Nelson, S. M. et al. (2020) Ethnic discordance in serum anti-Müllerian hormone in healthy women: a population study from China and Europe. *Reproductive BioMedicine Online*, 40(3), pp. 461-467.

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Deposited on 28 May 2021

Ethnic discordance in serum müllerian hormone (AMH) in healthy women; population study from China and Europe

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Key words: AMH, ethnicity

Word count: 1969

Abstract: 231

Highlights:

- We show that Chinese women have higher AMH levels than the European counterparts before age of 25, but after the age of 25 this is reversed and Chinese women have lower AMH levels.

- The disparity between the two populations widens with increasing age.

Objective: Chinese women are known to have an earlier age at natural menopause than their European counterparts, whether they also have a lower functional ovarian reserve is unknown. This study was designed to assess whether there are ethnic differences in Anti-Müllerian Hormone (AMH) in women of reproductive age.

Materials and Methods: Women with regular menstrual cycles, not on hormonal contraception or with any medical history of note, were recruited to provide a day 2-5 early follicular sample in China and Europe. AMH was determined using the Roche Elecsys assay. AMH decline was modelled with a linear, quadratic and quadratic with interaction on age equations to assess the impact of ethnicity.

Results: 887 European and 461 Chinese women participated in the study. Despite the Chinese population being slightly younger 34.1±8.4 years than their European counterparts 34.8±8.9 years, their median AMH was lower 1.87 (IQR 0.28, 3.64) as compared to 2.11 (IQR 0.73, 3.96), with evidence of increasing discordance from age 25 years. In all regression models of the AMH age–related decline, there was evidence of a difference between Chinese and European women. Whilst AMH was 28.1% (95% CI; 18.2, 36.7%) lower in the Chinese population at age 30, this decline increased to 79.4% (95% CI; 75.4, 82.9%) at age 45.

Conclusions: There were independent effects of age and ethnicity on serum AMH concentrations, with Chinese women having a substantially lower AMH in adult life than their European counterparts from age 25 onwards.

Keywords: AMH, ethnicity, ovarian reserve, healthy population

Introduction

The impact of ethnicity on sentinel reproductive events is increasingly recognised, with studies assessing the genetic architecture of reproductive aging identifying differences in allele frequency and effect estimates between European and Asian populations (Horikoshi, et al., 2018). The clinical impact of these differences may be substantial with the mean age at natural menopause being 1-2 years earlier in women of women of Chinese origin (Dorjgochoo, et al., 2008, Li, et al., 2012, Wang, et al., 2018), as compared to European populations (Cassou, et al., 2007, Hardy and Kuh, 2005, Parazzini, 2007). At earlier ages, ethnic differences in assisted conception outcomes have also been observed, with lower live-birth rates for non-European ethnicities, despite being treated in the same clinical setting (Dhillon, et al., 2015, Maalouf, et al., 2017). The mechanism underlying these observations is not clear, as differences in ovarian response and the functional ovarian reserve have been observed in some (Bleil, et al., 2014, Purcell, et al., 2007, Seifer, et al., 2009) but not all studies (Bhide, et al., 2015, Iglesias, et al., 2014, Olcha, et al., 2016, Randolph Jr, et al., 2003).

Several of these studies exploring ethnic differences, have primarily focused on patients attending infertility or medical clinics which may bias results, and attenuate differences due to the variable composition of underlying infertility diagnoses or the impact of disease on AMH concentrations (Bhide, et al., 2015, Olcha, et al., 2016, Purcell, et al., 2007). Infertility populations are also known to not be representative of the general population, as advanced ovarian aging is known to be overrepresented in infertile or diseased populations (Iliodromiti, et al., 2016). Existing studies have

also been dependent on self-reported ethnicity which may be less accurate and subject to reporting bias (Sucheston, et al., 2012). Despite these limitations, analysis of anti-müllerian hormone (AMH), a marker of the functional ovarian reserve, has been reported to be lower in women of black or Hispanic ethnicity (Bleil, et al., 2014, Seifer, et al., 2009), and also of Chinese origin (Bleil, et al., 2014) in some but not all studies (Olcha, et al., 2016). Although suggestive of ethnic differences in the functional ovarian reserve (Bleil, et al., 2014, Seifer, et al., 2009), these studies used the older AMH assays, that are known to have substantial variability, particularly when used on samples stored for variable time period, and required the use of conversion factors to be able to amalgamate the results over time (Iliodromiti, et al., 2014, Nelson, et al., 2014).

