8)	<0.001
Race ^{c,d}			0.001
Caucasian	15 (88%)	6 (33%)	
African American	2 (12%)	12 (67%)	
Current smoking ^{c,e}			
Yes	2 (11%)	9 (50%)	0.01
No	16 (89%)	9 (50%)	
# Cigarettes/week (median, range)	0 (0–140)	5 (0–140)	0.02
Current alcohol use ^{c,e}			
Yes	15 (83%)	4 (22%)	<0.001
No	3 (17%)	14 (78%)	
# Alcoholic drinks/week (median, range)	2.8 (0–42)	0 (0–6)	0.002

^a Student's t-test^b One subject with BMI = 28 kg/m² was grouped with obese women for analysis^c X² test^d One normal weight subject was Asian-American^e Wilcoxon rank sum test

Table 2 Measures of ovarian reserve^a

	Normal weight (BMI < 25) n = 18	Obese (BMI ≥ 30) n = 18	p-value
AFC ^b	7.6 (5–10.2)	6.3 (3.3–9.3)	0.35
Mean ovarian volume (cm ³) ^b	6.1 (4.7–7.5)	11.0 (6.2–15.8)	0.06
Ovarian cysts (%) ^b	44.4%	44.4%	1.0
AMH (ng/mL) ^c	0.30 (0.1–0.6)	0.07 (0.03–0.15)	0.014
Inhibin B ^c	13.4 (11.4–15.7)	9.3 (8.0–11.0)	0.006
FSH ^c	10.6 (8.26–13.6)	11.4 (8.9–14.6)	0.69
Estradiol ^c	38.1 (29.5–49.3)	31.0 (24.0–40.0)	0.28

^a Student's t-test^b Mean (95% CI)^c Geometric mean (95% CI). Geometric mean is back-transformed from the group mean of the log hormone levels.

Table 3
Unadjusted^a and adjusted^b associations of BMI and race with geometric means of AMH and inhibin B

	AMH (ng/mL) (95% CI)			Inhibin B (ng/mL) (95%CI)		
	Unadjusted	p-value	Adjusted	Unadjusted	p-value	Adjusted
BMI < 25	0.3 (0.14–0.63)	0.008	0.28 (0.12–0.67)	13.4 (11.4–15.7)	0.002	12.5 (10.1–15.4)
BMI ≥ 30	0.07 (0.03–0.15)		0.06 (0.03–0.13)	9.4 (8.0–11.0)		9.5 (8.0–11.3)
Race						
Caucasian	0.19 (0.1–0.38)	0.11	0.15 (0.08–0.29)	12.3 (10.6–14.3)	0.02	11.6 (9.9–13.7)
African American	0.08 (0.03–0.18)		0.12 (0.05–0.29)	9.3 (7.7–11.1)		10.2 (8.2–12.6)

^a Student's t-test

^b Multivariable linear regression model adjusting for age, BMI, race, smoking status and alcohol use