Original Article

Effect of Reproductive and Lifestyle Factors on Anti-Mullerian Hormone Levels in Women of Indian Origin

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Abstract

Background: Infertility is a world-wide problem and one third females. Over the years, anti-mullerian hormone (AMH) has emerged as a major marker of ovarian reserve. There is also increasing interest in determining the factors which can impact AMH levels. **Aims:** To correlate the association of reproductive and lifestyle factors on AMH levels in women of Indian origin. **Settings and Design:** Multicentric cross sectional study. **Materials and Methods:** The study was conducted using data extracted from the patient records of seven private fertility practices located in North India. Women who were attending these clinics for fertility treatment were requested to fill the questionnaire related to reproductive and lifestyle factors. **Statistical Analysis used:** Our outcome variable was level of AMH measured in the past 3 months, and was assessed as normal or low. All analyses were conducted using STATA 17. **Results:** We found a direct association of low AMH with increasing age, short cycles, amenorrhea and women with family history of premature menopause. We found a direct correlation of high AMH and women with polycystic ovary syndrome and those whose partners had Oligoasthenoteratozoospermia (OATS) or azoospermia. There was no correlation with smoking, sleep, diet, body mass index, cell phone or laptop use in our study. **Conclusion:** Reproductive and lifestyle factors may affect ovarian reserve and but there was a dearth of human studies in this area. To the best of our knowledge this is the first human study on the effect of AMH on Laptop and Cell phone use. We urgently need more studies to confirm or refute our findings so that we can counsel our patients well.

Keywords: *Anti-mullerian hormone levels, lifestyle factors, reproductive*

Introduction

The problem of infertility has been on the increase. Over the years, anti-mullerian hormone (AMH) has emerged as a major marker of ovarian reserve. There is also increasing interest in determining the factors which can impact AMH levels like obesity, level of physical activity, smoking, alcohol, race, ethnicity and socioeconomic status. The study of effect of these factors on AMH levels is difficult due to the effect of compounding factors like age, weight, family history of early menopause.[1] There is lack of sufficient data on this subject. This multicentre observational study aims to understand the relation of lifestyle factors and reproductive factors that can impact AMH levels in women of Indian origin.

AMH is a marker of ovarian reserve in women which is applied in Current clinical practice as a predictive and diagnostic tool. AMH is highly sensitive to age-related

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changes in the ovarian reserve. Reproductive factors such as age at menarche have been shown to predict serum AMH, with conflicting findings.

Genetic and environmental factors may contribute to variations in serum AMH in women. This study was aimed to evaluate the association between reproductive and lifestyle determinants of AMH among women of reproductive age. The hypothesis was set that selected reproductive and lifestyle factors will be significantly associated with serum AMH.

Comparable associations also have been reported in earlier studies for age at menarche. Bragg *et al*. reported that women with early age at menarche had significantly higher AMH as young adults, whereas the results from our study do not show a statistically significant association between age at menarche and low AMH in later life.[2] Women who experienced menarche at a younger age were not more likely to suffer from abnormal AMH. The results differ to previous studies done by Perhar *et al*. and Weghofer *et al*., have reported a Diminished Functional Ovarian Reserve associated with early menarche.[3,4]

AMH is predominantly known for its roles in the ovarian function. Few studies have reported a positive relationship between cycle length in days and serum AMH. Hu *et al*. have investigated the association of menstrual cycle length with AMH and ovarian response and reported that both short cycles and oligo/amenorrhea were associated with an increased risk of low response.[5] In another study, reported by Konishi *et al*., women with severe dysmenorrhoea had significantly lower serum AMH levels.[6] The results were similar to the study done by Dólleman *et al*. who reported that cycle irregularity was associated with a significantly less serum AMH level. It is likely that AMH levels may regulate the frequency of GnRH/LH pulse and affect the process of ovulation and menstrual cycle through the hypothalamus-pituitary-ovary axis. In our study we have found that there was significant association between shortened cycles/amenorrhea and low AMH.[7]

