AGE-SPECIFIC ANTI-MULLERIAN HORMONE REFERENCE RANGE FOR SAMOAN WOMEN AGE 25-51.

By:

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Thesis

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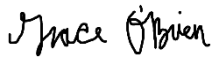

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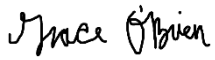

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This thesis by Grace O'Brien is accepted in its present form by the Department of Epidemiology in the School of Public Health at Brown University as satisfying the thesis requirements for the degree of Master of Science.

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Abstract Age-specific anti-mullerian hormone reference range for Samoan women age 25-51, by Grace O'Brien Sc.M, Brown University School of Public Health, May 2019.

Objective – To determine an age-specific anti-mullerian hormone (AMH) reference range for Samoan women and to examine the associations of AMH with adiposity, dietary, and reproductive factors.

Design – Cross-sectional study.

Setting – Community-based sample.

Participants – 681 Samoan women with no known reproductive disorders, reproductive surgeries, or contraceptive use, age 25-51.

Main Outcome(s) – AMH levels.

Results –AMH was negatively associated with age. AMH decline with age in Samoan women is best described by a cubic model. AMH was not associated with body mass index (BMI) and insulin resistance. It was negatively associated with percent calories from dietary protein and positively associated with total dietary sugars.

Conclusions – This study was the first to construct an age-specific AMH reference range for Samoan women. Our results contribute to the literature on population-specific AMH reference ranges. This study provides further evidence of the seemingly paradoxical relationship between adiposity and fecundity in Samoan women. Further longitudinal research needs to be done to examine these associations and non-associations more thoroughly.

INTRODUCTION

Anti-mullerian hormone (AMH) levels in serum, a marker for ovarian reserve, decline with advancing chronological age in women (1-3). AMH is produced by ovarian granulosa cells of preantral follicles. Since this pool of follicles is relatively constant over a short term time frame, AMH levels are stable throughout the menstrual cycle, making it a convenient indicator of fecundity (3, 4). AMH serum levels are increasingly used as a marker for this potential fertility in individuals and populations because of their robustness and high sensitivity (2, 5, 6).

AMH levels in serum are used to assess ovarian reserve in various settings. They are used in fertility assessments for family planning decisions and assisted reproduction treatment recommendations (7, 8). Since AMH levels decline with decreasing ovarian reserve and thus age, they are used as a crude estimator of time to onset of menopause (9-11). Finally, AMH levels serve as a surrogate marker for antral follicle count in the diagnosis of polycystic ovarian syndrome (PCOS). PCOS is a reproductive condition of infertility marked by high antral follicle count and anovulation. In the absence of the gold-standard clinical ultrasound, AMH levels are used as a surrogate to identify women with PCOS features (12-14). Despite AMH's widespread utility as a biomarker, there is still much unknown about the mechanisms behind AMH and its associations with adiposity, dietary, and reproductive measures (12, 14, 15).

Given these applications, it is important to study the normative characteristics and associations of AMH levels. Information on AMH levels continues to emerge and be refined. Age is the strongest predictor of AMH levels, but there are other factors that are reported to modify AMH levels' decline with age. It is well-established that hormonal contraceptives temporarily decrease AMH levels, but AMH levels return to normal six months after hormonal contraceptive cessation (16, 17). Smoking, another modifiable exposure, has been shown to alter

metabolic paths for reproductive hormone estradiol, but the effect it has on AMH levels is less clear. Some studies report smoking to a negative association (16, 18, 19), while others report no significant association (20-22).

Reproductive function is also hypothesized to be affected by diet. High-fat diets are thought to mediate AMH levels by causing increased inflammation. A study on predominately normal weight women of European ancestry found that AMH levels were negatively associated with the percentage of energy from fats and positively associated with energy from carbohydrates (23). Another study of a similar population, however, did not find any significant associations between dietary intake and AMH levels (24).

High body mass index (BMI) is a known risk factor for reproductive disorders (12, 25-27). Studies assessing the association between BMI and AMH levels, however, have also had conflicting results. Some studies found an inverse relationship (28, 29), while others found no significant relationship (30, 31). Further, there are reports that ancestry and ethnicity affect the relationship between AMH and BMI (25, 32). Ethnic disparities are well-demonstrated in reproductive medicine, with age of menarche, age of menopause, and prevalence of reproductive disorders differing by ancestry and ethnicity (33-35). AMH levels decrease with age and the rate at which AMH declines varies by ancestry and ethnicity (1-4, 16, 25, 32, 36-41).

Contemporary Samoan populations have very high levels of adiposity and cardiometabolic risk. A 2010 national survey revealed that over 91% of Samoan women were overweight or obese based on WHO criteria, making Samoa have one of the highest levels of obesity in the world (42). Concomitantly, Samoa has been experiencing a nutrition transition in recent decades towards a more modern and processed diet (43). Higher BMI is associated with negative reproductive outcomes such as PCOS, hyperandrogenemia, and menstrual irregularity

(12, 25-27). Given that the Samoan population has a high prevalence of obesity, it would be expected that many women in the population would have these adverse reproductive outcomes. The prevalence of hyperandrogenemia, menstrual irregularity, and estimated PCOS in Samoan women, however, is much lower compared to populations with similar obesity and overweight prevalence (44, 45). The 2014 Demographic and Health Survey (DHS) estimated that the total fertility rate in Samoan is 5.1, while the United Nations estimated that the current world total fertility rate is estimated to be less than 2.5 (46, 47).

There is currently no age-specific AMH reference range for the Samoan population. Understanding how AMH levels vary with age and BMI in Samoan women will provide further insight into why Samoan women remain relatively fertile given the high prevalence of obesity in the population. It is important to establish a standard AMH reference range to aid in the development of future research on Samoan women's reproductive health (12). Additionally, this study will contribute to the limited literature about population-specific AMH decline with age. This study will define an age-specific AMH reference range for Samoan women age 25-51, and examine the associations of AMH levels with adiposity, dietary, and reproductive measures.

MATERIALS AND METHODS

Source Population

The data for this study were from an adiposity and cardiometabolic risk factors population-based genome wide association study (GWAS) of Samoans in 2010. Subjects aged 25 to 65 years with self-reported Samoan descent were recruited for the GWAS. The GWAS consisted of anthropometric measurements, health questionnaire, and fasting reproductive serum samples. The health questionnaire was designed to obtain detailed information on the subjects' reproductive condition. Further details about recruitment, data collection, and specimen collection are described by prior papers (48, 49). This cross-sectional study consisted of Samoan women aged 25-51.

