Original Article

Geographical Diversity in the Age Specific Anti Müllerian Hormone Levels in Infertile Women: A Hospital based Cohort Study

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Abstract

Background: Serum anti-Mullerian hormone (AMH) is a significant determinant of ovarian reserve. It is still not clear about the rate at which AMH declines with age and varies across populations. **Aim:** The present study examined the AMH levels specific to the North and South Indian populations and attempted to establish an age-dependent reference parametrically. **Settings and Design:** This was a prospective study in a tertiary centre. **Materials and Methods:** Serum samples were collected apparently from 650 infertile women (327 from North and 323 from South Indians). AMH was measured using an electrochemiluminescent technique. **Statistical Analysis Used:** Comparison of the AMH data between North and South regions was done by independent *t*-test. For each age, seven empirical percentiles (3rd, 10th, 25th, 50th, 75th, 90th and 95th) were applied. AMH nomograms for the 3rd, 10th, 25th, 50th, 75th, 85th, 90th and 95th percentiles were produced using the lambda-mu-sigma method. **Results:** AMH levels remarkably decreased with increasing age in the North Indian population, but in the South Indian population, they did not decline beyond 1.5 ng/mL. Further, in the North Indian population, AMH levels were significantly higher in the age group of 22–30 years (4.4 ng/mL) than in the South Indian population (2.04 ng/mL). **Conclusion:** The present study suggests a major geographical difference in mean AMH levels according to their age and ethnic background, regardless of their subjacent pathologies.

Keywords: *Anti-Mullerian hormone, assisted reproductive technology, environment factors, folliculogenesis*

Introduction

Amongst the ovarian reserve markers used in the field of assisted reproductive technology (ART), the anti-Mullerian hormone (AMH) is of particular interest because of its low intra- and inter-cycle variability. AMH is a dimeric glycoprotein member of the transforming growth factor-beta family. The follicle-stimulating hormone stimulates the granulosa cells of growing follicles to release AMH until the ovary achieves an appropriate size and differentiation.[1,2] AMH is one of the most reliable tests to answer the infertile couple 'whether the reproductive window is still open'? and 'what chances do they have'?[3] Several studies have found a mathematical link between serum AMH levels and the age of women,[4-9] and to predict the onset of menopause.[10] Studies have shown that AMH diminishes steadily with age and eventually becomes undetectable during the transition to menopause.[11] van Disseldorp *et al*. reported that AMH is a more accurate predictor of reproductive age than biological age alone.[12] AMH increases until the age of 25 years, with a steady fall after 34 years of age that continues until menopause.[13,14] Moreover, studies have shown that AMH levels vary

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across races and geographical areas. AMH levels in African and Hispanic women are lower when compared to Caucasian women of the same age. However, Chinese women aged 25 years showed substantially higher AMH levels than Caucasian women.[15,16] In subpopulations within ethnicities, the study found that Maya women had lower AMH levels than other Hispanic women.[17] Furthermore, Gromski *et al*. reported that Indian fertile and infertile women had significantly lower AMH levels than European women. Additionally, the study found that the rate of decline in AMH levels was faster in Indian women than in European women.[18] Hence, there is a wide range of geographical differences in the age-related decline in the AMH pattern. The lack of consensual standardisation of the AMH level in different geographical settings may require specific AMH reference values in the ART unit so that infertile couples will have a more appropriate and personalised approach. Moreover, in the populations of the North and South Indians, it is still not clear the rate at which AMH declines with age and whether it varies across populations in different geographical settings. Hence, the present study aims to establish an accurate parametrical association between AMH serum values and age in a large group of women specific to North and South India.

Methodology

Blood samples were collected from 650 women (aged 18–41 years) belonging to Northern (Valley Fertility Centre, Kashmir) and Southern (GarbhaGudi IVF Centre, Bengaluru, Karnataka) India. The sampling was done between November 2020 and December 2021. The blood samples collected from median cubital vein at the Fertility Centre were processed in the biochemistry laboratory. Serum AMH levels were measured by using an electrochemiluminescent technique by means of an enzymatically based immunoassay (AMH Gen II ELISA Kit, Beckman Coulter) and a fully automated analyser (DSX analyser, DYNEX Technologies). AMH values were reported as ng/mL. The procedure of the present study was in accordance with the Ethical Standards of the 2013 Helsinki Declaration.[19] After obtaining Ethical Clearance (GGIRHR/22/RI-6/06), signed informed consent was obtained from all subjects and the data were analysed anonymously. No identification information was disclosed at any point. The total sample size of 650 was confirmed after sample size calculation.

