Age-related changes in serum anti-Müllerian hormone in women of reproductive age in Kenya

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Background. Anti-Müllerian hormone (AMH) is produced by the granulosa cells of ovarian antral follicles and plays a role in the recruitment of dominant follicles during folliculogenesis. The serum level of AMH is proportional to the number of developing follicles in the ovaries and reflects ovarian reserve. Nomograms of AMH variation with age exist from Caucasian populations, but there are none drawn from local African data.

Objectives. To establish age-specific median serum AMH levels in an unselected East African population of women of reproductive age.

Methods. We retrospectively analysed data on 1 718 women who underwent AMH testing using the Beckman Coulter AMH Gen II enzyme-linked immunosorbent assay during the period 2015 - 2019 at Aga Khan University Hospital, Nairobi, Kenya. Age-specific median AMH levels were derived and presented in 5-year age bands. AMH levels were then log-transformed and, using linear regression in a natural spline function, presented on a scatter plot to demonstrate variation across reproductive age.

Results. The median (interquartile range (IQR)) age of women who were tested for AMH was 38 (19 - 49) years. For the study population, the median (IQR) serum AMH level was 0.87 (0.01 - 17.10) ng/mL. The AMH concentration was inversely related to age, with a progressive decline whereby an increase of 1 year resulted in a corresponding decrease in AMH of 0.18 ng/mL. The proportion of women with decreased ovarian reserve increased exponentially with age from 14.9% in those aged 20 - 24 years to 48.7% at 35 - 39 years.

Conclusion. From a large dataset of mainly black African women, this study confirms that serum AMH declines with advancing age, as reported elsewhere in Caucasian populations. There was, however, a higher than expected number of women with diminished ovarian reserve for age. Future studies prospectively exploring ovarian reserve in the general population could unravel underlying biological, reproductive and environmental factors that may influence AMH levels and reproductive capacity in this indigenous population.

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Subfertility in developing countries, especially in sub-Saharan Africa, attracts little interest among policymakers in reproductive health, where the emphasis is on family planning and contraception. It has been reported that 26.1% of gynaecological consultations in Africa are for subfertility, the predominant diagnosis being tubal damage, implying a high demand for assisted reproductive treatment.[1] A key investigation undertaken by fertility specialists in assisted reproduction treatment is anti-Müllerian hormone (AMH).

AMH is a dimeric glycoprotein produced by granulosa cells of antral follicles in the ovaries and plays a role in recruitment of the dominant follicle during folliculogenesis.[2] A member of the transforming growth factor-β (TGF-β) family, AMH is specifically expressed in small growing follicles[3,4] and plays a role in primordial follicle recruitment and regulation of follicle growth initiation.[5] The serum AMH level is proportional to the number of resting antral follicles and therefore most suitable to determine ovarian reserve.[6]

The measurement of serum AMH has several clinical applications,[7] including assessment of ovarian reserve,[8,9] estimation of ovarian damage such as after gonadotoxic treatment or surgery,[10-12] and more recently in the diagnosis of polycystic ovarian syndrome.[5,13] However, an important and common use for AMH is in assisted conception as a predictor of response to controlled ovarian stimulation,[9,14] where serum levels <1.1 ng/mL and >4.8 ng/mL are predictive of poor ovarian response and ovarian hyperstimulation syndrome, respectively.[15] A woman's age and AMH level may also be combined to estimate the chance of success of assisted reproductive treatment.[10,16,17] Extremely low AMH levels may also reflect premature ovarian insufficiency in women aged <40 years.

Existing nomograms of age-specific AMH levels are derived from validated models and inform on the dynamics of changing ovarian reserve during a woman's reproductive life. These data show that the AMH level has an initial peak shortly after birth, rising steadily until the age of 9 years, when a declining trend is noted through puberty to 15 years before rising again to a peak at 25 years. This is followed by a steady decline to about 51 years, corresponding to the menopause.[18]

There is inter-individual variation in AMH levels that may be influenced by ethnicity and body mass index (BMI).[19,20] African Americans and Hispanics have been reported to have lower levels of circulating AMH than their Caucasian counterparts,[21,22] and women with a higher BMI have an inversely lower AMH concentration compared with those of corresponding age whose BMI is within the normal range.[20,23] These demographic variables indicate a need for regional nomograms and reference ranges for AMH. Data on AMH levels in a predominantly black African population are scarce, as this group is under-represented in studies conducted in the West. The objectives of the present study were to determine variations in serum

AMH levels with age and construct a local reference nomogram in an unselected population.

