Antimüllerian hormone levels and antral follicle counts are not reduced compared with community controls in patients with rigorously defined unexplained infertility

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Objective: To test the hypothesis that women with unexplained infertility demonstrate evidence of diminished ovarian reserve when compared with a population of community controls.
Design: Cross-sectional study.
Setting: Multicenter university-based clinical practices.
Patient(s): Study participants included 277 healthy, normo-ovulatory female partners with rigorously defined unexplained infertility randomly selected from a multicenter trial (Assessment of Multiple Intrauterine Gestations from Ovarian Stimulation). Controls included 226 healthy, normo-ovulatory women not seeking treatment for fertility from a community-based cohort (Ovarian Aging study).
Intervention(s): Serum antimüllerian hormone (AMH) assay at a central laboratory, FSH, fasting serum metabolic testing, transvaginal ultrasonography for antral follicle counts (AFCs), anthropometric measurements.
Main Outcome Measure(s): Average AMH, AFC, and AMH/AFC were compared between infertile and control women by age. Analyses of covariance compared these outcomes while controlling for confounders, including age, race, body mass index, smoking history, and study site.
Result(s): In our models, AMH, AFC, and AMH/AFC ovarian reserve indices did not differ between infertile women and community-based controls, after controlling for age, race, body mass index, smoking history, and study site.
Conclusion(s): Currently utilized predictors of ovarian reserve do not discriminate women with rigorously defined unexplained infertility from healthy community-based women of similar demographic characteristics. Contrary to our hypothesis, among women with FSH in the normal range ($\leq$12 IU/L), women with unexplained infertility did not show evidence of decreased ovarian reserve as measured by AMH and AFC. Ovarian reserve markers in isolation may not serve as predictors of future fertility. (Fertil Steril® 2017;108:1070–7. ©2017 by American Society for Reproductive Medicine.)
Key Words: AFC, AMH, ovarian reserve, unexplained infertility

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"Ovarian reserve" reflects "reproductive potential as a function of the number and quality of remaining oocytes" (1). Although ovarian reserve declines with increasing age, there is substantial variation among women, and as a result, ovarian reserve has been widely investigated as a marker of reproductive potential. The majority of studies on this topic investigate the use of ovarian reserve to predict success by infertility patients during fertility treatment. There has been minimal investigation of whether ovarian reserve predicts reproductive potential outside of an infertile population. The present study was designed to compare ovarian reserve markers in women with unexplained infertility (UI) compared to community controls (1). We hypothesized that ovarian reserve would be reduced in infertile women compared with reproductively healthy controls.

Presently, antral follicle count (AFC) and serum antimüllerian hormone (AMH) are regarded as the most informative markers of ovarian reserve (1). Antral follicle count is the sum of antral follicles, typically defined as those follicles measuring 2–10 mm in mean diameter in a 2-dimensional plane, in both ovaries, as observed on transvaginal ultrasound in the early follicular phase of the menstrual cycle. Antral follicle count has good interobserver reliability (2) and is associated with response to stimulation in IVF (3). Antimüllerian hormone is a member of the transforming growth factor-β superfamily and is produced by granulosa cells in preantral and small (<8 mm) antral follicles. Antimüllerian hormone functions in regulation of follicular recruitment and reflects primordial follicle pool size (4, 5). Given the gonadotropin-independent growth of these early follicles, AMH has minimal intra- and intercycle variability and may thus be measured at any point in the menstrual cycle (6–8).

In up to 30% of patients presenting with infertility, no cause is identified (9, 10). The reported prevalence of UI varies as a function of the intensity of evaluation and factors included in the etiology (11); reports range widely between 0 and 37% (12). The definition of UI, a diagnosis of exclusion, is failure to achieve pregnancy after 12 months of attempting conception in the absence of definable cause after thorough evaluation (13). Couples with UI are afflicted by diminished and delayed fecundity, between 2% and 4% per cycle on average (14).

Professional guidelines of the American College of Obstetricians and Gynecologists recommend ovarian reserve testing in all women aged >35 years who have not conceived after 6 months of attempting pregnancy (15). The American Society for Reproductive Medicine has no specific guideline. Women with neither proven fertility nor infertility, desirous of understanding their "fertility potential," frequently request and obtain measurements of AMH and/or AFC (16).

There is thus an assumption that egg quantity predicts reproductive trajectory. In the context of UI, when lower than expected ovarian reserve metrics are identified, an explanatory value is often imputed. In the context of fertility planning, recommendations to freeze eggs or proceed with aggressive treatments may be suggested on the basis of the results (16). However, if low ovarian reserve is a cause of failure to conceive, one would expect UI subjects to have lower ovarian reserve than a control population. To date there is a paucity of data characterizing markers of ovarian reserve in the UI population compared with the general fertile population (17). The objective of this study was thus to test the hypothesis that women with UI demonstrate evidence of diminished ovarian reserve, when compared with a population of community controls.

MATERIALS AND METHODS

Design

This was a cross-sectional cohort analysis of 277 women with unexplained infertility, compared with 226 control women from a community-based cohort. Written informed consent was provided by all participants. Institutional review board approval at each participating study site was secured.