The demographic details (age, childbearing, previous surgeries, history of infertility and history of polycystic ovary syndrome) of infertile women were documented. The females with ovariectomy, ovarian cancer, previous ovarian surgeries and those having any endocrinological disorders such as diabetes, hyperthyroidism/hypothyroidism and prolactinoma were excluded from the study.

Statistical analysis

The statistical analysis was carried out using IBM SPSS Statistics version 23. Continuous variables were subjected to descriptive analyses. Initially, the data were tested for Kolmogorov–Smirnov test to know the type distribution. Comparison of the AMH data between North and South regions was done by independent *t*-test. For each age, seven empirical percentiles (3rd, 10th, 25th, 50th, 75th, 90th and 95th) were applied.

Results

A total of 650 subjects met the inclusion criteria and participated in the present study; 327 were from North and 323 from South Indians. The mean value of serum AMH levels in North Indians was 3.2 ng/mL (range: 0.01–16.9 ng/mL), and in South Indians, it was 1.9 ng/mL (range: 0.009-2.9 ng/mL). As age advanced, the annual decline in AMH values (up to 0.25 ng/mL) was observed in North Indian population [Figure 1], whereas, in South Indian population, the lower limit of AMH was 1.5 ng/mL [Figure 2].

There was a statistically significant difference between the mean value of age and the AMH level amongst the North and South Indian populations [Table 1]. The overall mean age of the North Indian population (30.1 ± 4.8 years) was significantly lower as compared to the South Indian population (32.1 ± 4.7 years) [$P = 0.001$, Table 1]. On the contrary, the significant mean AMH level was higher in the North Indian population (3.2 ± 1.1 ng/mL) than in the South Indian population (1.9 ± 0.6 ng/mL) [$P = 0.001$, Table 1].

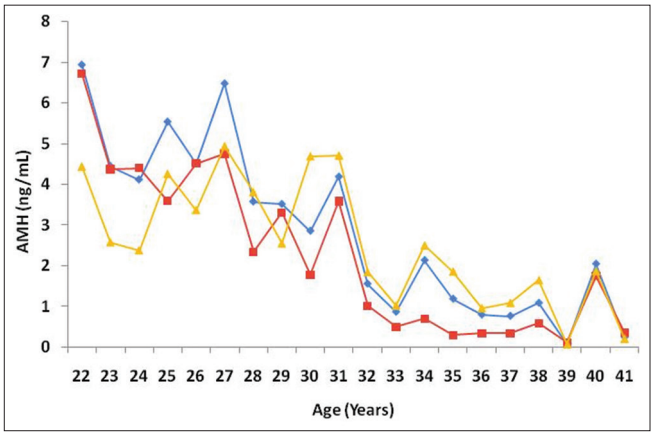

Figure 1: Representation of age-specific AMH median, mean and SD values at 1-year age intervals of North Indian population. Mean (blue), median (red) and SD (yellow) of AMH values are represented versus age (years). AMH = Anti-Mullerian hormone, SD = Standard deviation

The mean AMH of South Indian population was significantly lower (2.04 ± 0.6 ng/mL) than the mean AMH levels of the North Indian population, 4.4 ± 1.9 ng/mLbetween the age groups of 22 and 30 years. Further, in the age group of 36-41 years we observed a statistically significant decline in the mean AMH level among the North Indian population (0.9 ± 0.28 ng/mL) than the South Indian population (1.8 ± 0.6 ng/mL) ($P = 0.001$). However, the mean AMH levels were similar between the age groups of 31 and 35 years amongst the North and South Indian cohorts, 1.7 ng/mL [$P > 0.05$, Table 2].

In the South Indian population, the median AMH value decreased (2.1 ng/mL) in the age groups of 22–30 years as compared to the North Indian population. However, the decline in median AMH level (0.7 ng/mL and 0.4 ng/mL) was observed in North Indian population aged 31–35 years and 36–41 years, respectively [Table 2].