Methods

The study was a retrospective data analysis conducted at Aga Khan University Hospital, Nairobi, Kenya. This is a private 250-bed teaching hospital offering specialised healthcare services, including women's and reproductive health, to a diverse patient population in East Africa.

AMH levels were measured on Cobas e601 analyser (Roche, Germany) using an assay standardised against the AMH Gen II enzyme-linked immunosorbent assay (ELISA) (Beckman Coulter, USA) according to the manufacturer's instructions, with the values reported as nanograms per millilitre.[24] Test results from the analyser were fed directly into the hospital information system.

Aga Khan University Hospital uses CARE 2000 (Symphony, India), a commercial electronic health management system for data storage and archiving. The system is integrated with laboratory analyser software where data is interfaced automatically following analysis and linked to a unique patient identifier. These data are accessed on user interface computers via a virtual private network.

To retrieve data, Structured Query Language (SQL) with query names of 'anti-Müllerian hormone' or 'AMH', 'age' and 'AK (patient identification) number' was used. This was limited to the dataset between 1 January 2015 and 31 December 2019. The data were exported onto an Excel spreadsheet, version 17 (Microsoft, USA) for management and analysis. Data were excluded if the age was outside 15 - 49 years, the World Health Organization definition of women of reproductive age, or if there were multiple entries for the same patient identifier, as there may have been clinical reasons to perform repeated testing.

Statistical analysis was performed using Stata 16 (StataCorp, USA). Data were tested for normality using the Shapiro-Wilk test. Age and AMH, being continuous data, were described using medians and interquartile ranges (IQRs). A frequency distribution graph for age was plotted and depicted in bands of 5-year age brackets. Taking 34 years as the cut-off for younger reproductive age, women aged ≥35 were considered of advanced reproductive age. Median AMH and the proportion of women with AMH <1.1 ng/mL, defined as diminished ovarian reserve, were calculated. AMH was then log-transformed and, using linear regression in a natural spline function, presented on a scatter plot to demonstrate variation with age.

As the study involved analysis of already existing data with no patient identification, approval was granted by the Research and Ethics Committee at Aga Khan University Hospital to waive seeking patient consent (ref. no. 2019/IERC-143).

Results

Population characteristics

Between January 2015 and December 2019, there were 1 718 tests for AMH stored in the relational database CARE 2000. After exclusion of entries that fell outside the reproductive age range and multiple entries, a total of 1 678 entries were retained for analysis. As shown in Fig. 1, using the Shapiro-Wilk test for normality, age distribution was found to be skewed to the right. The median (IQR) age of women of reproductive age who underwent testing for AMH was 38 (19 - 49) years. Using 34 years as the cut-off age, a majority of women, representing 68.5% of the study population, were of advanced maternal age (≥35 years), of whom 17.5% were of very advanced maternal age (45 - 49 years).

AMH variation with age

The median (IQR) serum AMH level was 0.87 (0.01 - 17.10) ng/mL (Table 1). Median AMH decreased with increasing age, while the proportion of women with diminished ovarian reserve (AMH <1.1 ng/mL) increased sharply with age, reaching as high as 96.5% in those aged 45 - 49 years. As shown in Fig. 2, AMH and age depicted an inverse linear relationship whereby an increase in age of 1 year resulted in a corresponding decrease in AMH level of 0.18 ng/mL. However, age in this linear model accounted for ~21% of the decline in serum AMH. For women aged <40 years, the number with diminished ovarian reserve was disproportionately high, increasing from 14.9% in those aged 20 - 24 years to 48.7% at 35 - 39 years.

Discussion

We report on a large dataset of serum AMH levels in an unselected population over a 5-year study period. This is the first study to report on age-related AMH levels in a largely black African population using the Gen II ELISA assay. Our study confirms an age-related decline in AMH, as previously reported by several other studies in different populations and geographical areas. In contrast, a derived linear nomogram did not fit the quadratic model of AMH changes with age as reported by others, where there was an initial increase in AMH levels from the age of 15 years, peaking at ~25 years and progressively declining to undetectable levels towards the menopause.[18,25,26] Instead, we found an inverse linear relationship with a steady decline in AMH with advancing age.