Methods

Study design and data source

It is a multicentric cross sectional study using data extracted from the patient records of seven private fertility practices located in North India. These included Delhi, Agra and Meerut. 602 women who were attending these clinics for fertility treatment were requested to fill the questionnaire [Annexure 1]. Ethical clearance for the study was obtained from Indian Fertility Society, Ethical Clearance Board, New Delhi, Ethical Clearance Board, with ethical clearance number F.1/IEC/IFS/2020/No. 68. Our study adhered to the principles of Helsinki Declaration (2013). Anonymized data were extracted to create the analytic dataset for research or educational purposes and no formal sample size calculation was done. All precautions were taken while using the patient data for current research and confidentiality was maintained throughout. Patient identifiable data (name, contact, address, etc.) will not be presented in journal or any public forum.

Outcomes and variable specification

Our outcome variable was level of AMH measured in the past 3 months, and was assessed as normal or low. AMH was tested by electrochemiluminescence immunoassay by using cobase immunoassay analyzers.

We assessed the correlation of a large number of independent variables with the outcome based on the literature.[8-10] Diet was coded as vegetarian or non-vegetarian/both, and snacking between meals was reported as once only, more than once, or does not snack between meals. Infertility duration was categorized as <2 years, between 2 and 5 years or >5 years. Lifestyle factors (hours spent on laptop/day, on phone/day and sitting/day) were reported as <2 h, 2–5 h and 5 h per day. Menstrual cycle was categorized as normal, subnormal or short/amenorrhoeic. Other potential clinical correlates included a male factor (normal, subnormal, or OATS/azoospermia), other known factors (polycystic ovary syndrome [PCOS], none, other), family history of premature menopause (yes, no), sleep quality (good, disturbed) and tubal status (open, one blocked or two blocked).

Data analyses

The unit of analysis was the individual woman. Summary statistics, including means and standard deviations were calculated to examine sample characteristics. Respondent's age, body mass index (BMI) and age at menarche were measured as continuous variables. Differences between the two groups (of the outcome variable) were characterized using Chi square and *t*-tests. In order to construct a parsimonious analytic model, we used a variable selection procedure, where all independent variables associated with the outcome at $P \leq 0.2$ were entered into the logistic regression. As the data were clustered at the physician level, we used a clustering correction to adjust for this. All analyses were conducted using STATA 17 (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, Texas, USA: StataCorp LLC).

Results

Table 1 depicts the sample characteristics. Among the study sample of 602 women, the mean age was

Table 1: Sample description (*n*=602)

	n (%)
Age	32 (4.7)[a]
Age at menarche	13 (1.4)[a]
BMI	25.4 (4.1)[a]
Alcohol	
Does not drink alcohol	414 (79)
Drinks alcohol	109 (21)
Smoking	
Does not smoke	568 (94)
Smokes/chews tobacco	36 (6)
AMH (in last 3 months)	
Normal	332 (71)
Low	132 (29)
Diet	
Vegetarian	283 (47)
Non-vegetarian/both	317 (53)
Snacking between meals	
Once only	215 (37)
More than once	264 (45)
Does not snack between meals	108 (18)
Infertility duration (years)	
<2	109 (21)
2-5	217 (42)
>5	189 (37)
Hours on laptop/day	
<2	196 (45)
2-5	120 (27)
>5	124 (28)
Hours on phone/day	
<2	174 (31)
2-5	270 (47)
>5	125 (22)
Hours sitting/day	
<2	49 (9)
2-5	281 (49)
>5	239 (42)
Menstrual cycle	
Regular	411 (69)
Irregular	124 (21)
Short/amenorrhoea	62 (10)
Male factor	
Normal	374 (71)
Subnormal	80 (15)
OATS/azoospermia	72 (14)
Other known factors	
PCOS	114 (19)
Other	248 (41)
None	246 (40)
Family history of premature menopause	
Yes	18 (3)
No	560 (97)
Sleep quality	
Good	428 (74)
Disturbed	154 (26)
Tubal status	
Open	226 (63)
One blocked	68 (19)
Both blocked	62 (17)