In the present study, we calculated the mean AMH level for individuals of varying ages (from 22 to 41 years). In the South Indian population, the mean AMH level ranged from 1.4 ng/mL to 2.29 ng/mL, and the data did not show an annual decrease in AMH level, even at 41 years of age (the mean AMH level was 1.58 ng/mL, which was similar to the age group of 25 years). However, in the North Indian population, the mean AMH level ranged from 0.01 ng/mL to 6.94 ng/mL, and there was an annual decline in the mean AMH level (i.e. at the age of 41 years, the mean AMH level was 0.25 ng/mL). Furthermore, in North Indian population, the infertile women aged 39 years (0.09 ± 0.27 mg/mL) and 41 years (0.25 ± 0.1 ng/mL) had a very poor ovarian reserve [Figure 1].

Women aged 22 and 24–28 years showed a statistically significant increase in AMH level in North Indian population than South Indian population ($P < 0.05$) whereas women aged 33–41 years showed a significant increase in AMH level in South Indian population than North Indian population [Figures 1 and 2].

Furthermore, we calculated the 3rd, 10th, 25th, 40th, 50th, 75th, 85th, 90th and 95th percentiles for AMH in association with women's age for North and South Indian populations [Tables 3 and 4]. Figures 3-5 depict the nomograms for AMH levels in infertile women.

Discussion

The present study relies on the AMH levels of infertile female patients aged between 22 and 41 years from the North ($n = 327$) and South ($n = 323$) Indian populations. The study establishes an accurate mathematical relationship between AMH values and age in the North and South Indian populations. This potential peculiarity may apply to the respective geographical regions. The North Indian cohort showed a decline in AMH levels with advancing age, whereas, in the South Indian cohort,

Table 1: Overall association of anti-Mullerian hormone levels (ng/mL) and age amongst North and South Indian cohorts

Parameters	*n*	Mean±SD	*P*
Age			
North	327	30.1±4.8	0.001
South	323	32.1±4.7	
AMH			
North	327	3.2±1.1	0.001
South	323	1.9±0.6	

$P<0.05$ is considered significant. *n*=Number of participants, SD=Standard deviation, AMH=Anti-Mullerian hormone

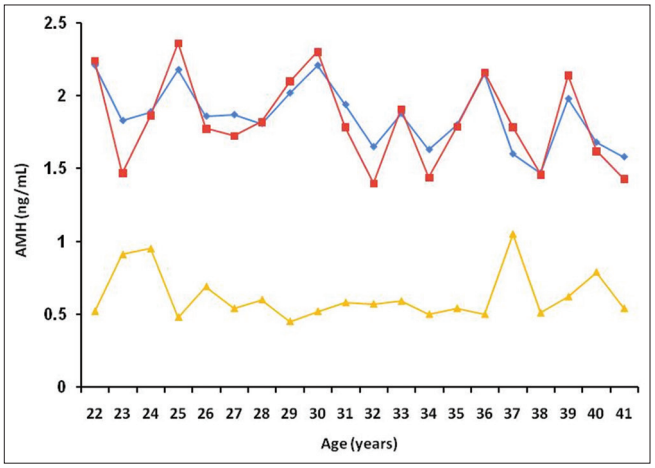

Figure 2: Representation of age-specific AMH median, mean and SD values at 1-year age intervals of South Indian population. Mean (blue), median (red) and SD (yellow) of AMH values are represented versus age (years). Serum AMH values decline in direct relationship to ageing. AMH = Anti-Mullerian hormone, SD = Standard deviation

Table 2: Association of mean anti-Mullerian hormone levels and grouped age amongst North and South Indian cohorts

Grouped age	North Indian population			South Indian population			SE	*P*
	Number of patients	AMH		Number of patients	AMH			
		Mean±SD	Median		Mean±SD	Median		
22-30	189	4.4±1.9	3.3	132	2.04±0.6	2.1	0.4	0.001
31-35	94	1.7±0.47	0.7	111	1.7±0.57	1.7	0.2	0.6
36-41	44	0.9±0.28	0.4	80	1.8±0.56	1.7	0.2	0.001

$P<0.05$ is considered significant. SD=Standard deviation, SE=Standard error, AMH=Anti-Mullerian hormone

Table 3: Correlation between the 3rd, 10th, 25th, 40th, 50th, 75th, 90th and 95th percentiles of serum anti-Mullerian hormone level and age amongst North Indian population (*n*=327, age between 22 and 41 years)