Participants

Unexplained infertility cohort. The study population included 277 female patients aged 25–40 years with UI. Patients were randomly selected from the AMIGOS (Assessment of Multiple Intrauterine Gestations from Ovarian Stimulation) study, a multicenter, prospective, partially blinded clinical trial comparing gonadotropins vs. clomiphene citrate vs. aromatase inhibitors (18). Inclusion criteria for the UI study cohort have been previously described (18–20). Participants were recruited between 2010 and 2013 at 12 participating Reproductive Medicine Network clinical sites throughout the United States. Women were included if they had 1 or more years of infertility, were desirous of conceiving, and were regularly ovulating, with a normal uterine cavity and at least one patent fallopian tube. Regular ovulation was considered as nine or more menses per year. To rigorously define UI, normal ovarian function was required by cycle day 3 ($\pm$2 days) FSH $\leq$12 IU/L within 1 year before study initiation. Normal uterine cavity and tubal patency were confirmed by hysterosalpingography, sonohysterography, or laparoscopy/hysteroscopy. Normal thyroid hormone and PRL serum testing were required within 1 year of study initiation. The male partner needed 5×10^6 sperm per milliliter in the ejaculate for inclusion in the UI cohort.

Exclusion criteria for the study cohort consisted of medical comorbidities, such as diabetes and heart, liver, and renal diseases. A washout period of 2 months was required for women taking oral contraceptives, depo-progestins, or hormonal implants, before initial baseline screening assessment. Further details regarding participant selection are publicly available (21).

Controls. The control population comprised 226 ovulatory women aged 25–40 years not seeking fertility treatment. Subjects were randomly selected from a community-based cohort, the OVA (Ovarian Aging) study (22), a prospective observational study designed to investigate reproductive aging. The OVA subjects were evaluated at one of the AMIGOS study sites (University of California, San Francisco). Participants were recruited between 2006 and 2010 from a sampling frame of all age-eligible female members of the Kaiser Permanente of Northern California Health Plan, within a reasonable travel distance to the research center. Kaiser

Permanente is an integrated healthcare delivery system covering 30% of the regional population. Inclusion criteria for the control population included regular menses at 22- to 35-day intervals and self-identification in one of four racial/ethnic groups categorized as Caucasian (27%), Asian (27%), Hispanic (23%), or African American (23%). Women reporting multiethnic origin were not enrolled.

Exclusion criteria for the OVA cohort included estrogen- or progestin-containing medication use in the 3 months before enrollment, history of endometriosis, or any history of uterine or ovarian surgery. To parallel the requirement of normal ovarian function as measured by cycle day 3 FSH in the UI group, women with FSH >12 IU/L were excluded. Further details regarding the study design and methodology for OVA have been previously published (22–24).

Ultrasound, Anthropomorphic Measurements, and Serum Testing

Unexplained infertility. Transvaginal ultrasonography for AFC was performed during an index visit at 1 of the 12 participating sites between menstrual cycle day 3 and 5 using standard clinical machines. Anthropometric baseline measurements were conducted within 6 months before trial start. Serum was obtained for assays at a core laboratory (University of Virginia, UVA), including AMH, FSH, fasting glucose, insulin, lipids, and high-sensitivity C-reactive protein before initiating ovulation induction (21).

Controls. Control patients were evaluated at one of the AMIGOS sites for all procedures. Anthropometric measurements and transvaginal ultrasound scans for AFC were performed at an index visit between cycle day 2 and 4. Antral follicle counts were determined by transvaginal ultrasound using a Shimadzu SDU-450XL machine with a variable transducer frequency of 4–8 mHz. Serum was collected at this baseline visit and banked for future use. Laboratory assays (other than AMH) were performed at a single commercial laboratory. Insulin results were converted to the UVA assay according to a previously published calibration curve (22, 23).

Serum AMH Measurements

Serum AMH for subjects from both cohorts was assayed at the single central core laboratory, the Ligand Assay and Analysis Core Laboratory at UVA, using the Ansh assay (ELISA; lower limit of detection 23 pg/mL; intra-assay coefficient of variation 3.9%; interassay coefficient of variation 6.2%; Ansh Labs) (25). Subset sizes were determined by funding and captured roughly one-third of each study cohort. The infertile patients were randomly selected for UVA assay by the SAS random number system and hence included as the study cohort. The control subset included all patients participating in the lngitudinal component of the study (n = 106), as well as 54% selected randomly by an SAS random number generator (n = 126), who participated in the baseline visit only.

The Ansh assay is resistant to AMH degradation, with stable AMH results demonstrated in 75 samples after 2.5 years of frozen storage ($R^2 = 0.97$ for AMH at t = 0 vs. t = +2.5 years; unpublished data from the Reproductive Medicine Network and Ligand Assay and Analytic Core Laboratory at UVA).

Statistical Analysis

The three primary endpoints considered were AMH, AFC, and AMH/AFC ratio. Considering UI as the predictor, analyses of covariance (ANCOVAs) were performed for the primary endpoints, while controlling for age, body mass index (BMI), smoking history, race, and study site. Statistical analyses were performed with SAS, version 9.4.

RESULTS

The infertile women were slightly younger on average compared with the control cohort (32.4 vs. 33.2 years). Distribution of socioeconomic factors is shown in Table 1. The community control cohort was more diverse than the infertile group (44% vs. 73% Caucasian). Mean BMI was the same in the two groups; however, the infertile women had larger waist circumference. Fasting glucose and total cholesterol were also

TABLE 1

Subject baseline characteristics.