[a]Indicates mean (SD). BMI=Body mass index, AMH=Anti mullerian hormone, OATS=Oligoasthenoteratozoospermia, PCOS=Polycystic ovary syndrome, SD=Standard deviation

32 years with the mean age of menarche being 13 years; the mean BMI was 25.4. Nearly three-quarters (71%) of respondents had normal AMH levels. A large majority (94%) were non-smokers, and the sample was approximately evenly split between vegetarians and non-vegetarians; nearly half (45%) reported snacking more than once between meals. A majority reported spending 2–5 h on the phone or sitting per day and spent <2 h on the laptop per day. Sleep quality was good in 74% of respondents. In terms of clinical parameters, more than two thirds (66%) had a normal menstrual cycle, and PCOS was reported in 14%. A majority (97%) had no family history of premature menopause and 63% had patent tubal status. Among those reporting infertility, 42% reported a 2–5 years duration.

Table 2 shows the bivariate associations between the outcome and the independent variables. Nine variables were associated at $P \leq 0.2$ (age, age at menarche, hours sitting/day, menstrual cycle, male factor, other known factors, family history of premature menopause, and sleep quality) and these were entered into a logistic regression model.

The regression model [Table 3] showed that the odds of reporting low AMH were associated with increasing age (odds ratio [OR] = 1.21, 95% confidence interval [CI] 1.16–1.25). Compared to women reporting regular menstrual cycles, women with short cycles or with amenorrhea had greater odds (OR = 4.03, 95% CI 1.52–10.66) of having low AMH. Women whose male partners had OATS or azoospermia had 67% lower odds of having low AMH (OR = 0.33, 95% CI 0.17–0.65) compared to partners who had normal male factor. Women who had PCOS had very low odds (OR = 0.05, 95% CI 0.01–0.19) of low AMH; having premature menopause was associated with significantly greater odds (OR = 23.64, 95% CI 8.95–62.41) of having low AMH.

Discussion

Anti-mullerian hormone and reproductive factors

Our study reveals a strong correlation between familial history of menopause and low AMH level in women

Table 2: Characteristics of women with low anti mullerian hormone versus normal anti mullerian hormone in past 3 months

	Normal AMH, *n* (%)	Low AMH	*P*
Age	31.7[a]	35.2[a]	0.000
Age at menarche	13.2[a]	12.9[a]	0.07
BMI	25.4[a]	25.5[a]	0.81
Alcohol			
Does not drink alcohol	224 (76)	97 (79)	0.50
Drinks alcohol	69 (24)	25 (21)	
Smoking			
Does not smoke	302 (94)	121 (92)	0.58
Smokes/chews tobacco	20 (6)	10 (8)	
Diet			
Vegetarian	155 (48)	64 (49)	0.89
Non-vegetarian/both	167 (52)	67 (51)	
Snacking between meals			
Once only	104 (33)	36 (28)	0.49
More than once	163 (52)	71 (55)	
Does not snack between meals	47 (15)	23 (17)	
Infertility duration (years)			
<2	58 (20)	21 (18)	0.91
2-5	132 (44)	50 (43)	
>5	107 (36)	44 (39)	
Hours on laptop/day			
<2	114 (45)	48 (46)	0.99
2-5	75 (30)	31 (30)	
>5	62 (25)	25 (24)	
Hours on phone/day			
<2	106 (34)	29 (23)	0.09
2-5	149 (47)	70 (56)	
>5	61 (19)	27 (24)	
Hours sitting/day			
<2	31 (10)	11 (9)	0.11
2-5	172 (54)	58 (45)	
>5	113 (36)	60 (46)	
Menstrual cycle			
Regular	229 (72)	82 (63)	0.000
Irregular	70 (22)	14 (11)	
Short/amenorrhoea	18 (6)	35 (27)	
Male factor			
Normal	205 (69)	91 (75)	0.10
Subnormal	43 (15)	20 (17)	
OATS/azoospermia	48 (16)	10 (8)	
Other known factors			
PCOS	77 (24)	2 (2)	0.00
Other	141 (44)	80 (61)	
None	104 (32)	50 (37)	
Family history of premature menopause			
Yes	3 (1)	12 (9)	0.000
No	317 (99)	120 (91)	
Sleep quality			
Good	228 (73)	84 (66)	0.15
Disturbed	84 (27)	43 (34)	
Tubal status			
Open	135 (63)	47 (61)	0.45
One blocked	38 (18)	18 (23)	
Both blocked	43 (19)	12 (16)	