The aim of the current study was to determine whether there were ethnic differences in serum AMH concentrations using a current automated laboratory assay in contemporary non-select adult women from Europe and China.

Materials and Methods

Study Participants

Women from a European population living in the Netherlands, Belgium, Germany, France, Turkey and a Chinese population living in Beijing, were included. All participants were between 20 and 50 years of age. European women were recruited to a multicentre study to evaluate the analytical performance of the Elecsys® AMH assay and to facilitate determination of a reference range (Anckaert, et al., 2016). European participants were self-reported as apparently healthy, with a regular menstrual cycle (length 21–35 days). Women with a BMI exceeding 30 and/or receiving hormone replacement therapy or using hormonal contraceptives in the preceding 3 months were excluded from the study. Furthermore, women with infertility, gonadal disorder/dysfunction, diagnosed endometriosis, known previous or current endocrine or metabolic disorders were excluded. Chinese women were recruited in 8 communities in Beijing and had a regular menstrual cycles (24-35 days) for the previous 3 months, had not taken oral contraceptives or any drug containing hormones within the past 3 months, had no history of ovarian surgery, and were required to provide a health report within 1 year confirming no concurrent comorbidities. Women with a history of polycystic ovary syndrome (PCOS), endocrine or metabolic abnormalities (i.e., diabetes, pituitary, adrenal, pancreas, liver, or kidney disturbances), current or past smokers were excluded. Early follicular serum samples were collected on day 2-5 for all participants.

All investigation and sample collection sites followed International Conference on Harmonization guideline for Good Clinical Practice E6 and conducted the study in accordance with the Declaration of Helsinki (as amended in Tokyo, Venice, Hong

Kong, and Edinburgh). Where required, Ethics Committee approval of the respective institutions was obtained. Specifically; ethical approval for the European cohort is on file at Roche Switzerland and was obtained from the following institutions; UZ Brussels, Free University of Brussels (VUB), Belgium; Duzen Laboratories, Ankara, Turkey; Laboratoire Eylau, Paris, France; Limbach Laboratory, Heidelberg and MVZ wagnerstibbe für Laboratoriumsmedizin and Pathologie GmbH, Hannover, Germany. Ethical approval for the Chinese cohort was obtained from the Peking Union Medical College Hospital.

Sample measurements

Three ml serum aliquots for each participant were stored at −80°C. AMH was measured on first thaw of stored samples using the Elecsys® AMH automated method on a clinically validated platform (cobas e 411, e 601 and E 170 Roche Diagnostics, Germany)(Anckaert, et al., 2016). The assay was calibrated and quality controlled using the manufacturer's reagents. All AMH samples from the European and Chinese population were measured in the respective laboratories (Peking Union Medical College Hospital and Laboratory of Hormonology and Tumour Markers, Universitair Ziekenhuis Brussel) using the same AMH assay. The within-run imprecision was investigated by the study sponsor providing each laboratory with spiked serum sample material in five levels covering the entire measuring interval. Each sample level was analysed 21 times in one analytical run. Mean, standard deviation and the coefficient of variation (CV) were calculated from these data. The repeatability and intermediate imprecision were investigated using two levels of quality control material (Elecsys® PreciControl AMH 1 and 2 assay, Roche Diagnostics GmbH, Germany) and the same five levels of spiked serum pool

material covering the lower and upper measuring interval. The spiked serum material was provided to the sites in frozen aliquots.

Across the range of 0.24ng/ml (1.71pmol/l) to 19.17ng/ml (136pmol/l) the within-run imprecision was 0.7 to 3.4%, the repeatability CVs ranged from 1.2 to 1.7% and the intermediate CVs ranged from 2.2% to 4.4%. The limit of quantitation (LoQ) was 0.03 ng/ml. All values between the two sites can therefore be compared uniformly.