Characteristic	Unexplained infertility (n = 277)	Control (n = 226)
Age (y)	32.3 (0.2)	33.1 (0.3)
BMI (kg/m^2)	26.3 (0.4)	26.3 (0.5)
Waist circumference (cm)	85.1 (0.9)	82.2 (1.0)
Income ($)		
<25,000	2.9	9.7
25,000 to 49,999	10.1	35.0
50,000 to 74,999	20.6	22.6
75,000 to 100,000	22.4	11.0
>100,000	21.7	20.8
Decline to state	22.4	–
Education (%)		
8th grade or less	–	2.2
Some high school	2.2	1.3
High school graduate	5.4	8.8
Some college	20.6	22.6
College graduate	44.8	43.8
Graduate degree	27.1	21.2
Race		
Caucasian	73.3	43.8
African American	7.2	19.5
Hispanic	11.1	17.7
Asian	8.3	19.0
History of smoking	33.9	25.2
Fasting glucose (mg/dL)	85.0 (0.7)	86.5 (0.6)
Fasting insulin (mg/dL)	9.24 (0.80)	7.79 (0.56)
HOMA-IR	2.03 (0.19)	1.73 (0.14)
Total cholesterol (mg/dL)	168.7 (2.0)	173.0 (2.0)
Triglycerides (mg/dL)	93.3 (3.4)	85.1 (3.4)
CRP (mg/L)	2.81 (0.21)	2.76 (0.48)
FSH (mIU/mL)	6.82 (0.11)	6.45 (0.10)
AMH (ng/mL)	5.87 (0.31)	5.18 (0.26)
AFC	20.6 (0.69)	17.3 (0.62)
AMH/AFC	0.32 (0.03)	0.30 (0.01)

Note: Values are mean (standard error) or percentage prevalence for categorical variables. AFC = antral follicle count; AMH = antimüllerian hormone; BMI = body mass index; CRP = C-reactive protein; FSH = follicle-stimulating hormone; HOMA-IR = homeostatic model assessment of insulin resistance.

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TABLE 2

Predictors of ovarian reserve outcomes in analysis of covariance multivariate model.

Factor	Coefficient	95% CI	*P* Value
AMH			
Infertility diagnosis (Y/N)	−1.71	−6.05, 2.64	.44
Age (y)	−0.30	−0.40, −0.21	<.01
Race			.02
Caucasian	Referent		
African American	1.41	0.13, 2.70	
Hispanic	0.74	−0.48, 1.97	
Asian	1.69	0.47, 2.92	
BMI (kg/m^2)	−0.10	−0.16, −0.04	<.01
Smoking history (Y/N)	0.01	−0.84, 0.87	.97
AFC			
Infertility diagnosis (Y/N)	−5.01	−14.32, 4.32	.29
Age (y)	−0.96	−1.17, −0.75	<.01
Race			.04
Caucasian	Referent		
African American	2.08	−0.68, 4.84	
Hispanic	2.89	0.25, 5.52	
Asian	−1.47	−4.11, 1.17	
BMI (kg/m^2)	−0.07	−0.20, 0.07	.35
Smoking history (Y/N)	−0.45	−2.28, 1.39	.63
AMH/AFC			
Infertility diagnosis (Y/N)	0.01	−0.31, 0.32	.97
Age (y)	−0.004	−0.011, 0.003	.27
Race			.04
Caucasian	Referent		
African American	−0.01	−0.10, 0.09	
Hispanic	−0.01	−0.10, 0.08	
Asian	0.13	0.04, 0.22	
BMI (kg/m^2)	−0.003	−0.007, 0.002	.24
Smoking history (Y/N)	0.05	−0.01, 0.11	.13

Note: Controlled for study site. AFC = antral follicle count; AMH = antimüllerian hormone; BMI = body mass index; CI = confidence interval; N = no; Y = yes.

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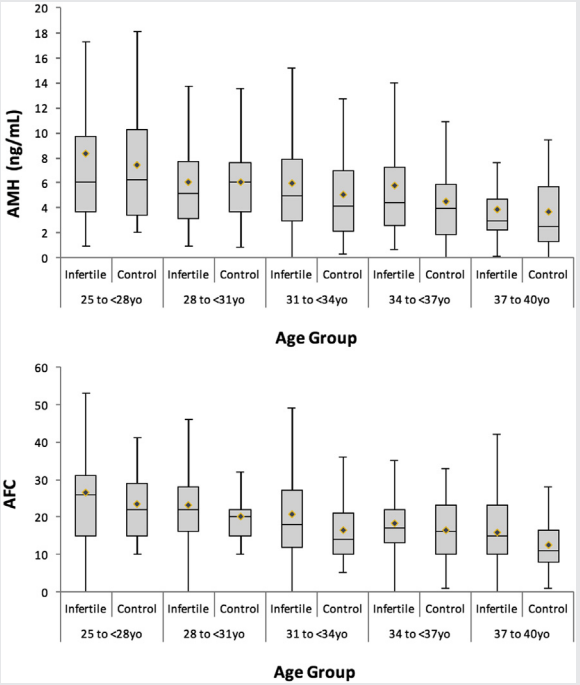
FIGURE 1



Antimüllerian hormone and AFC by age. Unadjusted whisker plots of ovarian reserve measures by age group.