Our linear model also showed that age only accounted for ~21% of the variance seen in serum AMH, suggesting that other factors such as the number of AMH-producing follicles and the rate of follicle loss may affect AMH levels. These findings were similar to those reported in a cohort study of healthy females.[25] However, we studied an unselected population attending hospital for various medical reasons, and a more reliable determination of AMH variation with age may be obtained from a healthy population of women with no history of infertility or other factors known to affect serum AMH levels.

Derived from a linear regression natural spline and log-transformed model, AMH levels showed a 0.18 ng/mL decline per year with increasing age. Similar declines have been reported in other studies that have fitted both a quadratic and a linear model to age-related AMH changes.[9] Whereas other population nomograms show an initial increase in serum AMH from 15 to 25 years before a steady decline, determination of this pattern was untenable, as only 3% of our study population was aged <25 years. As our study was hospital based, it was not surprising that this age group was under-represented, as it may not be a clinical concern to test for AMH in younger women. Furthermore, the general usefulness of AMH for ovarian reserve determination is greatest after the age of 25 years, when women are more likely to seek medical attention regarding fertility, and a steady decline with age is expected.[9,17,25]

A surprising finding was the large proportion of women aged <40 years with serum AMH levels <1.1 ng/mL, rising from ~15% in the 20 - 24 years age bracket to just under half in those aged 35 - 40 years. This is an unexpectedly high number of women with diminished ovarian reserve at a relatively young reproductive age, as most studies report that an estimated 10% of women in the general population will have accelerated loss of ovarian reserve prior to age 40.[27] As this was a laboratory-based study, it is likely that the hospital-based population may already be at increased