Years	Number of patients	Centiles (North Indian population)								
		3rd	10th	25th	40th	50th	75th	85th	90th	95th
22	16	2	2.13	2.9	4.8	6.7	10.2	10.9	13.2	0
23	11	0.7	1.02	2.5	3.6	4.4	5.06	8.6	9.3	0
24	12	0.02	0.6	2.3	3.6	4.4	6.63	7.3	7.3	0
25	28	0.9	1.8	2.9	3.3	3.6	7.55	9.2	11.9	16.9
26	24	0.2	0.5	1.3	3.13	4.5	7.36	8.9	9.7	11.3
27	16	0.5	1.8	3.2	4.3	4.7	8.9	15.5	15.5	0
28	18	0.02	0.08	1.3	1.5	2.3	4.4	5.9	10.7	0
29	12	0.01	0.3	1.07	2	3.3	5.9	7.02	7.02	0
30	52	0.01	0.02	0.4	1	1.8	3.08	4.2	5.3	12.09
31	10	0.01	0.01	0.2	3	3.6	5.4	10	15	0
32	36	0.01	0.13	0.3	0.7	1.02	1.9	3.45	4	7.1
33	11	0.01	0.01	0.1	0.39	0.5	1.12	2.6	3	0
34	6	0.31	0.31	0.5	0.7	0.7	4.8	6	0	0
35	31	0.02	0.04	0.06	0.21	0.3	1.2	2.8	5.6	6.2
36	8	0.05	0.05	0.08	0.2	0.34	1.7	2	0	0
37	9	0.01	0.01	0.04	0.09	0.34	1.07	2.3	0	0
38	14	0.01	0.01	0.03	0.5	0.6	0.9	3.9	4.9	0
39	3	0.01	0.01	0.01	0.08	0.12	0	0	0	0
40	7	0.02	0.02	0.03	1.2	1.75	3.3	4.7	0	0
41	3	0.01	0.01	0.01	0.23	0.37	0	0	0	0

Table 4: Correlation between the 3rd, 10th, 25th, 40th, 50th, 75th, 90th and 95th percentiles of serum anti-Mullerian hormone level and age amongst South Indian population (*n*=323, age between 22 and 41 years)

Years	Number of patients	Centiles (South Indian population)								
		3rd	10th	25th	40th	50th	75th	85th	90th	95th
22	8	1.35	1.4	1.8	2.05	2.2	2.7	2.8	0	0
23	3	1.15	1.2	1.2	1.3	1.5	0	0	0	0
24	8	0.009	0.009	1.4	1.7	1.9	2.8	2.9	0	0
25	11	1.2	1.3	1.9	1.9	2.4	2.5	2.6	2.9	0
26	12	1.04	1.06	1.2	1.4	1.8	2.6	2.7	2.8	0
27	14	1.08	1.2	1.6	1.7	1.7	2.2	2.8	2.8	0
28	15	1	1.04	1.2	1.6	1.8	2.3	2.6	2.8	0
29	14	1.21	1.3	1.7	1.9	2.1	2.3	2.6	2.6	0
30	47	1	1.4	1.9	2.3	2.3	2.6	2.7	2.8	2.9
31	8	1.32	1.3	1.5	1.6	1.8	2.5	2.8	0	0
32	36	1.007	1.06	1.2	1.3	1.4	2	2.4	2.6	2.9
33	19	1.07	1.1	1.2	1.7	1.9	2.4	2.5	2.7	0
34	22	1.04	1.06	1.2	1.4	1.4	2	2.3	2.5	2.6
35	26	1.06	1.08	1.3	1.6	1.8	2.4	2.5	2.5	2.7
36	17	1.23	1.5	1.7	2	2.2	2.6	2.8	2.8	0
37	10	0.009	0.009	0.8	1.4	1.8	2.4	2.9	2.9	0
38	21	0.009	1.002	1.2	1.4	1.5	1.9	2	2.1	2.2
39	14	1.02	1.08	1.3	2	2.1	2.5	2.6	2.7	0
40	11	0.009	0.2	1.2	1.6	1.6	2.3	2.5	2.8	0
41	7	1.07	1.07	1.2	1.2	1.4	2.3	2.4	0	0

the women had a minimum AMH level of 1.5 ng/mL. A low level of mean AMH level was observed in the