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slightly lower in infertile women. Smoking history was more common in infertile women (Table 1).

In ANCOVAs controlling for age, race, BMI, smoking, and study site, infertility was not an independent predictor of AMH, AFC, or AMH/AFC ratio (Table 2). In this model, age and BMI demonstrated a negative effect on AMH (coefficient [Coeff] −0.32, 95% confidence interval [CI] −0.42, −0.22, $P<.0001$; Coeff −0.10, 95% CI −0.16, −0.03, $P<.01$, respectively). Antimüllerian hormone also varied by race ($P=.02$). Compared with Caucasians as the referent, African American and Asian women had higher AMH levels (Coeff 1.30 and 1.73, respectively). Age, but not BMI or race, maintained a statistically significant relationship with AFC (Coeff −0.99, 95% CI −1.20, −0.78, $P<.0001$). Race alone was an independent predictor of AMH/AFC ratio, with Asian women having higher AMH/AFC vs. white women (Coeff 0.13, 95% CI 0.03, 0.22, $P=.04$). Smoking history was not an independent predictor of ovarian reserve metrics in this model (Table 2).

By age, AMH and AFC demonstrated wide variation (Fig. 1). Infertile patient ovarian reserve markers did not differ significantly from control patients. Predictive models showed a decline in AMH of 0.32 (95% CI −0.41, −0.22) per year (Fig. 2). Antral follicle count declined by 1.0 by year. These rates of decline were the same in each group. When comparing per-follicle AMH, we again noted a wide range of values, which did not differ between the two study groups (Fig. 2).

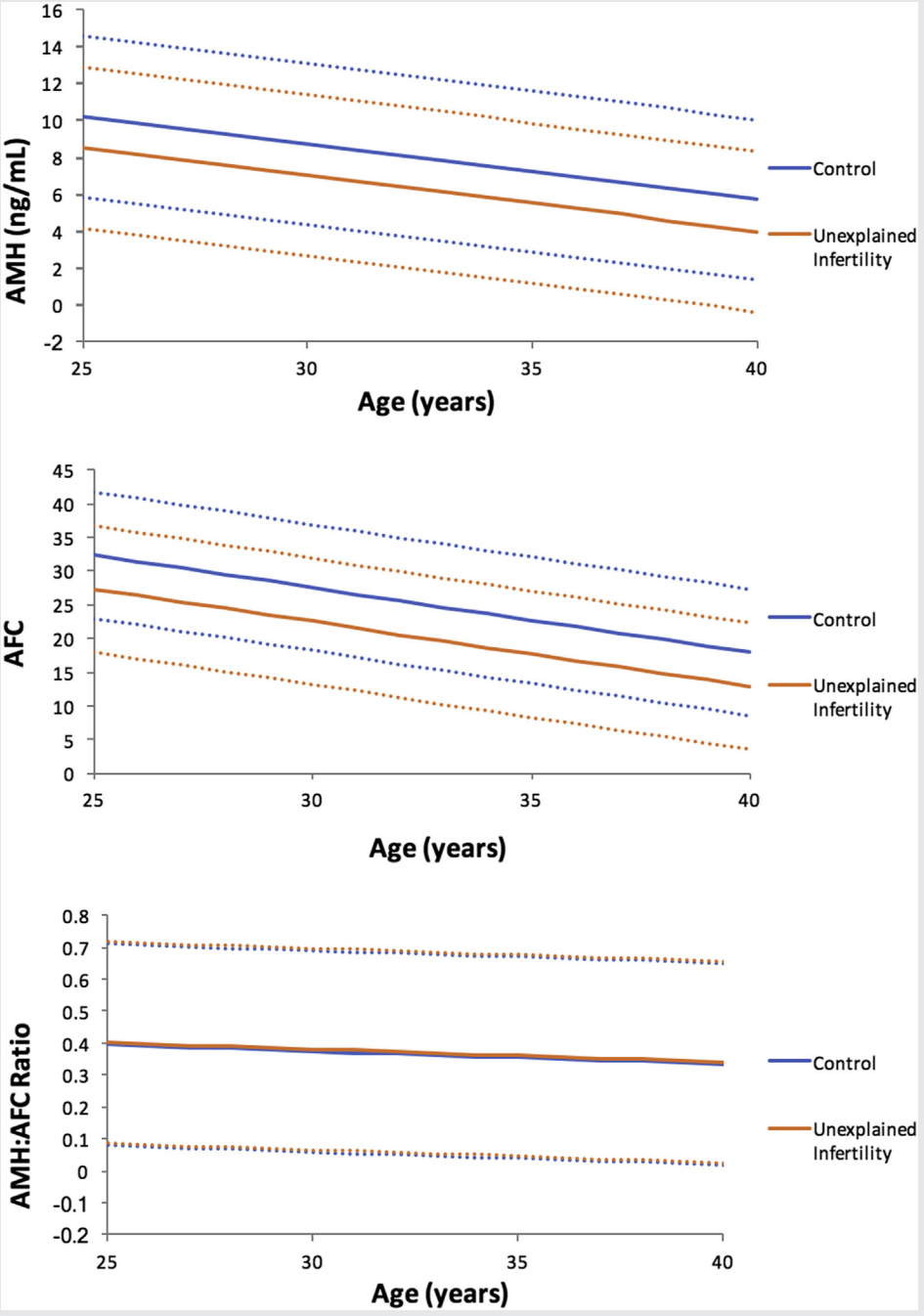
DISCUSSION

In a large population of women with unexplained infertility, we demonstrate that markers of ovarian reserve do not differ from those in a community-based cohort of reproductively healthy controls. To our knowledge, our study is the largest to compare such markers in the UI population with healthy community controls.