Analytic Sample

The original sample size was 1,019 Samoan women aged 25-51. The target population for this study was Samoan women aged 25-51 with no reproductive surgical procedures and no hormonal contraceptive use that may alter their normal AMH serum levels (50, 51). Therefore, 30 participants who had reproductive surgeries that may alter AMH serum levels (uterus removal, ovary removal, tubal ligation, pelvic surgery, and female sterilization) were removed from the original sample of 1,019 women. Two hundred forty-eight women were removed that reported ever using hormonal contraceptive methods (oral contraceptive, contraceptive patch, injection, intrauterine devices (IUD), other methods). An additional 60 participants were excluded for missing homeostasis model assessment-estimated insulin resistance (HOMA-IR), BMI, sex hormone binding globulin (SHBG), total testosterone or menstrual cycle regularity information. Of the 1,019 women whose GWAS health questionnaires were reviewed, 681 Samoan women met the above inclusion criteria and were included in the analysis.

AMH Measurement

The serum measurement of AMH was performed by an enzyme-linked immunosorbent assay (pico or ultrasensitive AMH, Ansh Labs, Webster, TX). Inter- and intra-assay coefficients of variation were less than 15%, as previously described by Maredia et al (45). AMH values that were below the limit of detection (0.006 ng/mL) were imputed with 0.005 ng/mL.

Androgen and Cardiometabolic Measures

The serum measurement of total testosterone and SHBG were performed by automated chemiluminescent immunoassay (Siemens, Los Angeles, California) at Women and Infants Hospital, as previously described by Maredia et al (49). Total testosterone values that were below the limit of detection (20 nmol/L) were imputed with 19 nmol/L (52). The free androgen index (FAI) was calculated for each sample where FAI is total testosterone multiplied by 100 and then divided by sex hormone binding globulin (53, 54).

Insulin and glucose assays were performed by Northwest Lipid Labs in Seattle, USA. Methods for assays for described by Hawley et. al. (48). HOMA-IR was defined as: HOMA-IR = fasting plasma insulin (μU/mL) * glucose (mg/dL)/ 405 (55). Insulin resistance was defined as HOMA-IR > 3.80 (56).

Anthropometric and Health Questionnaire Measures

Standard procedures for anthropometric measures are described by Hawley et. al. (48). The BMI categorical variable was defined by Polynesian cut-offs that define overweight (BMI 26-32 kg/m^2) and obesity (BMI>32 kg/m^2) (57). Normal and underweight were collapsed into one category due to the low prevalence of underweight women in the Samoan population.

Other important variables used in this study, including smoking status, contraceptive use, menstrual cycle regularity, and age, were obtained from the GWAS health questionnaire.

Women were classified as having a regular menstrual cycle if they reported having their latest period in the last 0-3 months. Oligomenorrhea was defined as having their latest period in the last 3-12 months. Amenorrhea was defined as not having a period in the last 12 months (44). Oligomenorrhea and amenorrhea groups were collapsed into one category due to the small sample in each category. For contraceptives, participants were asked if they had ever used that form of contraception.

Due to the low prevalence of smoking and contraceptive use in the Samoan population, participants with missing data for those variables were assumed to be non-smokers and not be using hormonal contraceptives, respectively (44, 48). Smoking was evaluated as a categorical covariate as current cigarette smokers and noncurrent smokers.

Women were classified with having features consistent with PCOS if they had two of the following three criteria: irregular menstrual cycle, hyperandrogenism (FAI >8.6), and high AMH serum levels (AMH >5.6 ng/mL) (45, 49). In the absence of ultrasound diagnostic testing, this modified criterion was used with high AMH serum levels as a surrogate marker for high antral follicle count (58).

A 104-item food-frequency questionnaire (FFQ) was used to obtain dietary intake. Participants reported, on average, how often they ate a standard portion of each food. For each of the 28 prespecified food groups, the residual method was used to compute the sum of the individual food frequencies adjusted for total energy intake. We used these residual-adjusted amounts of individual nutrients and percent calories for analysis. Total calories were measured as portion-size fixed calories (total/day), and total sugars were measured as residual-adjusted portion-fixed sugar (g/day). Further details on dietary intake assessment are described by Wang et. al. (43).

Statistical Analyses

Baseline characteristics of the sample population were determined using mean and standard deviation if normally distributed and median and log10-transformed standard deviation if not normally distributed for continuous variables and using frequency and percentage tables for categorical variables. The AMH variable was not normally distributed. It was log10-transformed for all subsequent analyses to approximate a normal distribution. Because we imputed AMH levels for those whose values were below the lower limit of the assay detection, we performed sensitivity analyses with and without the imputed AMH values.

Spearman correlation tests and Kruskal-Wallis tests were conducted to analyze the associations between AMH levels and continuous and categorical variables, respectively. Differences of means of categorical variables were assessed using the Wilcoxon rank sum test. We computed AMH mean, median, and 95^{th} percentile for ten-year incremented age groups: 25-34 years old, 35-44 years old, and 45-51 years old.

Models assessing the relationship between AMH and age were evaluated, and the model with the largest adjusted R^2 was chosen as the best fit model. Models of AMH's decline with age stratified by BMI group and menstrual regularity status were constructed to assess how the rate of AMH decline with cross-sectional age varied by these different groups. To further assess if the model of the rate of AMH decline in irregular cycling women was different by age, we constructed a piece-wise model with different rates of decline for 25-39 years old and 40-51 years old.

The association of the percentage of energy intake for the three macronutrients, carbohydrates, fats, and protein, with AMH levels was assessed by including each macronutrient percent as a linear covariate in separate age-adjusted models with age as a cubic covariate and

multivariable models with age as a cubic covariate and total calories per day as a linear covariate. To assess how substituting calories from one macronutrient with another affects AMH levels, each combination of two macronutrients with the third macronutrient being omitted was put into a linear regression model with log10-transformed AMH as the dependent variable (23). This nutrient substitution modeling method allowed us to estimate the effect replacing one macronutrient with another has on AMH levels. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using R (59).

Ethical Approval

The Brown University Institutional Review Board and the Health Research Committee of the Samoan Ministry of Health approved the parent 2010 GWAS and its informed consent process and this study's analysis (48).