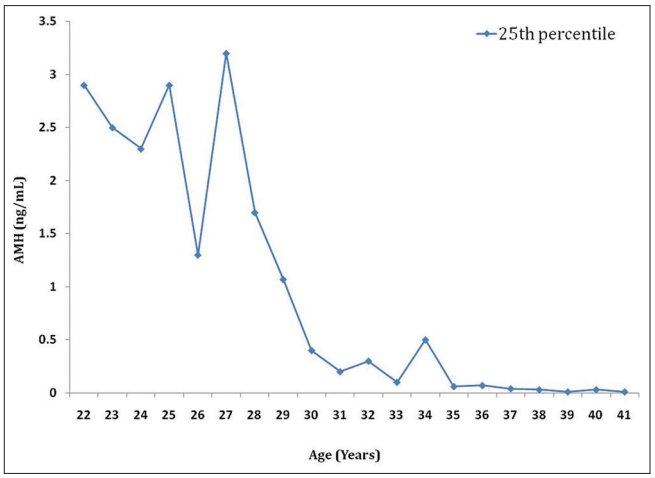

Figure 3: Twenty-fifth percentile of serum AMH level (ng/mL) and age (years) amongst North Indian population. AMH = Anti-Mullerian hormone

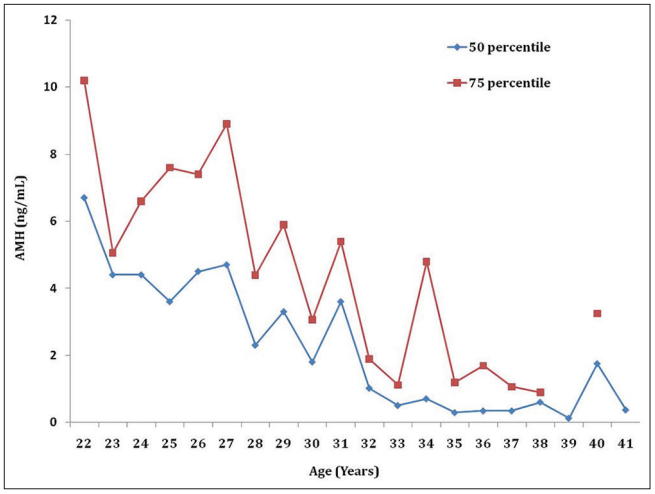

Figure 4: Correlation between the 50th and 75th percentiles of serum AMH level and age amongst North Indian population. AMH = Anti-Mullerian hormone

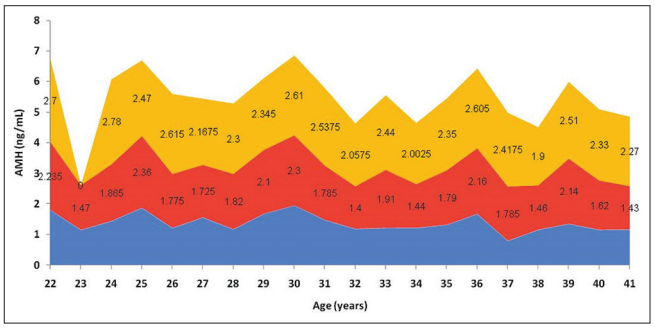

Figure 5: Correlation between the 25th (blue), 50th (red) and 75th (yellow) percentiles of serum AMH level and age amongst South Indian population. AMH = Anti-Mullerian hormone

North Indian population at the ages of 39 (0.09 ng/mL) and 41 (0.25 ng/mL) years, indicating a significant age-specific decline in fertility and suggesting that after 35 years of age, there is a drop in the female

fertility of the North Indian population. The most comprehensive investigation involving 17,120 women older than 36 years of age revealed a decline in mean and median AMH values of 0.2 ng/mL/year.[8] In the Asian population, the 6700 Chinese women showed a rise in AMH level at 18 years of age and a consistent decline over 50 years.[20] The study on AMH over the age of 40 years showed a drastic decrease in AMH level, and 1.0 ng/mL was the cut-off level to predict at least 4 oocytes.[21] Furthermore, Nelson *et al*. reported that Chinese women showed a higher level of AMH until the age of 25 years. After 25 years of age, there was an intense decrease in AMH levels in Chinese women compared with Caucasian women.[22] The present study showed that women >37 years had a 0.2 ng/mL/year decline in AMH level and Doroftei *et al*. reported 0.1 ng/mL/year.[23] A similar observation was observed in the age group of >36 years in the North Indian population.