We noted difference in baseline characteristics (Table 1) between infertile and control groups. These differences are perhaps attributable to geographic variability or differences in the ethnic makeup of the two cohorts, rather than a direct relationship with infertility diagnosis. The baseline differences identified have little clinical impact. There were no differences in our main outcome variables, AMH and AFC, while controlling for study site.

The results of our study are novel and challenge the assumption that ovarian reserve is an indicator of fertility. Despite being a common theory, the physiologic evidence associating UI and ovarian reserve is relatively limited. Prior studies in women with unexplained infertility detected elevations in early follicular serum gonadotropins relative to

FIGURE 2



Antimüllerian hormone, AFC, and AMH/AFC predicted models. Solid lines display predicted outcome effects. Models are controlled for age, race, BMI, smoking history, and study site. The 95% confidence intervals, depicted by dotted lines, are calculated for infertility effect only.

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parous controls (26, 27). This work focused on FSH, a metric limited by substantial inter- and intracycle variability (1). Antimüllerian hormone and AFC exhibit less variability and have widespread clinical use today. Although they predict response to ovarian hyperstimulation, they have not been shown to predict pregnancy after treatment or in natural circumstances (1, 28, 29).

Antral follicle count and AMH are common components in the evaluation of a female infertile patient. Among IVF populations, low AFC (threshold three to four follicles) is highly specific for predicting cycle cancellation or recovery of fewer than three to four oocytes (30–32). Antimüllerian hormone correlates well with AFC in normo-ovulatory IVF populations (33, 34), is also strongly associated with poor

ovarian response to stimulation when low (35–37), predicts excessive response (38, 39), and may further convey information regarding oocyte or embryo quality (40, 41). Although AMH and AFC are closely correlated, some investigators have reported further utility in the per-follicle AMH level (AMH/AFC ratio) in predicting pregnancy (42). Elevated per-follicle AMH production has been additionally implicated in the ovulatory dysfunction characteristic of polycystic ovary syndrome (43), implying potential value as an independent biologic parameter.

Egg quality and egg quantity might vary together, and at present we do not have a good way to measure egg quality other than IVF; thus egg quantity is often used as a surrogate for egg quality. The utility of AMH in differentiating between fertile and infertile has been assumed from extrapolated contexts. Antimüllerian hormone predicts age at menopause (44, 45). Given the posited fixed period of time between the end of natural fertility and menopause (46), AMH level might thus inform individual women about their reproductive lifespan and current reproductive capacity. However, no prior studies have directly compared AMH levels in couples with unexplained infertility vs. normal controls. These data demonstrate that AMH levels are no different between women with unexplained infertility and healthy community-dwelling women of similar age and BMI. Our findings are consistent with other reports of inability of AMH to discriminate fertility status between various infertility diagnoses compared with fertile controls (47) and within infertility diagnoses (UI vs. male factor) (48).

We did not detect a difference in AFC between infertile and control women. This is consistent with a prior analysis of 1,107 unstimulated timed donor insemination cycles in 549 presumably fertile women, in which AFC was not predictive of pregnancy (49). Alternatively, one prior study reported lower AFC among unexplained infertile women when compared with community controls by 5-year age strata, suggesting decreased AFC "may be a marker of both oocyte quantity and quality" (50). The reason for our conflicting findings is unclear. Possibly by only comparing median AFC values between 5-year age strata, this prior study failed to account for the potential contribution of other confounders, such as BMI and race. Age itself might have varied between groups using this stratified approach in a statistically meaningful way. Our study uses an ANCOVA model using age as a continuous predictor, negating the impact of these potential pitfalls. An alternate explanation for our contrasting results is perhaps that the enrollment of subjects into the AMIGOS trial may have been biased toward patients perceived as having a good prognosis, whereas patients perceived as having a poorer prognosis on the basis of ovarian reserve were encouraged to proceed with more aggressive assisted reproductive technology therapies. Although we are unable to confirm these hypotheses, the findings that AMH levels did not differ between UI and control groups on a single assay, and the established association of AMH and AFC, suggests AFC too is no different between groups. In our study, the AMH/AFC outcome also did not differ, a likely consequence of the covariance of these two outcomes.

The implications of our results have value beyond challenging the common assumption that female patients with UI have reduced ovarian reserve. Understanding comparability of ovarian reserve markers is essential for investigations of various clinical populations. Recent interest in using AMH as a biomarker in the diagnosis of polycystic ovary syndrome has relied on convenience data from infertile populations as the control (51). Selection of infertile women as control subjects has been criticized on the basis of the underlying assumption that markers of ovarian reserve should be, theoretically, lower in women with infertility than in their reproductively healthy counterparts. However, our data suggest that in fact women with unexplained infertility have AMH levels and AFC values no different than in community-based controls and may thus afford a proper comparison.