Statistical analyses

AMH was compared between the two ethnicity groups using linear regression. As may be expected the distribution of AMH in each ethnicity group follows a right skewed distribution reflecting that a few women have high values whilst the majority of women have lower values, with some closer to zero. We therefore present the median and IQR of the untransformed data in the descriptive tables and model the (natural) log transformed AMH data. Differences between the ethnicity groups and confidence intervals were calculated on the log-scale. These values were then back-transformed and are interpreted as the ratio of geometric means (GM/GMR). Graphs displayed are in original units and values were derived by back transforming from the log scale.

Age and ethnicity were included in each of the models and the European group used as the reference group. Age was also be centered to avoid interpretation at age 0. Quadratic age (or other powers) were included if they improved model fit (using likelihood ratio tests). If an age x ethnicity interaction term was present, then separate ethnicity effect estimates at appropriate age categories are presented. If the interaction

was not statistically significant (at 10% level) an overall difference in ethnicity is reported.

Model fit was assessed via standard methods (e.g. graphical plots). This included checking the relationship between the observed and predicted values was linear, checking there was constant variance between the predicted values and residuals and that the residuals were normally distributed. The R^2 statistic, the proportion of variation explained by the model, was also reported. A sensitivity analysis was conducted to assess the impact of values less than the LoQ by uniformly imputing values from between a fixed lower value of 0.01 ng/ml and the LoQ of 0.03 ng/ml. Analyses were performed in Stata V.15.0 (StataCorp, College Station, Texas, USA).

Results

A total of 1348 subjects met the inclusion criteria and participated in the study; 887 European and 461 Chinese women. Despite the Chinese population being slightly younger 34.1 ± 8.4 years than their European counterparts 34.8 ± 8.9 years, their median AMH was lower 1.87 (IQR 0.28, 3.64) as compared to 2.11 (IQR 0.73, 3.96). These differences were apparent at each age group (Table 1).

Of the three models fitted, the model with a quadratic age term and an interaction term between age and ethnicity explained the most variability in AMH (R-squared=67.75) In all regression models of the AMH age–related decline, there was evidence of a difference in AMH by ethnicity (lower values in Chinese women; Table 2).There was evidence of an ethnicity age interaction and therefore separate ethnicity effects are presented for each age group (Table 3). Despite a slightly higher AMH value at age 20, overall lower AMH levels were found in the Chinese population compared to the European population: at age 30 AMH was 28.1% (95% CI, 18.2 to 36.7%) lower; at age 35 AMH was 52.6% (95% CI, 46.9 to 57.8%) lower; at age 40 AMH was 68.8% (95% CI, 64.2 to 72.8%) lower; at age 45 AMH was 79.4% (95% CI, 75.4 to 82.9%) lower and at age 50 AMH was 86.5% (95% CI, 82.8 to 89.4%) lower.

To further assess the impact of ethnicity on AMH we plotted the actual values and modelled the GM of AMH for any given age using the best model which incorporated a quadratic term of log(AMH) on age with ethnicity and interaction between age and ethnicity is shown in Figure 1. This model was then used to display the predicted log AMH and associated 95% CI, which clearly shows that the Chinese population

despite a similar starting point, exhibited a faster decline in functional ovarian

reserve as measured by AMH as compared to the Chinese population (Figure 2).

Conclusions

In this large cohort of healthy adult Chinese and European women, we demonstrate that Chinese women initially exhibit higher circulating serum AMH concentrations than their European counterparts. However, after age 25 this pattern was reversed, with Chinese women exhibiting substantially lower AMH, and the apparent disparity widening with advancing age. These findings are consistent with previous smaller analyses of infertile or medical patient populations (Bleil, et al., 2014, Seifer, et al., 2009), and support epidemiological observations that Chinese adult women exhibit an accelerated age-related decline in their ovarian reserve and an earlier age at natural menopause (Cassou, et al., 2007, Dorjgochoo, et al., 2008, Hardy and Kuh, 2005, Li, et al., 2012, Parazzini, 2007, Wang, et al., 2018).