RESULTS

Baseline characteristics of the 681 women in the sample population are displayed in Table 1. The mean age was 37.88 $\pm$ 7.70 years, and the median (IQR) was 38.46 (13.82) years. 90.6% of the participants were overweight or obese by Polynesian BMI standards. We found Samoan women in our study consumed a median of 2,847 calories per day and a mean of 4,463 calories per day, which is much higher than the WHO recommendation of 2,000 calories per day. Their diet consisted of 49.43% calories from carbohydrates, 36.68% calories from fat, and 16.17% calories from protein. The 36.68% calories from fat is just beyond the WHO recommended range of 20-35% calories from fat (60). Presence of reproductive disorders was low with 8.81% having hyperandrogenemia, 13.36% having menstrual irregularity, and 3.82% having features consistent with PCOS. The study population's median AMH was 0.86 ng/mL and mean was 1.87 ng/mL with a 95% confidence interval of [0.005, 10.17]. The log10-transformation of the AMH variable violated the normality tests the least and was used for subsequent analyses (Supplementary Figures 1 and 2). The log10 transformed AMH had a mean value of -0.86 with a 95% confidence interval of [-5.30, 2.32].

AMH was negatively correlated with age. Linear and cubic models of log-transformed AMH decline with age (Figure 1) show that the cubic model best fits the data with an adjusted R^2 value of 0.687. AMH decreased by 9.9% for every cross-sectional year increase in age. The 95^{th} percentile of AMH decreased across the three age groups (Table 3).

Women with irregular menstrual cycles had statistically significantly lower mean AMH values than women with regular menstrual cycles (Table 2). The AMH decline with age significantly differs by menstrual regularity status (Figure 2). From visual inspection of Figure 2, irregular cycling women have a higher AMH levels compared to regularly cycling women until

around age 35, then AMH begins to decrease at a faster rate for irregular cycling women. Regular cycling women follow a rate of decline similar to that of the entire sample population. Separating the irregular cycling women to observe different rates of decline with age did not fit the data well (Supplementary Figure 3). It was best to treat the rate of AMH decline in irregular cycling women with a single model for all ages. AMH was positively correlated with FAI and hyperandrogenemia (Table 2).

AMH was not significantly associated with BMI, smoking, or insulin resistance (Table 2). Rate of AMH decline with age does not differ significantly by BMI group (Figure 3). Insulin resistance and BMI were positively correlated, as expected. BMI was not associated with any of the dietary intake variables.

Percent of calories from protein was negatively associated with AMH. In nutrient density models, replacing energy from carbohydrates and fat with energy from proteins was associated with lower AMH concentrations. The magnitude and direction of association were similar and significant when energy from protein was replaced with carbohydrates and fat (Table 4). Participants in the highest quartile of total sugar intake had higher AMH levels (Table 2). There was no significant association between AMH and total calories per day (Table 2).

DISCUSSION

This is the first study of Samoan women to assess the associations between AMH and age and to investigate the impact of reproductive, adiposity, and dietary characteristics. We constructed an age group-specific AMH reference range for Samoan women age 25-51.

Consistent with reports from a variety of ethnic groups, our study shows a non-linear AMH decline with cross-sectional age (1-4, 16, 25, 32, 36-41). From unadjusted linear models, we note that every one year increase in age was associated with a 9.9% decrease in AMH in Samoan women, while it is associated with a 9.9%, 9.9%, 10.2%, and 6.3% in AMH in white, Latina, Chinese, and African-American women, respectively (1). AMH decline in the Samoan population can be characterized as slow until around age 35 and then getting steeper as cross-sectional age increases (Figure 1). One interesting observation is that the first occurrence of AMH levels below the limit of detection (0.006 ng/mL) does not occur in Samoans until around 45 years of age, whereas individuals in prior reports of African-Americans and European ancestry populations experience this AMH level as early as 35 years old (1). It is difficult to compare population-specific AMH studies due to varying methods and exclusion criteria, such as contraceptive use and age range. In general, the Samoan AMH curve and trajectory of decline with age was within the range of other studies (1-4, 16, 25, 32, 36-41).

We did not find AMH to be associated with BMI or smoking, which further contributes to conflicting findings in literature on the subject (16, 18-22, 28-32). Some studies included BMI and smoking in their models, but we found they did not improve the fit (model with cubic age, BMI, and smoking adjusted R^2= 0.687 and model with cubic age adjusted R^2 = 0.688), and given the conflicting reports one these associations, we did not think there was sufficient evidence of a biological mechanism for the associations to warrant including them in the model.

One of the most recent covariates of serum AMH levels to be reported is dietary fat intake (23, 24). Dietary intake is of particular interest in this population given its high adiposity levels and recent shifts to a more processed and modern diet (43). Diets high in fat intake are hypothesized to reduce reproductive function in women. This is because dietary fat causes lipotoxicity which leads to a lower functioning female hypothalamic-pituitary-ovarian axis and thus fertility (61). Evidence for this hypothesis is limited, but the Samoan population, with its high fat diet and high rates of obesity, would again be a good candidate population for testing this hypothesis. This study, however, found no association between AMH levels and dietary fat intake as a proportion of total energy and in total grams per day. In exploratory analyses, we found that saturated fat, monounsaturated fat, and polyunsaturated fat did not have significant associations with AMH levels. Given the high levels of adiposity and the higher than recommended daily intake of fat, it was surprising that dietary fat was not associated with AMH levels. These preliminary dietary intake results demonstrate a more thorough analysis of dietary factors on AMH levels is needed in both Samoan women and other populations.

Diet is a modifiable exposure and is thus an important factor to consider in fertility research. WHO recommends keeping sugar intake to less than 10% of total energy intake or 25 grams, which is about 100 kcal of sugar per day (60). Our study population reported a median sugar intake of 161.4 grams of sugar per day. We found a positive association between total sugars per day and AMH levels, while Anderson et. al found no association in a sample of Caucasian normal weight women (N=296). Anderson et. al examined this further by also finding no AMH association with glycemic index and glycemic load (23). Assessing these two indices in Samoan women could provide further insight into the mechanisms behind the association between AMH levels and sugar intake.