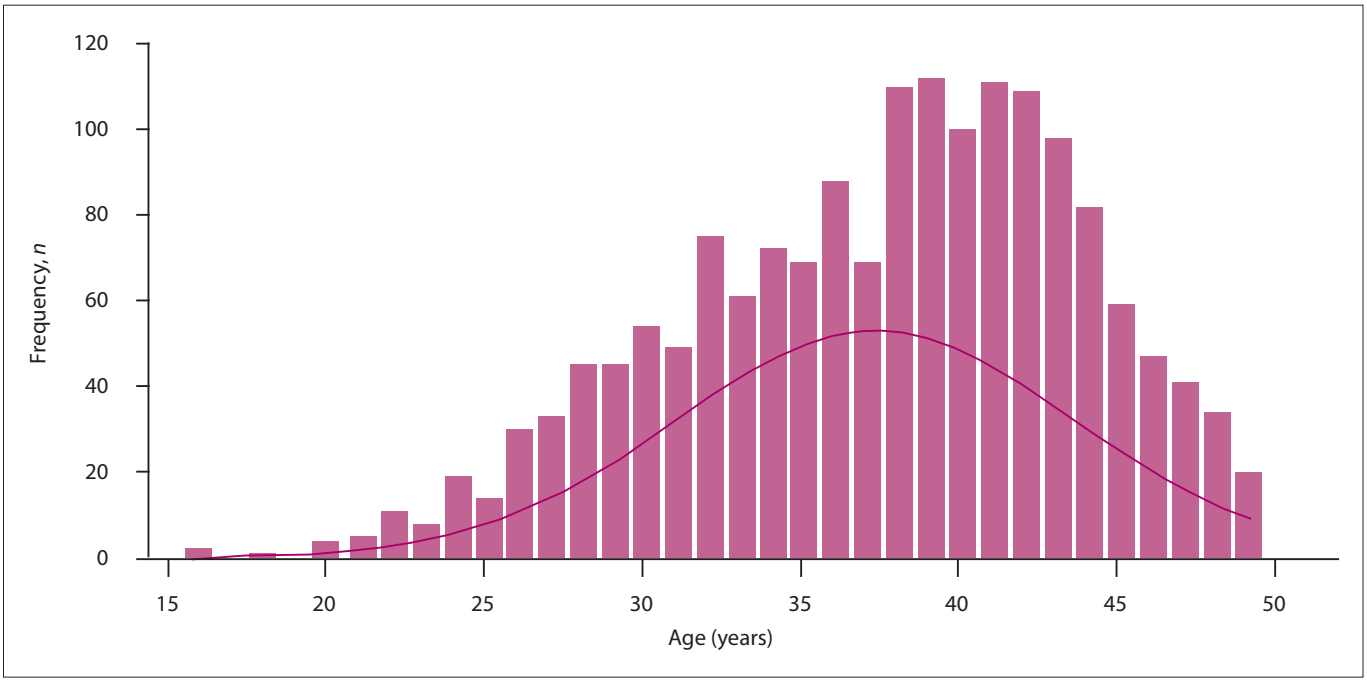

Fig. 1. Frequency distribution of age of the study population.

Table 1. Median AMH levels and proportion of women with AMH <1.1 ng/mL, grouped in 5-year age sets

Age group (years)	Frequency, *n* (%)	AMH level (ng/mL), median (IQR) centiles	AMH <1.1 ng/mL (% within age group)
15 - 19	4 (0.2)	4.62 (2.92 - 6.03)	0
20 - 24	47 (2.8)	3.22 (0.42 - 9.99)	14.9
25 - 29	167 (10.0)	2.81 (0.01 - 14.26)	23.4
30 - 34	311 (18.5)	1.90 (0.01 - 15.16)	33.1
35 - 39	448 (26.7)	1.13 (0.01 - 10.13)	48.7
40 - 44	500 (29.8)	0.41 (0.01 - 6.75)	74.8
45 - 49	201 (12.0)	0.07 (0.01 - 1.45)	96.5

AMH = anti-Müllerian hormone; IQR = interquartile range.

risk of diminished ovarian reserve and therefore seeking fertility assessment. Low serum AMH at an early age may be considered a warning sign for premature ovarian insufficiency, and serial AMH measurement may be useful in assessing the rate of decline in ovarian reserve.[26] Compared with other biomarkers such as follicle-stimulating hormone and inhibin B, AMH has consistently been shown to be a reliable marker of ovarian reserve and hence reproductive capability of women.[8,20] Having a population nomogram may be helpful in predicting the estimated time to diminished ovarian reserve and unfavourable reproductive consequences, and therefore in counselling women appropriately on important life decisions such as childbearing plans or fertility preservation. In most instances, AMH testing takes place in the context of fertility investigations to predict response to ovarian stimulation; however, other indications include assessment of ovarian reserve prior to ovarian endometriosis surgery, or fertility preservation in cancer treatment programmes.[10,28] Ovarian reserve testing in these situations provides valuable insights to aid decision-making during treatment planning. For instance, a decision to pursue fertility treatment or oocyte cryopreservation prior to surgical excision of endometrioma may be taken when ovarian reserve is likely to be severely compromised.[29] Although diminished ovarian reserve does not imply difficulty in conceiving naturally, a finding of low serum AMH may be invaluable in

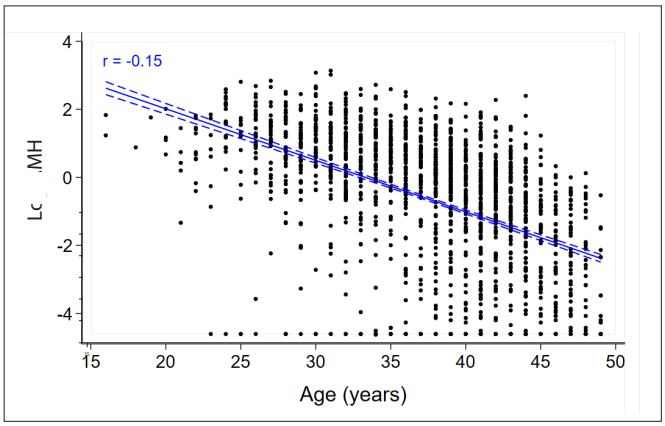

Fig. 2. Log-transformed AMH linear regression and correlation with age using natural spline function. (AMH = anti-Müllerian hormone; r = correlation coefficient.)

deciding when to consider pregnancy, especially around the age of 35 years, when a decline in natural fertility is to be expected.[30]