Previous studies, have suggested that there are racial disparities in ovarian reserve, despite a similar age at menarche, with Chinese women have an age at natural menopause that is 1- 2 years younger than Europeans (Cassou, et al., 2007, Dorjgochoo, et al., 2008, Hardy and Kuh, 2005, Li, et al., 2012, Parazzini, 2007, Wang, et al., 2018). Although AMH is principally recognised as a biomarker of the functional ovarian reserve, and is primarily used to predict ovarian response to gonadotrophin stimulation (Iliodromiti, et al., 2014), it has been shown to be strongly correlated with primordial follicle counts(Hansen, et al., 2011). That menopause represent a terminal depletion of primordial follicles (Depmann, et al., 2015), the earlier age of menopause in Chinese women would be consistent with our observed premature decline in AMH in this population. Determining whether this excessive drop in AMH in adulthood reflects fewer follicles at birth or excessive follicular recruitment prepubertally and therefore fewer follicles in later life (Kelsey, et al.,

2012), is limited by our cross-sectional design. However, in support of the former, is our observation that the AMH peak at age 25 years is very similar for the two ethnicities, with 25 years previously noted to be the peak plateau of AMH across the lifespan (Kelsey, et al., 2011). The ability of the ovary to manage follicular recruitment rates in adult life, depending on the number of follicles available, is also supported by the observation that unilateral oophorectomy also only reduces the age at natural menopause by 1-2 years (Rosendahl, et al., 2017).

The observation that AMH was higher at age 20 and then lower throughout the adult lifespan in Chinese women is interesting. We have previously shown that AMH levels, reflect follicular recruitment levels throughout life (Fleming, et al., 2012), rise to a maximum at age 25 and then decline thereafter(Kelsey, et al., 2011). At present it not however known whether an excessive follicular recruitment and thereby higher AMH in adolescent and early adult life is detrimental to the overall reproductive lifespan. Longitudinal prospective studies with repeat measures in diverse ethnic populations will be helpful in determining whether women with increased AMH in childhood or adolescence have impaired ovarian reserve and a shorter time to menopause.

There has been considerable interest in whether ethnic specific reference ranges should be determined for ovarian biomarkers (Du, et al., 2016, Lee, et al., 2017). There is however, no evidence that ethnicity modifies the association between circulating AMH concentrations and ovarian response, with recent multicentre trials reporting similar strengths of association irrespective of ethnicity (Nyboe Andersen, et al., 2016). The role of reporting an ethnic specific reference range would therefore

be more useful if there was agreement that AMH could be used for counselling regarding the reproductive lifespan particularly given the earlier age at natural menopause in Chinese populations (Cassou, et al., 2007, Dorjgochoo, et al., 2008, Hardy and Kuh, 2005, Li, et al., 2012, Parazzini, 2007, Wang, et al., 2018). However, although lower age-specific AMH is associated with an increased risk of premature ovarian insufficiency(Bertone-Johnson, et al., 2018), wider application for counselling regarding reproductive status and future fertility potential has been criticised by professional bodies (Opinion, 2019). It may therefore be detrimental to report ethnic specific reference ranges, particularly if patients were not aware of the overall shorter reproductive lifespan and earlier age at natural menopause, as they may be falsely reassured by being placed on the equivalent centile but at a much lower actual value than their European counterparts.

Although our study has a number of strengths including its large sample size inclusion of healthy women not on any form of contraception or with medical disorders, timed early follicular sampling and robust statistical analysis we do acknowledge a number of limitations. Firstly the AMH samples although similarly collected were processed in different laboratories. However, both laboratories in Europe and China were part of ongoing regulatory quality control processes, with cross-measurement of control samples provided by Roche, and the Elecsys AMH assay has previously been shown to be robust across different scale of machines and sites with an overall inter-laboratory CV of <10%, the observed differences clearly exceed this (Anckaert, et al., 2016). The increased performance, reliability and sensitivies of the automated AMH assay platforms over previous manual ELSIA iterations have also been well documented (Iliodromiti, et al., 2014, Nelson, et al.,