AMH is proposed as a surrogate marker for antral follicle counts used in the diagnosis of PCOS (58, 62). There is debate as to the appropriate AMH cut-off level to best define high ovarian follicle number characteristic of PCOS (63, 64). Further, there has been a push in recent years to establish an international standard AMH assay cut-off for PCOS and other reproductive disorders (65). A standard AMH cut-off is appealing because, unlike antral follicle counts, it does not require an ultrasound to assess ovarian reserve. This is especially attractive to low-resource settings, like Samoa, that do not readily have access to ultrasound equipment. Current Samoan research applies an AMH cut-off of 5.6 ng/mL as a surrogate marker for PCOS features. This cut-off was obtained from a 2016 study that used five serum assay methods (66, 67).

There are, however, several technical issues with creating an international AMH serum assay. Assay variability and lack of a standardized assay method make it difficult to establish an international assay across methods (68). Another issue is the persisting controversy of the association between AMH levels and BMI. Our null findings contribute to this conflicting literature. The lack of consensus on this relationship indicates that there are still unknown mechanisms of AMH, making it difficult to defend any standardized assay that is produced. Lastly, deciding what populations are included in the normative population for the standardization is unclear (66). There have been several attempts at creating an international AMH assay, with a recent 2018 study preparing a reference using recombinant AMH serums, but due to the limitations mentioned, none have been accepted by WHO (65). An international standard AMH assay would greatly impact research on Samoan women. While we currently use AMH as a surrogate marker for antral follicle count, having an established standard AMH assay would provide more confidence in our findings and allow us to more easily synthesize and validate our results with similar studies in other populations.

The primary strength of this study is that it was the first study to evaluate AMH and age in Samoan women. As AMH becomes considered as an ovarian reserve marker in Samoan clinical practice and as a prognostic tool, it is critical that we assess possible factors that influence AMH levels in this unique population. Our study contributes to the limited literature on how AMH levels are affected by ancestry and ethnicity because it is the first one to characterize the rate of AMH decline with age in a Pacific Islander population and a population with high rates of overweight and obesity. Our extended age range allowed us to create an inclusive reference range and to characterize the rate of AMH decline with age in the Samoan population.

One study limitation was that AMH measurements were performed during two different time periods, with the older aged women (40-51 years) added later when a more sensitive AMH assay method (picoAMH) became available. In both cases, however, samples were thawed from long term freezer storage. Furthermore, despite the enhanced sensitivity AMH assay, 84 values were below the lower limit of detection and were imputed with 0.005 ng/mL. Sensitivity analyses showed that imputing with 0.005 ng/mL, 0.006 ng/mL, and removing AMH values below the lower limit of detection yielded similar results and models. We used nonparametric tests and were careful in our interpretations to ensure the validity of our statistical analyses.

Our study was also limited in that exclusion criteria may have removed women that were actually eligible. Due to the way hormonal contraceptive use was asked in the health questionnaire, we were unable to discern which participants were currently using hormonal contraceptives at the time of study and had to eliminate anyone who has ever used a hormonal contraceptive. Further, by including women up to age 51, it is possible that the irregular menstrual cycle group includes women experiencing normal menopause. This limits our interpretations of the menstrual cycle regularity variable.

Our results may also be limited in generalizability to other groups because of the high level of overweight and obesity in our Samoan sample and population. More than 90% were overweight or obese (Table 1). Given the continuing global rise in obesity, however, the Samoan findings may be relevant to other women as the nutrition transition proceeds (42, 69). We may have failed to discern the effect of BMI on AMH levels because of the relatively narrow range of and high levels of BMI values.

Finally, due to the cross-sectional design of the study, we cannot make causal inferences on the effect of age and other variables on AMH levels. A prospective longitudinal study would provide information about the mechanisms behind AMH decline with age and the effects factors have on that rate of decline. Another next step is to perform a GWAS to evaluate genetic associations with AMH and other hormone markers of fertility (70, 71).

We have recently reported a novel rs373863828 (p.Arg457Gln) mutation in the CREBRF gene in the Samoan population, which is positively associated with adiposity but negatively associated with diabetes and glucose levels (72). Collectively, our studies of reproductive health in Samoan women suggest that this mutation may also have a protective effect against reproductive disorders typically detrimental to obese populations. Specifically, we have reported relatively low rates of menstrual irregularity and PCOS features in Samoan women and published fertility rates that align with age-related expectations (44, 45). In the present study, AMH levels, an indicator of ovarian reserve, are as good as or better than those seen in other groups of women with lower rates of overweight and obesity.

Conclusions

This study provides an age-specific AMH reference range that can be used in future reproductive research on Samoan women. The results of this study add to the research on

reproductive hormones and outcomes in Samoan women and provide further evidence of the paradoxical nature of this population and the possible protective effect the CREBRF gene variant has on reproductive disorders. Our results add to the limited number of ethnic-specific studies on AMH levels. In this study, we presented an age-specific reference range of AMH in a sample of women age 25-51 in the Samoan population.

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Table 1. Baseline Characteristics of Samoan women age 25-51

Characteristics	N (%)	Summary of Results
Mean Age in years (SD)		37.88 (7.70)
25-34	264 (38.77)	
35-44	233 (34.21)	
45-51	184 (27.02)	
Mean BMI in kg/m^2 (SD)		34.63 (6.83)
Underweight/Normal (<26 kg/m^2)	64 (9.40)	
Overweight (26≤x≤32 kg/m^2)	191 (28.05)	
Obese (>32 kg/m^2)	426 (62.55)	
Smoking Status		
No	496 (72.83)	
Yes	185 (27.17)	
OM/AM		
No	590 (86.64)	
Yes	91 (13.36)	
Hyperandrogenemia		
No	621 (91.19)	
Yes	60 (8.81)	
PCOS features		
No	655 (96.18)	
Yes	26 (3.82)	
Insulin resistance		
No	443 (65.05)	
Yes	238 (34.95)	
Median AMH in ng/mL (log10 SD)		0.86 (1.00)
Range		(0.005- 16.82)
Median FAI (log10 SD)		2.51 (0.39)
Range		(0.14- 42.57)
Median HOMA-IR (log10 SD)		2.79 (0.36)
Range		(0.33- 40.76)
Median total calories per day in g/d (log10 SD)		2,847 (0.41)
Range		(611-33,604)
Median total sugars per day in g/d (log10 SD)		161.38 (0.11)
Range		(67.52- 306.06)
Daily caloric intake (log10 SD)		
Median % calories from carbohydrates		49.43 (0.07)
Median % calories from fat		36.68 (0.08)
Median % calories from protein		16.17 (0.11)