In the present study, most of the women were of advanced maternal age, of whom 17.5% were of very advanced maternal age, reflecting the changing demographic of reproductive age. Studies have reported that since the 1970s, women in developed countries are continuing to delay childbearing until a later age, with most having their first

child in their 30s, with a consequent decrease in the number of births in those under 20 years old and an increase in first-time mothers aged >40.[31] This pattern of older first-time mothers is also starting to emerge in developing countries.[32] The consequences of this demographic shift are decreased ovarian reserve as well as an increase in reproductive complications, with a resultant increase in the use of assisted reproductive treatments.[33] Whereas assisted reproductive treatment may not reverse age-related reproductive complications, there is growing evidence that oocyte cryopreservation at a younger age may obviate the consequences of diminished ovarian reserve and offer a chance for biological offspring.[34]

To the best of our knowledge, this is the first study to demonstrate age-related AMH changes in a predominantly black African population. Whereas other studies report ethnic variations in AMH levels, these have largely been in Western countries where various environmental factors such as BMI and vitamin D concentration may affect interpretation of results.[21,22]

The present study benefits from a large sample size of unselected patients aged between 15 and 49 years, which may be difficult to recruit in a normal clinical set-up, strengthening its external validity. Data storage and archiving in the relational database, which is easily accessible and verifiable, eliminates the limitation of missing information. AMH testing has undergone various protocol iterations over the past 20 years, with the current Gen II ELISA assay considered most robust, a protocol that was used for this study over a 5-year period. It therefore compares well with other studies using the Gen II ELISA assay protocol, which has since been standardised.[24] As reported in other studies, the derived linear model for the AMH nomogram is comparable to a quadratic model after the age of 25 years. This is helpful clinically, as assessment of ovarian reserve is useful after this age.[25,26]

Our results should be interpreted with caution, however, because the study population may not be generalisable to the normal population without fertility concerns. As this was a laboratory-based analysis, a lack of clinical data such as prior fertility outcomes, BMI, medical conditions and smoking status may limit the general application in advising women in routine practice, especially on the rate of decline in AMH concentration over time. Indeed, the study showed that only 21% of AMH variance was due to age, so other factors should be considered in a multivariable model. A future prospective study in healthy women, starting from puberty to menopause and used to derive a population-based nomogram, may shed some light on changes in serum AMH in the local general population. The findings may then be compared with validated models from other populations to understand the impact of race, ethnicity or genetics on AMH and the relationship with reproductive outcomes in the local population. It would be useful to conduct further research to establish whether there are biological or environmental factors associated with diminished ovarian reserve in women aged <40 years, as this has significant reproductive implications.

Conclusion

Our study confirms that AMH declines in a linear fashion in a local predominantly black African cohort. It appears that most women who were tested for ovarian reserve were of advanced maternal age, although this may have been influenced by physician choice. There was, however, an unexpectedly high proportion of women with diminished ovarian reserve prior to 40 years of age, indicating an increased risk of premature ovarian insufficiency. Future studies based on a general population cohort and conducted prospectively are needed to understand the relationship between serum AMH and biological, reproductive and environmental factors.

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Author contributions. MA: performed the data analysis, contributed to the first draft of the manuscript and proofread the final draft; DM: extracted data from CARE 2000, contributed to the first draft and proofread the final draft; AM: designed the study protocol and proofread the final draft; CM: performed data analysis, wrote the first draft and proofread the final draft.