2014). Secondly, we did not have details on other potential determinants of AMH concentrations such as smoking in the European population, with smoking known to deplete antral follicles and thereby AMH (Freour, et al., 2008). However, smoking is substantially more prevalent in European women of reproductive age than their Chinese counterparts (Hermalin and Lowry, 2010), and as such any discordance in smoking behaviour would only attenuate the observed ethnic differences. Similarly we did not have information on adiposity, but previous large studies (de Kat, et al., 2016) including those with DXA determined fat mass (Anderson, et al., 2013), have not shown an impact of BMI on AMH concentrations. We also did not have information on socioeconomic class, such that if there was a systematic discordance in socioeconomic status between the two populations this may have contributed to the observed differences, as lower socioeconomic class has been associated with lower AMH levels (Barut, et al., 2016). Thirdly, our study was cross-sectional in design, which precludes the examination of trajectories of the decline in AMH, although our limited extrapolation of the decline in AMH is consistent with longitudinal studies with repeat measures. Lastly, we are unable to dissect whether these observed discordances are solely due to ethnicity or due to living in quite different settings with different environment, lifestyles, early life exposures or other potential confounders. Further well powered studies examining healthy unselected participants resident in a similar setting with unified inclusion criteria would begin to address some of these issues.

In conclusion, our study suggests that in healthy premenopausal women, there are substantial racial/ethnic difference in the functional ovarian reserve as determined by AMH. Such that Chinese women resident in China have significantly lower AMH

concentrations, and potentially ovarian reserve compared to their European counterparts consistent with their known earlier age at natural menopause. Development of racial/ethnic specific AMH reference ranges should be made with caution and with reference to the outcome of interest.

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Table 1: Serum AMH concentrations by Age Groups

	European (N=887)				Chinese (N=461)			
	Number of participants	(%)		AMH values	Number of participants	(%)		AMH values
Overall (Mean, SD)			2.69	2.45			2.38	2.40
Overall (Median, IQR)			2.11	(0.73, 3.96)			1.87	(0.28, 3.64)
Overall years (Range)				(0.01, 15.73)				(0.01, 11.69)
20-24 years (Mean, SD)	150/887	16.9%	4.62	2.61	63/461	13.7%	5.68	2.33
20-24 years (Median, IQR)			4.00	(2.87, 6.01)			5.84	(3.80, 7.12)
20-24 years (Range)				(0.48, 15.73)				(1.32, 11.69)
25-29 years (Mean, SD)	150/887	16.9%	4.05	2.29	107/461	23.2%	3.54	1.56
25-29 years (Median, IQR)			3.31	(2.40, 5.39)			3.31	(2.48, 3.94)
25-29 years (Range)				(0.49, 11.34)				(0.81, 9.66)
30-34 years (Mean, SD)	138/887	15.6%	3.39	2.10	79/461	17.1%	2.86	2.12
30-34 years (Median, IQR)			2.80	(1.57, 4.72)			2.10	(1.40, 3.86)
30-34 years (Range)				(0.26, 9.72)				(0.66, 10.30)
35-39 years (Mean, SD)	138/887	15.6%	2.55	2.03	78/461	16.9%	1.22	1.22
35-39 years (Median, IQR)			2.00	(1.14, 3.55)			0.82	(0.37, 1.59)
35-39 years (Range)				(0.05, 10.91)				(0.10, 5.72)
40-44 years (Mean, SD)	142/887	16.0%	1.32	1.34	64/461	13.9%	0.44	0.63
40-44 years (Median, IQR)			0.88	(0.32, 1.83)			0.13	(0.04, 0.65)
40-44 years (Range)				(0.01, 6.76)				(0.02, 2.58)
45-50 years (Mean, SD)	169/887	19.1%	0.47	0.69	70/461	15.2%	0.13	0.35
45-50 years (Median, IQR)			0.19	(0.06, 0.59)			0.01	(0.01, 0.01)
45-50 years (Range)				(0.01, 4.16)				(0.01, 2.60)