BMI= body mass index, OM/AM= oligomenorrhea/amenorrhea, PCOS= polycystic ovary syndrome, AMH= anti-mullerian hormone, FAI= free androgen index, HOMA-IR= homeostatic model assessment of insulin resistance, SD= standard deviation, log10 SD= standard deviation computed after logarithmic 10 transformation

Table 2. Bivariable Analysis of log10 anti-mullerian hormone for Samoan women age 25-51

Variables	Result Estimates‡	p-value¥
FAI	0.32	<0.0001*
Age (years)	-0.83	<0.0001*
BMI (kg/m^2)	-0.04	0.3382
HOMA-IR	-0.05	0.2299
Total calories (g/d)	0.07	0.05809
Total sugars (g/d)	0.13	0.0008523*
% Calories from carbohydrates	0.05	0.1764
% Calories from fat	0.03	0.2684
% Calories from protein	-0.13	0.0007371*
Age (years)		<0.0001*§
25-34	3.77 (0.406)	
35-44	1.11 (0.717)	
45-51	0.132 (0.784)	
BMI (kg/m^2)		0.5091
Underweight/Normal (< 26 kg/m^2)	2.08 (1.051)	
Overweight (26 $\leq$ x $\leq$ 32 kg/m^2)	1.97 (1.044)	
Obese (>32 kg/m^2)	1.81 (0.978)	
Smoking status		0.3027
No	1.91 (0.990)	
Yes	1.79 (1.035)	
OM/AM		<0.0001*
No	2.00 (0.863)	
Yes	1.06 (1.179)	
Hyperandrogenemia		<0.0001*
No	1.61 (1.00)	
Yes	4.62 (0.630)	
Insulin Resistance		0.668
No	1.83 (0.977)	
Yes	1.97 (1.050)	
Total calories quartiles (g/d)		0.2971
<1,180	1.75 (0.98)	
1,180-2,849	1.84 (1.05)	
2,850-6,719	1.82 (1.03)	
$\geq$6,720	2.10 (0.95)	
Total sugars quartiles (g/d)		0.0006812*£
<140	1.89 (1.06)	
140-160	1.62 (1.07)	
161-188	1.84 (0.93)	
$\geq$189	2.15 (0.91)	

BMI= body mass index, OM/AM= oligomenorrhea/amenorrhea, PCOS= polycystic ovary syndrome, AMH= anti-mullerian hormone, FAI= free androgen index, HOMA-IR= homeostatic model assessment of insulin resistance, SD= standard deviation, log10 SD= standard deviation computed after logarithmic 10 transformation

*Statistically significant

‡Correlation coefficient from spearman correlation test for continuous variables, mean AMH (standard deviation of log10 AMH) for categorical variables

¥ P-value obtained from Spearman correlation test for continuous variables and Krustal-Wallis test for categorical variables

§All age groups and FAI quartiles have statistically significant different AMH means using Wilcoxon rank sum test
£ The following total sugars per day quartiles have significantly different mean AMH values: <140 and ≥189, 140-160 and 161-188, and 140-160 and ≥189.

Table 3. Anti-mullerian hormone in ng/mL by 10 year age increments

Age group (years)	N(%)	Median (Range)	Mean (log10 SD)	95th percentile
25-34	264 (38.77)	2.57 (0.080-16.82)	3.77 (0.406)	11.0
35-44	233 (34.21)	0.64 (0.005-9.53)	1.11 (0.717)	3.75
45-51	184 (27.02)	0.0244 (0.005-1.59)	0.132 (0.784)	0.648

log10 SD= standard deviation computed after logarithmic 10 transformation

Table 4. Log10 anti-mullerian hormone and macronutrients associations from nutrient density models‡

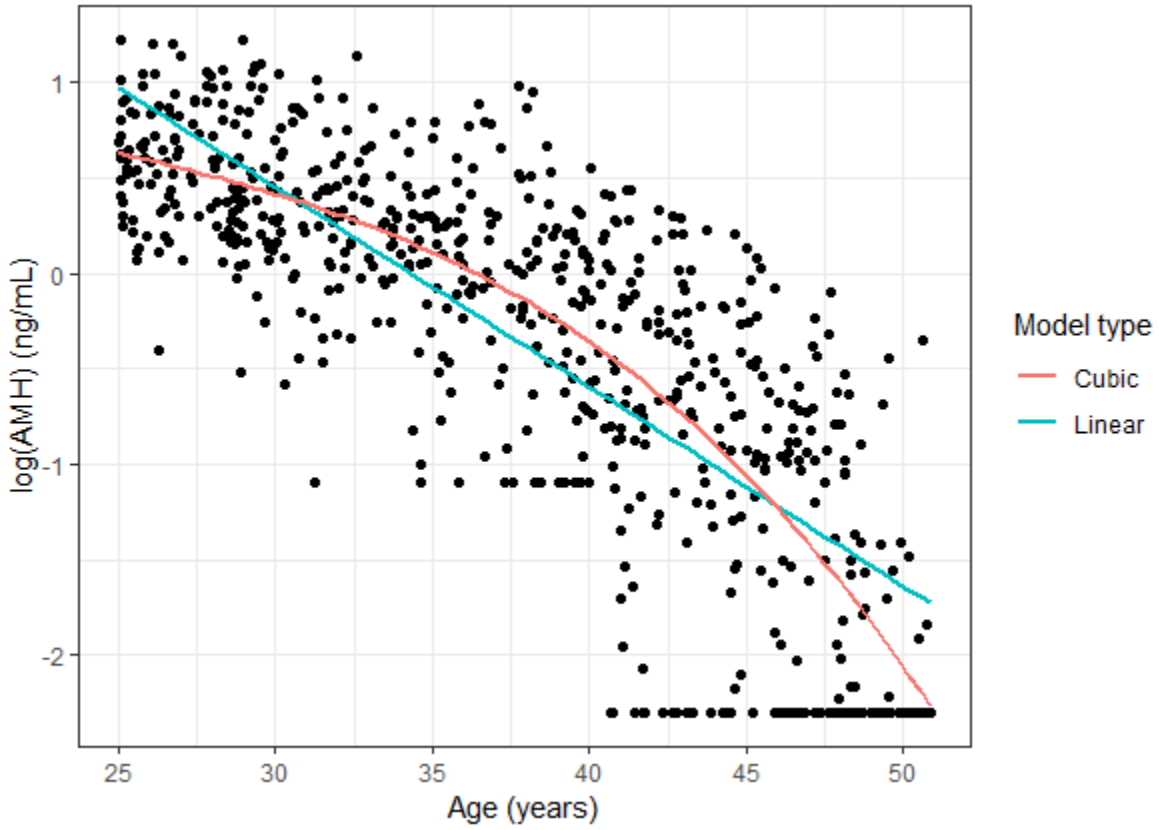
Macronutrient	β	p-value
Omitting carbs		
Replace carbs with fat	0.007	0.231
Replace carbs with protein	-0.035	0.001*
Omit fat		
Replace fat with carbs	-0.007	0.277
Replace fat with protein	-0.043	0.001*
Omit protein		
Replace protein with carbs	0.029	<0.001*
Replace protein with fat	0.035	<0.001*