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1. Murage A, Muteshi MC, Githae F. Assisted reproduction services provision in a developing country: Time to act? Fertil Steril 2011;96(4):966-968. https://doi.org/10.1016/j.fertnstert.2011.07.1109
2. Visser JA, de Jong FH, Laven JSE, Themmen APN. Anti-Müllerian hormone: A new marker for ovarian function. Reproduction 2006;131(1):1-9. https://doi.org/10.1530/rep.1.00529
3. Cate RL, Mattaliano RJ, Hession C, et al. Isolation of the bovine and human genes for Müllerian inhibiting substance and expression of the human gene in animal cells. Cell 1986;45(5):685-698. https://doi.org/10.1016/0092-8674(86)90783-x
4. Durlinger ALL, Visser JA, Themmen APN. Regulation of ovarian function: The role of anti-Müllerian hormone. Reproduction 2002;124(5):601-609. https://doi.org/10.1530/rep.0.1240601
5. Dewailly D, Pigny P, Soudan B, et al. Reconciling the definitions of polycystic ovary syndrome: The ovarian follicle number and serum anti-Müllerian hormone concentrations aggregate with the markers of hyperandrogenism. J Clin Endocrinol Metab 2010;95(9):4399-4405. https://doi.org/10.1210/jc.2010-0334
6. Kevenaar ME, Meerasahib MF, Kramer P, et al. Serum anti-Mullerian hormone levels reflect the size of the primordial follicle pool in mice. Endocrinology 2006;147(7):3228-3234. https://doi.org/10.1210/en.2005-1588
7. Hansen KR, Hodnett GM, Knowlton N, Craig LB. Correlation of ovarian reserve tests with histologically determined primordial follicle number. Fertil Steril 2011;95(1):170-175. https://doi.org/10.1016/j.fertnstert.2010.04.006
8. Broer SL, van Disseldorp J, Broeze KA, et al. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: An individual patient data approach. Hum Reprod Update 2013;19(1):26-36. https://doi.org/10.1093/humupd/dms041
9. Nelson SM, Messow MC, Wallace AM, Fleming R, McConnachie A. Nomogram for the decline in serum antimüllerian hormone: A population study of 9,601 infertility patients. Fertil Steril 2011;95(2):736-741.e1-3. https://doi.org/10.1016/j.fertnstert.2010.08.022
10. Anderson RA, Rosendahl M, Kelsey TW, Cameron DA. Pretreatment anti-Müllerian hormone predicts for loss of ovarian function after chemotherapy for early breast cancer. Eur J Cancer 2013;49(16):3404-3411. https://doi.org/10.1016/j.ejca.2013.07.014
11. Lie Fong S, Schipper I, de Jong FH, Themmen APN, Visser JA, Laven JSE. Serum anti-Müllerian hormone and inhibin B concentrations are not useful predictors of ovarian response during ovulation induction treatment with recombinant follicle-stimulating hormone in women with polycystic ovary syndrome. Fertil Steril 2011;96(2):459-463. https://doi.org/10.1016/j.fertnstert.2011.05.084
12. Su HI, Flatt SW, Natarajan L, DeMichele A, Steiner AZ. Impact of breast cancer on anti-mullerian hormone levels in young women. Breast Cancer Res Treat 2013;137(2):571-577. https://doi.org/10.1007/s10549-012-2361-5
13. Li HWR, Anderson RA, Yeung WSB, Ho PC, Ng EHY. Evaluation of serum antimullerian hormone and inhibin B concentrations in the differential diagnosis of secondary oligoamenorrhea. Fertil Steril 2011;96(3):774-779. https://doi.org/10.1016/j.fertnstert.2011.06.016
14. Broer SL, Dólleman M, Opmeer BC, Fauser BC, Mol BW, Broekmans FJM. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: A meta-analysis. Hum Reprod Update 2011;17(1):46-54. https://doi.org/10.1093/humupd/dmq034
15. Broer SL, Dólleman M, van Disseldorp J, et al. Prediction of an excessive response in in vitro fertilization from patient characteristics and ovarian reserve tests and comparison in subgroups: An individual patient data meta-analysis. Fertil Steril 2013;100(2):420-429.e7. https://doi.org/10.1016/j.fertnstert.2013.04.024
16. Yates AP, Rustamov O, Roberts SA, et al. Anti-Mullerian hormone-tailored stimulation protocols improve outcomes whilst reducing adverse effects and costs of IVF. Hum Reprod 2011;26(9):2353-2362. https://doi.org/10.1093/humrep/der182
17. Jeppesen JV, Anderson RA, Kelsey TW, et al. Which follicles make the most anti-Mullerian hormone in humans? Evidence for an abrupt decline in AMH production at the time of follicle selection. Mol Hum Reprod 2013;19(8):519-527. https://doi.org/10.1093/molehr/gat024
18. Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WHB. A validated model of serum anti-müllerian hormone from conception to menopause. PLoS ONE 2011;6(7):e22024. https://doi.org/10.1371/journal.pone.0022024
19. Gougeon A. Ovarian follicular growth in humans: Ovarian ageing and population of growing follicles. Maturitas 1998;30(2):137-142. https://doi.org/10.1016/s0378-5122(98)00069-3
20. La Marca A, Spada E, Grisendi V, et al. Normal serum anti-Müllerian hormone levels in the general female population and the relationship with reproductive history. Eur J Obstet Gynecol Reprod Biol 2012;163(2):180-184. https://doi.org/10.1016/j.ejogrb.2012.04.013
21. Seifer DB, Golub ET, Lambert-Messerlian G, et al. Variations in serum Müllerian inhibiting substance between white, black, and Hispanic women. Fertil Steril 2009;92(5):1674-1678. https://doi.org/10.1016/j.fertnstert.2008.08.110
22. Schuh-Huerta SM, Johnson NA, Rosen MP, Sternfeld B, Cedars MI, Reijo Pera RA. Genetic variants and environmental factors associated with hormonal markers of ovarian reserve in Caucasian and African American women. Hum Reprod 2012;27(2):594-608. https://doi.org/10.1093/humrep/der391