Strengths of our study include the use of a rigorously defined cohort of women with unexplained infertility and a well-characterized community-based cohort of prospectively recruited multiethnic, healthy, normo-ovulatory women not seeking fertility treatment. Study subjects and controls alike were required to abstain from hormonal contraceptives for at least 3 months before index measurements, allowing avoidance of this known confounder of AMH. Finally, the utilization of a single, reliable AMH assay (Ansh) known for its excellent performance (25) for all individuals in our study is a unique strength of our design.

Limitations of our study include its retrospective ancillary design. We were unable to send all banked serum specimens from individuals in our study to the central laboratory for analysis owing to funding constraints. However, random selection among our study groups mitigated the statistical implications of this limitation. The baseline characteristics of our study and control cohorts differed in various ways (Table 1). Our infertile group also had larger waist circumference, though similar BMI. Obesity is known to adversely affect AMH through a suspected negative effect on granulosa cell function (52), but the impact of waist circumference, an index of central adiposity and risk factor for insulin resistance, is less clear. Nonetheless, metabolic parameters, including fasting insulin and glucose levels, were similar between groups and in the normal range. Differences in waist circumference might reflect the different ethnic makeup of the two cohorts. Smoking was more common in our infertile group. The community-based OVA cohort is believed to represent a sample of women with relatively normal fertility. Although 46% of the OVA cohort were gravid, we do not know how many had ever attempted pregnancy, a potential limitation of the study. Subjects were excluded from the control population if they had a history of uterine or ovarian surgery. Had such subjects been included it may have further biased our results toward the null, given the association of ovarian surgery with decreased ovarian reserve. Finally, the measurement of AFC may vary substantially between study sites, given differences in ultrasound machines and technique. Our regression analysis controlled for study site to offset this potential source of bias. Further, both AMH and AFC outcomes were similar between infertile and fertile women, corroborating our findings.

Another potential limitation of our study is the exclusion of women with cycle day 3 FSH >12 IU/L. This cutoff was set to rigorously define UI; a grossly elevated FSH level was not considered consistent with a true "unexplained" diagnosis owing to its association with premature ovarian failure, diminished ovarian reserve, and/or abnormal ovarian function. It is possible that a study comparing women with UI with a control population, without excluding women with FSH >12 IU/L, might have yielded different results. Nevertheless, our results indicate that among women with an FSH in the normal range, there is no difference in AMH and AFC. The study design characterized UI in a rigorous manner and thus may not be generalizable to a population with less rigorously defined UI, nor does it apply to the broader group of patients with infertility related to other causes.

Notably, our results do not preclude a functional difference in the follicular machinery that may relate to fertility potential. Frequencies of genetic polymorphisms in AMH and AMHRII, encoding the receptor, may differ between women according to fertility status (53). Such differences might not be captured by variation in hormone levels or follicle counts.

In conclusion, to our knowledge this is the largest study comparing predictors of ovarian reserve using a single AMH assay in women with rigorously defined unexplained infertility against a well-characterized control population. Contrary to our hypothesis, among women with normal FSH, women with UI did not show evidence of decreased markers of ovarian reserve. Our findings suggest that ovarian reserve testing in isolation may not be predictive of future fertility. Caution should be exercised when applying results of ovarian reserve testing to individual women.