AMH= anti-mullerian hormone

*Statistically significant

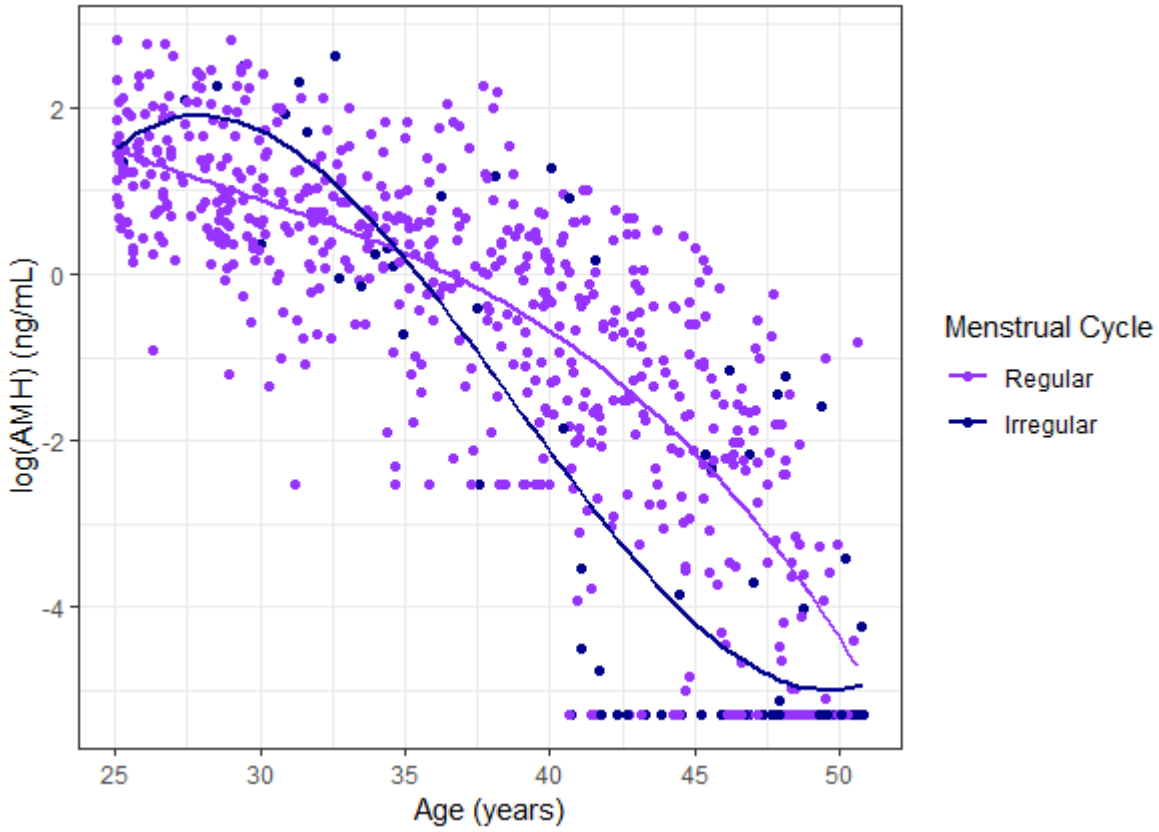
‡ β coefficients are the effect of substituting calories of the omitted nutrient with that macronutrient for equal energy in model log10 AMH ~ linear(% calorie carbs) + linear(% calorie fat) + linear(% calorie protein)

Figure 1. Linear and cubic models of log10 anti-mullerian hormone levels decline with age



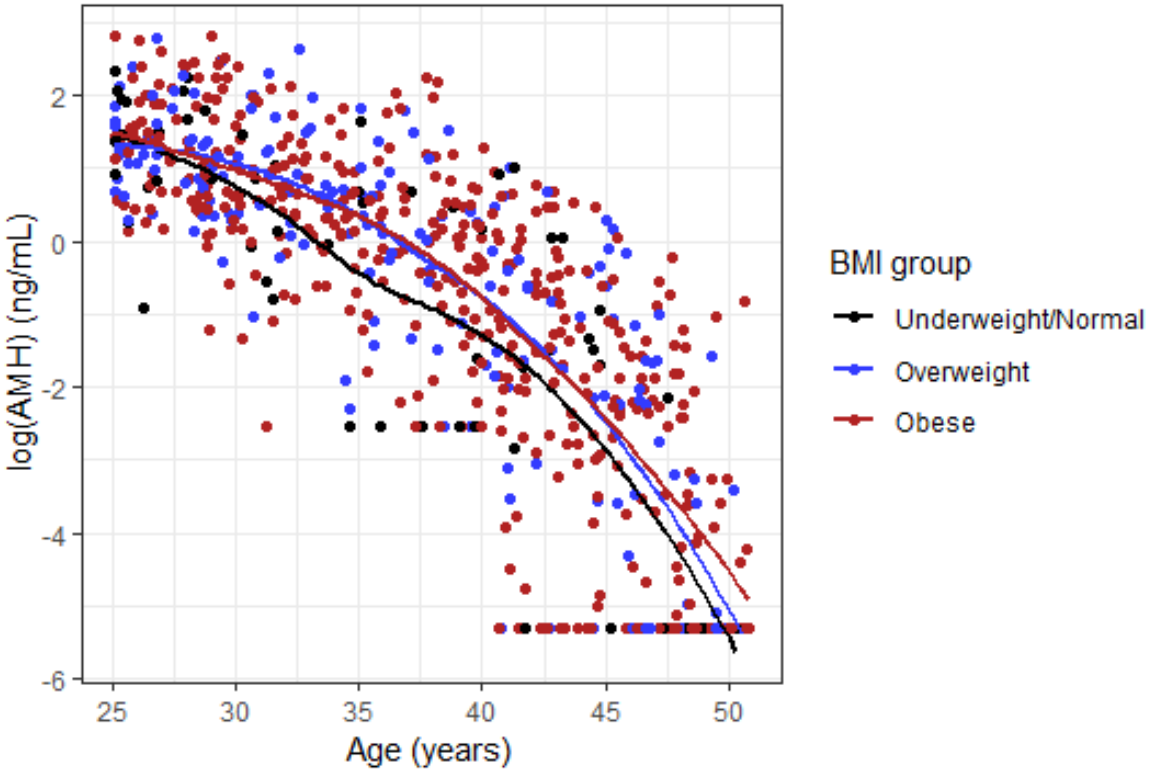
AMH= anti-mullerian hormone
AMH rate of decline with age is described by the linear model: log10AMH = - 0.104469*Age + 3.584454 (adjusted R^2 = 0.6438) and the cubic model: log10AMH = - 0.85156*Age^3 – 5.45672*Age^2 – 20.98548*Age – 0.37255 (adjusted R^2 = 0.6876)