The study showed a decrease in AMH levels in 237 Black and 213 Chinese women of younger and middle ages compared to 227 Caucasian women. Nonetheless, when compared to Black women, there was a decline in AMH in Latina and Chinese women as they aged.[15] Marsh *et al*. studied 1654 African American women to determine the variation in AMH levels. The median AMH level was 3.18 ng/mL, which was comparable to the 31 years of North region population of the present study. Furthermore, they showed that thyroid disorders and hormonal pills had a negative influence on AMH levels, whereas a history of abnormal bleeding during menses and oligomenorrhea was linked with higher AMH levels.[24] In Caucasian women, the study showed that being obese at 18 years of age had a significant negative correlation with AMH levels.[25] In addition, the cross-sectional study of 671 post-menopausal women revealed no significant association between race and AMH levels.[26] However, in the present study, as age increased, there was a decrease in AMH level and it was significantly associated with geographical setting, where the mean AMH level of South Indian population was significantly higher than North Indian population. Furthermore, Bleil *et al*. found that in a cohort of 947 women, AMH levels varied more consistently across ethnicities amongst older women, especially as they approached perimenopause.[15] However, in Caucasians, a study of AMH levels showed no statistically significant difference between the AMH levels of fertile and infertile Black women, providing further evidence that AMH is a better marker for ovarian reserve than it is for fertility. Bleil *et al*. also reported that all age groups of 220 Hispanic women showed lower AMH as compared to 227 Caucasian women.[15] However, in the present study, after the age of 31 years both the North and South Indian populations showed a decline in AMH in infertile women. In the South Asian population, Bhide *et al*. studied AMH levels in 865 women and reported that despite higher AMH levels seen in South Asian patients compared to Caucasians, the difference was insignificant in multivariable analysis.[27] A study showed that AMH was at its peak, at the age of 15.8 years and remained stable until 25 years of age. Then, there was a gradual decline in AMH levels until the women reached menopause.[7,13,28] Lie *et al*. also concluded that older women had low AMH levels.[7] Overall, the authors also suggest that the associations between AMH and age are not linear across all age groups. When considering ovarian reserve and fertility, the decline in AMH becomes more precipitous. In Western societies, women of 30 years plan to have a baby. By 30 years of age, around 90% of the initial primordial follicles are lost, which illustrates that the formation and maintenance of ovarian reserve is a wasteful process in humans.[29] The study has shown that delayed motherhood increases the probability of obstetric complications and perinatal problems such as declined fertility, damaged DNA in germ cells, poor oocyte quality and weakened placenta, resulting in birth defects and chromosomal abnormalities.[30] Therefore, differences between the present study and those already published may explain racial or ethnic peculiarities, genetic, socioeconomic and environmental factors.[31-33] Furthermore, the difference in geographical setting may be due to the lack of international standardisation for AMH measurement.[34]

In the 3rd percentile, there is a decline and rise in AMH levels amongst the North and South Indian populations compared to the Romanian population where the range was constant. In North Indians, the AMH level at the 25th percentile showed that the infertile women at 29 years of age had an AMH level of 1 ng/mL, and then the AMH level decreased drastically (0.01 ng/mL). In South Indian population, the AMH level was constant in all age groups. In the 50th percentile, 33–41-year-old North Indian women had <1 ng/mL of AMH, whereas, in the South Indian population, AMH was >1 ng/mL at all ages.

Conclusion

The present study showed that females in the age group of 22–30 years had significantly higher AMH levels in the North Indian population when compared to those in the South Indian population. These young women were negative for the risk of miscarriage and chromosomal aberrations. The decline in AMH over age was generally linear amongst subfertile North Indian women. Therefore, the study serves as a reference guide

to subfertile North Indian women to tailor their ovarian stimulation regimen. However, future validation of the nomograms with longitudinal data is required.

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AMH test was done as part of infertility assessment.

Conflicts of interest

There are no conflicts of interest.

Data availability statement

The data is available wiht the corresponding author and will be made available upon reasonable request.

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