REFERENCES

1. Practice Committee of the American Society for Reproductive Medicine. Testing and interpreting measures of ovarian reserve: a committee opinion. Fertil Steril 2012;98:1407–15.
2. Scheffer GJ, Broekmans FJ, Bancsi LF, Habbema JD, Looman CW, Te Velde ER. Quantitative transvaginal two- and three-dimensional sonography of the ovaries: reproducibility of antral follicle counts. Ultrasound Obstet Gynecol 2002;20:270–5.
3. Hendriks DJ, Mol BW, Bancsi LF, Te Velde ER, Broekmans FJ. Antral follicle count in the prediction of poor ovarian response and pregnancy after in vitro fertilization: a meta-analysis and comparison with basal follicle-stimulating hormone level. Fertil Steril 2005;83:291–301.
4. Durlinger AL, Gruijters MJ, Kramer P, Karels B, Ingraham HA, Nachtigal MW, et al. Anti-Mullerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. Endocrinology 2002;143:1076–84.
5. Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, et al. The physiology and clinical utility of anti-Mullerian hormone in women. Hum Reprod Update 2014;20:370–85.
6. Fanchin R, Taieb J, Lozano DH, Ducot B, Frydman R, Bouyer J. High reproducibility of serum anti-Mullerian hormone measurements suggests a multi-staged follicular secretion and strengthens its role in the assessment of ovarian follicular status. Hum Reprod 2005;20:923–7.
7. Tsepelidis S, Devreker F, Demeestere I, Flahaut A, Gervy C, Englert Y. Stable serum levels of anti-Müllerian hormone during the menstrual cycle: a prospective study in normo-ovulatory women. Hum Reprod 2007;22:1837–40.
8. Hehenkamp WJ, Looman CW, Themmen AP, de Jong FH, Te Velde ER, Broekmans FJ. Anti-Müllerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. J Clin Endocrinol Metab 2006;91:4057–63.
9. Aboulghar MA, Mansour RT, Serour GI, Al-Inany HG. Diagnosis and management of unexplained infertility: an update. Arch Gynecol Obstet 2003;267:177–88.
10. Dodson WC, Whitesides DB, Hughes CL Jr, Easley HA 3rd, Haney AF. Superovulation with intrauterine insemination in the treatment of infertility: a possible alternative to gamete intrafallopian transfer and in vitro fertilization. Fertil Steril 1987;48:441–5.
11. Moghissi KS, Wallach EE. Unexplained infertility. Fertil Steril 1983;39:5–21.
12. Collins JA, Crosignani PG. Unexplained infertility: a review of diagnosis, prognosis, treatment efficacy and management. Int J Gynaecol Obstet 1992;39:267–75.
13. Practice Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss: a committee opinion. Fertil Steril 2013;99:63.
14. Guzick DS, Sullivan MW, Adamson GD, Cedars MI, Falk RJ, Peterson EP, et al. Efficacy of treatment for unexplained infertility. Fertil Steril 1998;70:207–13.
15. Committee on Gynecologic Practice. Committee opinion no. 618: ovarian reserve testing. Obstet Gynecol 2015;125:268–73.
16. Kushnir VA, Barad DH, Gleicher N. Ovarian reserve screening before contraception? Reprod Biomed Online 2014;29:527–9.
17. Steiner AZ. Biomarkers of ovarian reserve as predictors of reproductive potential. Semin Reprod Med 2013;31:437–42.
18. Diamond MP, Legro RS, Coutifaris C, Alvero R, Robinson RD, Casson P, et al. Assessment of multiple intrauterine gestations from ovarian stimulation (AMIGOS) trial: baseline characteristics. Fertil Steril 2015;103:962–73.e4.
19. Diamond MP, Mitwally M, Casper R, Ager J, Legro RS, Brzyski R, et al. Estimating rates of multiple gestation pregnancies: sample size calculation from the assessment of multiple intrauterine gestations from ovarian stimulation (AMIGOS) trial. Contemp Clin Trials 2011;32:902–8.
20. Diamond MP, Legro RS, Coutifaris C, Alvero R, Robinson RD, Casson P, et al. Letrozole, gonadotropin, or clomiphene for unexplained infertility. N Engl J Med 2015;373:1230–40.
21. Diamond MP, Zhang H, RMN Data Coordinator Center. Assessment of Multiple Intrauterine Gestations from Ovarian Stimulation. Version 6.0, 2011. http://c2s2.yale.edu/rmn/resource/AMIGOS%20Protocol%20v6.0_246516_284_24481.pdf.
22. Bleil ME, Adler NE, Pasch LA, Sternfeld B, Gregorich SE, Rosen MP, et al. Depressive symptomatology, psychological stress, and ovarian reserve: a role for psychological factors in ovarian aging? Menopause 2012;19:1176–85.
23. Rosen MP, Johnstone E, McCulloch CE, Schuh-Huerta SM, Sternfeld B, Reijo-Pera RA, et al. A characterization of the relationship of ovarian reserve markers with age. Fertil Steril 2012;97:238–43.
24. Rosen MP, Johnstone E, McCulloch CE, Schuh-Huerta SM, Sternfeld B, Reijo-Pera RA, et al. A characterization of the relationship of ovarian reserve markers with age. Fertil Steril 2012;97:238–43.
25. Welsh P, Smith K, Nelson SM. A single-centre evaluation of two new anti-Mullerian hormone assays and comparison with the current clinical standard assay. Hum Reprod 2014;29:1035–41.
26. Leach RE, Moghissi KS, Randolph JF, Reame NE, Blacker CM, Ginsburg KA, et al. Intensive hormone monitoring in women with unexplained infertility: evidence for subtle abnormalities suggestive of diminished ovarian reserve. Fertil Steril 1997;68:413–20.
27. Randolph JF, Ginsburg KA, Leach RE, Blacker CM, Moghissi KS, Diamond MP, et al. Elevated early follicular gonadotropin levels in women with unexplained infertility do not provide evidence for disordered gonadotropin-releasing hormone secretion as assessed by luteinizing hormone pulse characteristics. Fertil Steril 2003;80:320–7.
28. Hansen KR, He AL, Styer AK, Wild RA, Butts S, Engmann L, et al. Predictors of pregnancy and live-birth in couples with unexplained infertility after ovarian stimulation-intrauterine insemination. Fertil Steril 2016;105:1575–83.e2.
29. Casadei L, Manicuti C, Puca F, Madrigale A, Emidi E, Piccione E. Can anti-Mullerian hormone be predictive of spontaneous onset of pregnancy in women with unexplained infertility? J Obstet Gynaecol 2013;33:857–61.