23. Overbeek A, Broekmans FJ, Hehenkamp WJ, et al. Intra-cycle fluctuations of anti-Müllerian hormone in normal women with a regular cycle: A re-analysis. Reprod Biomed Online 2012;24(6):664-669. https://doi.org/10.1016/j.rbmo.2012.02.023
24. Nelson SM, Pastuszek E, Kloss G, et al. Two new automated, compared with two enzyme-linked immunosorbent, antimüllerian hormone assays. Fertil Steril 2015;104(4):1016-1021.e6. https://doi.org/10.1016/j.fertnstert.2015.06.024
25. Lie Fong S, Visser JA, Welt CK, et al. Serum anti-Müllerian hormone levels in healthy females: A nomogram ranging from infancy to adulthood. J Clin Endocrinol Metab 2012;97(12):4650-4655. https://doi.org/10.1210/jc.2012-1440
26. Dewailly D, Andersen CY, Balen A, et al. The physiology and clinical utility of anti-Müllerian hormone in women. Hum Reprod Update 2014;20(3):370-385. https://doi.org/10.1093/humupd/dmt062
27. Tal R, Seifer DB. Ovarian reserve testing: A user's guide. Am J Obstet Gynecol 2017;217(2):129-140. https://doi.org/10.1016/j.ajog.2017.02.027
28. Seyhan A, Ata B, Uncu G. The impact of endometriosis and its treatment on ovarian reserve. Semin Reprod Med 2015;33(6):422-428. https://doi.org/10.1055/s-0035-1567820
29. Llarena NC, Falcone T, Flyckt RL. Fertility preservation in women with endometriosis. Clin Med Insights Reprod Health 2019;13:1179558119873386. https://doi.org/10.1177/1179558119873386
30. Crawford NM, Steiner AZ. Age-related infertility. Obstet Gynecol Clin North Am 2015;42(1):15-25. https://doi.org/10.1016/j.ogc.2014.09.005
31. Mills M, Rindfuss RR, McDonald P, te Velde E; on behalf of the ESHRE Reproduction and Society Task Force. Why do people postpone parenthood? Reasons and social policy incentives. Hum Reprod Update 2011;17(6):848-860. https://doi.org/10.1093/humupd/dmr026
32. Kahveci B, Melekoglu R, Evruke IC, Cetin C. The effect of advanced maternal age on perinatal outcomes in nulliparous singleton pregnancies. BMC Pregnancy Childbirth 2018;18(1):343. https://doi.org/10.1186/s12884-018-1984-x
33. Schmidt L, Sobotka T, Bentzen JG, Nyboe Andersen A; on behalf of the ESHRE Reproduction and Society Task Force. Demographic and medical consequences of the postponement of parenthood. Hum Reprod Update 2012;18(1):29-43. https://doi.org/10.1093/humupd/dmr040
34. Cimadomo D, Fabozzi G, Vaiarelli A, Ubaldi N, Ubaldi FM, Rienzi L. Impact of maternal age on oocyte and embryo competence. Front Endocrinol 2018;9:327. https://doi.org/10.3389/fendo.2018.00327

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