Figure 2. Unadjusted cubic model of log10 anti-mullerian hormone levels decline with age stratified by menstrual regularity



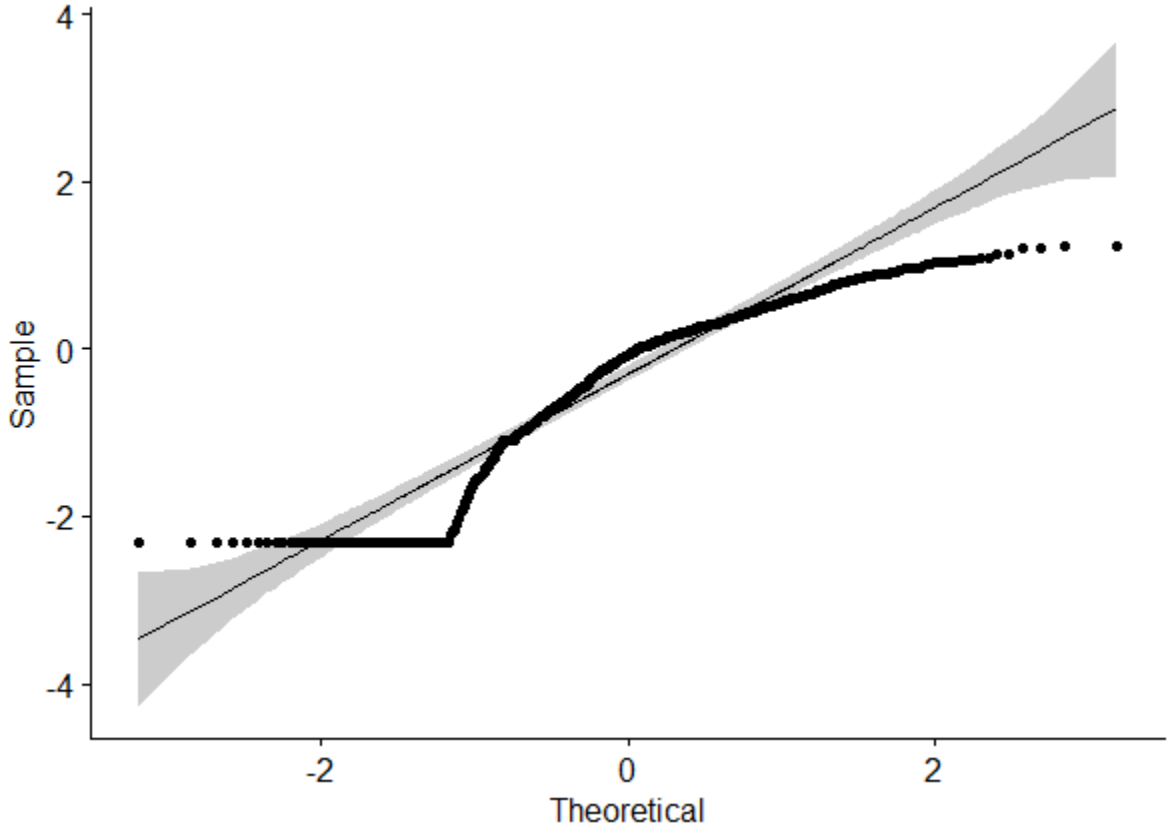
AMH= anti-mullerian hormone
Women with regular menstrual cycles' rate of AMH decline is described by the cubic model: log10AMH = -2.556*Age^3 -9.374*Age^2 -37.232*Age -0.479 (adjusted R^2=0.635). Women with irregular menstrual cycles' rate of AMH decline is described by the cubic model: log10AMH = 4.224*Age^3 +0.8297*Age^2 -21.892*Age -3.315 (adjusted R^2=0.742).

Figure 3: Unadjusted cubic model of log10 anti-mullerian hormone levels decline with age stratified by Polynesian BMI groups