Table 2: Regression models describing the decline in AMH with age and impact of ethnicity
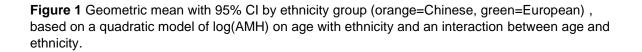
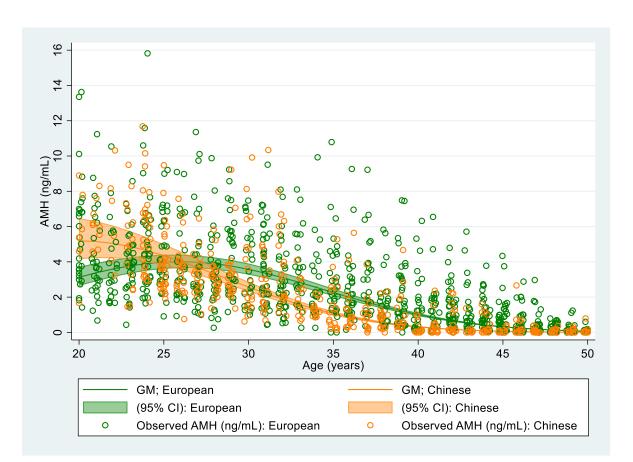| Model	Algebraic form	Parameter	GMR (95% CI)	R^2
Linear (1)	$\ln(AMH) = a + bAge + cEthnicity^*$	a	1.394 (1.291, 1.506)	
		b	0.859 (0.853, 0.866)	56.85
		c	0.531 (0.466, 0.606)	
Quadratic (2)	$\ln(AMH) = a + bAge + cEthnicity + dAge^2$	a	2.463 (2.234, 2.715)	
		b	0.864 (0.858, 0.870)	64.15
		c	0.502 (0.445, 0.566)	
		d	0.993 (0.992, 0.994)	
Quadratic, interaction on age (3)	$\ln(AMH) = a + bAge + cEthnicity + dAge^2 + eAgeEthnicity$	a	2.401 (2.188, 2.633)	
		b	0.887 (0.880, 0.894)	
		c	0.493 (0.440, 0.553)	67.75
		d	0.993 (0.992, 0.994)	
		e	0.920 (0.908, 0.932)	

Age is centred at 34.5 years in each model. Ethnicity reference group is European. GMR=Geometric mean ratio.

Table 3 Geometric mean ratio of AMH by age band and effect of ethnicity

Age	Ethnicity	Geometric mean	Geometric mean ratio (95% CI)
20	European	3.13 (2.63, 3.72)	1.66 (1.33, 2.07)
	Chinese	5.19 (4.16, 6.48)	
25	European	3.99 (3.61, 4.40)	1.09 (0.92, 1.29)
	Chinese	4.35 (3.79, 5.00)	
30	European	3.58 (3.27, 3.91)	0.72 (0.63, 0.82)
	Chinese	2.57 (2.31, 2.87)	
35	European	2.26 (2.06, 2.48)	0.47 (0.42, 0.53)
	Chinese	1.07 (0.96, 1.20)	
40	European	1.01 (0.92, 1.10)	0.31 (0.27, 0.36)
	Chinese	0.31 (0.28, 0.35)	
45	European	0.32 (0.29, 0.35)	0.21 (0.17, 0.25)
	Chinese	0.06 (0.06, 0.08)	
50	European	0.07 (0.06, 0.08)	0.14 (0.11, 0.17)
	Chinese	0.01 (0.01, 0.01)	

Differences between the ethnicity groups and confidence intervals were calculated on the log-scale. These values were then back-transformed and are interpreted as the ratio of geometric means (GM/GMR).
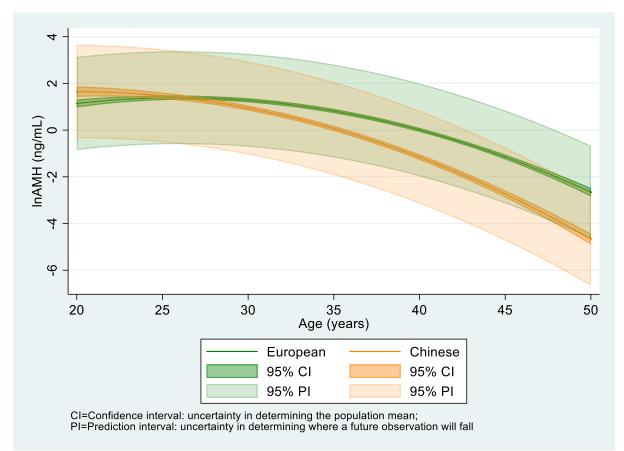
Figure 1 Geometric mean with 95% CI by ethnicity group (orange=Chinese, green=European) , based on a quadratic model of log(AMH) on age with ethnicity and an interaction between age and ethnicity.

Figure 2 Predicted log(AMH), based on a quadratic model of log(AMH) on age with ethnicity and an interaction between age and ethnicity. Shown is the predicted logAMH value versus age, with 95% CI and PI.

CI=Confidence interval: uncertainty in determining the population mean;
PI=Prediction interval: uncertainty in determining where a future observation will fall