30. McIlveen M, Skull JD, Ledger WL. Evaluation of the utility of multiple endocrine and ultrasound measures of ovarian reserve in the prediction of cycle cancellation in a high-risk IVF population. Hum Reprod 2007;22:778–85.
31. Bancsi LF, Broekmans FJ, Eijkemans MJ, de Jong FH, Habbema JD, te Velde ER. Predictors of poor ovarian response in in vitro fertilization: a prospective study comparing basal markers of ovarian reserve. Fertil Steril 2002;77:328–36.
32. Frattarelli JL, Lauria-Costab DF, Miller BT, Bergh PA, Scott RT Jr. Basal antral follicle number and mean ovarian diameter predict cycle cancellation and ovarian responsiveness in assisted reproductive technology cycles. Fertil Steril 2000;74:512–7.
33. van Rooij IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong FH, et al. Serum anti-Müllerian hormone levels: a novel measure of ovarian reserve. Hum Reprod 2002;17:3065–71.
34. Nardo LG, Christodoulou D, Gould D, Roberts SA, Fitzgerald CT, Laing I. Anti-Mullerian hormone levels and antral follicle count in women enrolled in in vitro fertilization cycles: relationship to lifestyle factors, chronological age and reproductive history. Gynecol Endocrinol 2007;23:486–93.
35. Muttukrishna S, McGarrigle H, Wakim R, Khadum I, Ranieri DM, Serhal P. Antral follicle count, anti-mullerian hormone and inhibin B: predictors of ovarian response in assisted reproductive technology? BJOG 2005;112:1384–90.
36. Gnoth C, Schuring AN, Friol K, Tigges J, Mallmann P, Godehardt E. Relevance of anti-Mullerian hormone measurement in a routine IVF program. Hum Reprod 2008;23:1359–65.
37. Penarrubia J, Fabregues F, Manau D, Creus M, Casals G, Casamitjana R, et al. Basal and stimulation day 5 anti-Mullerian hormone serum concentrations as predictors of ovarian response and pregnancy in assisted reproductive technology cycles stimulated with gonadotropin-releasing hormone agonist–gonadotropin treatment. Hum Reprod 2005;20:915–22.
38. Nardo LG, Gelbaya TA, Wilkinson H, Roberts SA, Yates A, Pemberton P, et al. Circulating basal anti-Mullerian hormone levels as predictor of ovarian response in women undergoing ovarian stimulation for in vitro fertilization. Fertil Steril 2009;92:1586–93.
39. Broer SL, Dolleman M, Opmeer BC, Fauser BC, Mol BW, Broekmans FJ. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: a meta-analysis. Hum Reprod Update 2011;17:46–54.
40. Silberstein T, MacLaughlin DT, Shai I, Trimarchi JR, Lambert-Messerlian G, Seifer DB, et al. Mullerian inhibiting substance levels at the time of HCG administration in IVF cycles predict both ovarian reserve and embryo morphology. Hum Reprod 2006;21:159–63.
41. Ebner T, Sommergruber M, Moser M, Shebl O, Schreier-Lechner E, Tews G. Basal level of anti-Müllerian hormone is associated with oocyte quality in stimulated cycles. Hum Reprod 2006;21:2022–6.
42. Grynberg M, Genro V, Arbo E, Frydman N, Frydman R, Fanchin R. Serum anti-müllerian hormone (AMH) level/antral follicle count ratio: an alternative approach to optimize the predictability of AMH as a marker of IVF-ET outcome. Fertil Steril 2009;92:S177.
43. Alebic MS, Stojanovic N, Duhamel A, Dewailly D. The phenotypic diversity in per-follicle anti-Mullerian hormone production in polycystic ovary syndrome. Hum Reprod 2015;30:1927–33.
44. Broer SL, Eijkemans MJ, Scheffer GJ, van Rooij IA, de Vet A, Themmen AP, et al. Anti-mullerian hormone predicts menopause: a long-term follow-up study in normoovulatory women. J Clin Endocrinol Metab 2011;96:2532–9.
45. Tehrani FR, Shakeri N, Solaymani-Dodaran M, Azizi F. Predicting age at menopause from serum antimullerian hormone concentration. Menopause 2011;18:766–70.
46. Broekmans FJ, Soules MR, Fauser BC. Ovarian aging: mechanisms and clinical consequences. Endocr Rev 2009;30:465–93.
47. Hvidman HW, Bentzen JG, Thuesen LL, Lauritsen MP, Forman JL, Loft A, et al. Infertile women below the age of 40 have similar anti-Müllerian hormone levels and antral follicle count compared with women of the same age with no history of infertility. Hum Reprod 2016;31:1034–45.
48. Murto T, Bjuresten K, Landgren BM, Stavreus-Evers A. Predictive value of hormonal parameters for live birth in women with unexplained infertility and male infertility. Reprod Biol Endocrinol 2013;11:61.
49. Ripley M, Lanes A, Leveille MC, Shmorgun D. Does ovarian reserve predict egg quality in unstimulated therapeutic donor insemination cycles? Fertil Steril 2015;103:1170–5.e2.
50. Rosen MP, Johnstone E, Addauan-Andersen C, Cedars MI. A lower antral follicle count is associated with infertility. Fertil Steril 2011;95, 1950–1954, 4.e1.
51. Iliodromiti S, Kelsey TW, Anderson RA, Nelson SM. Can anti-Mullerian hormone predict the diagnosis of polycystic ovary syndrome? A systematic review and meta-analysis of extracted data. J Clin Endocrinol Metab 2013;98:3332–40.
52. Freeman EW, Gracia CR, Sammel MD, Lin H, Lim LC, Strauss JF 3rd. Association of anti-mullerian hormone levels with obesity in late reproductive-age women. Fertil Steril 2007;87:101–6.
53. Rigon C, Andrisani A, Forzan M, D'Antona D, Bruson A, Cosmi E, et al. Association study of AMH and AMHRII polymorphisms with unexplained infertility. Fertil Steril 2010;94:1244–8.