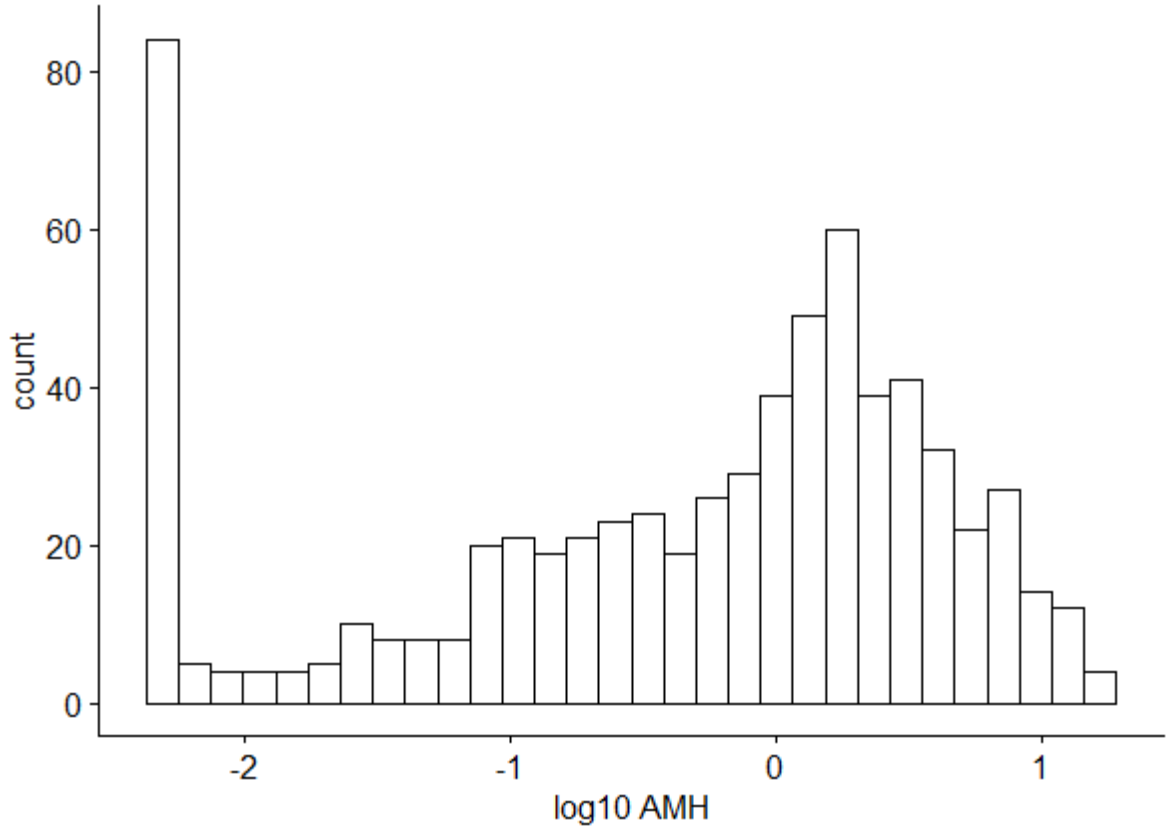
AMH= anti-mullerian hormone, BMI= body mass index
BMI group does not significantly affect AMH rate of decline with age. All BMI groups can be described by the general unadjusted cubic model: log10AMH = - 0.85156*Age^3 – 5.45672*Age^2 – 20.98548*Age – 0.37255 (adjusted R^2 = 0.6876)

Supplementary Figure 1. QQplot of log10 anti-mullerian hormone



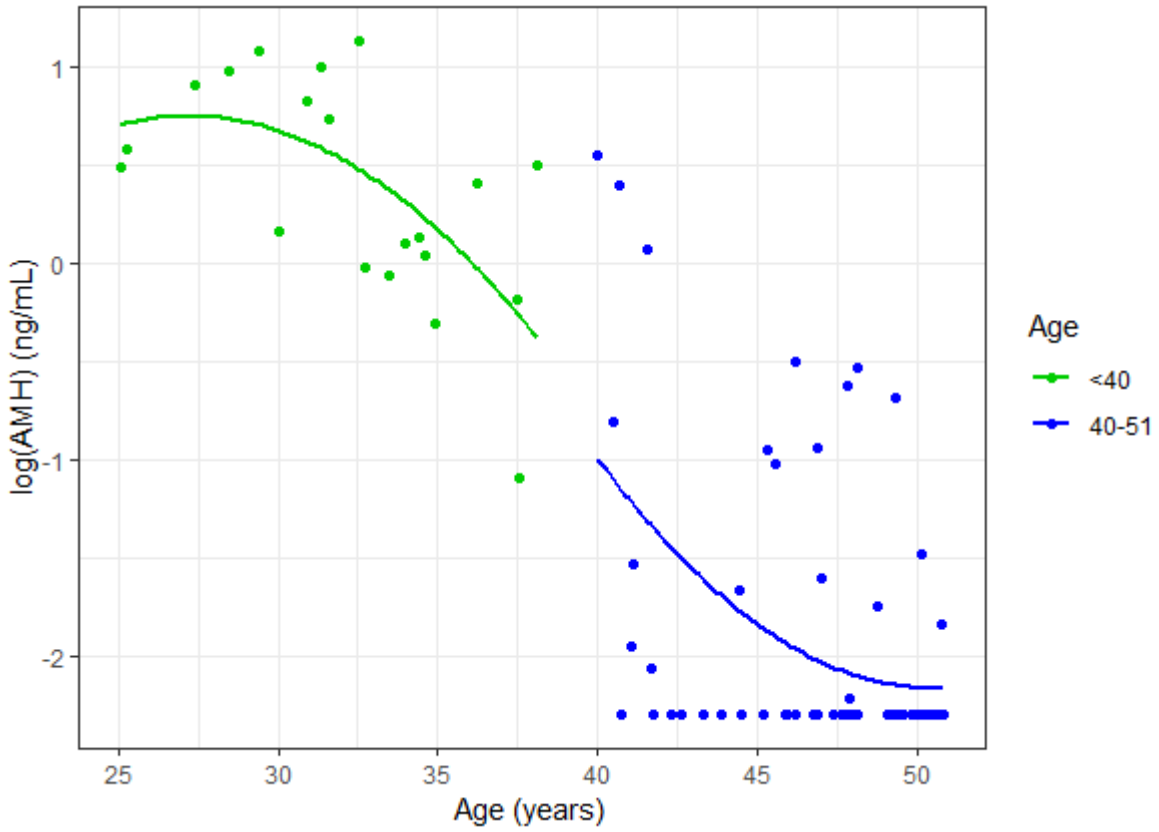
AMH= anti-mullerian hormone

Supplementary Figure 2. Histogram of log10 anti-mullerian hormone



AMH= anti-mullerian hormone

Supplementary Figure 3. Piece-wise model of log10 anti-mullerian hormone levels decline with age in irregular cycling women



AMH= anti-mullerian hormone
AMH rate of decline of irregular cycling women <40 is described by the quadratic model: log10AMH = -1.498* Age^2 -3.304*Age +0.860 (adjusted R^2 =0.334). AMH rate of decline of irregular cycling women 40-51 is described by the quadratic model: logAMH = 1.843* Age^2 -6.045*Age -4.492 (adjusted R^2 = 0.195).