[a]Indicates continuous variable. BMI=Body mass index, AMH=Anti mullerian hormone, OATS=Oligoasthenoteratozoospermia, PCOS=Polycystic ovary syndrome

Table 3: Logistic regression results of correlates of low anti mullerian hormone (*n*=346)

	OR (95% CI)
Age	**1.21 (1.16-1.25)**
Age at menarche	0.94 (0.74-1.19)
Hours on phone/day	
<2	1.00 (base)
2-5	0.97 (0.51-1.85)
>5	0.62 (0.23-1.72)
Hours sitting/day	
<2	1.00 (base)
2-5	0.79 (0.46-1.36)
>5	1.39 (0.75-2.59)
Menstrual cycle	
Regular	1.00 (base)
Irregular	1.39 (0.57-3.39)
Short/amenorrhoea	**4.03 (1.52-10.66)**
Male factor	
Normal	1.00 (base)
Subnormal	0.78 (0.47-1.30)
OATS/azoospermia	**0.33 (0.17-0.65)**
Family history of premature menopause	
Yes	**23.64 (8.95-62.41)**
No	1.00 (base)
Other known factors	
PCOS	**0.05 (0.01-0.19)**
Other	1.09 (0.31-3.85)
None	1.00 (base)
Sleep quality	
Good	1.00 (base)
Disturbed	0.90 (0.45-1.79)

Bold indicates $P<0.05$; model was adjusted for clustering at the physician level. OATS=Oligoasthenoteratozoospermia, PCOS=Polycystic ovary syndrome, CI=Confidence interval, OR=Odds ratio

of reproductive age. The results were similar with previous research done by Ruth *et al*. who investigated the genetic determinants of female reproductive lifespan associated with pre-menopausal AMH levels and reported that genetically predicted age at menopause was associated with lower AMH levels.[11] They identified a genome-wide significance in their analyses as rs16991615 in *MCM8*, a published menopause timing

variant, with the same allele associated with earlier menopause and lower AMH levels. Genome-wide analyses of menopause timing, a proxy measure for ovarian reserve, have identified 56 genetic variants and highlighted the importance of DNA damage response pathways during follicle formation *in utero* and for follicle maintenance during a woman's lifetime. Torgerson *et al*. and Cramer *et al*. also reported a strong correlation of a woman's age of onset of menopause with their mother's age of onset.[12,13]

Another interesting observation was the correlation of high AMH and male partners having oligoasthenoteratozoospermia and azoospermia. A possible explanation could be that couples with male factor usually seek treatment early. The female partner is usually young with good AMH levels. We found few papers which studied the correlation of female AMH levels with male factor infertility which revealed no correlation with same.[14,15]

Antimullerian hormone has been proposed as a diagnostic tool for polycystic ovary (PCO) patients over years. It has been vastly supported in literature that women with PCO have on an average higher AMH values as compared to their non PCO counterparts. Serum AMH levels has a sensitivity of 76.1% and specificity of 74.6% in predicting PCOS.[16] Dewailly *et al*. proposed the role of AMH in pathogenesis of polycystic ovaries. They found that AMH is 2–4 folds higher in PCO than their healthy counterpart.[17] We found a strong correlation of high AMH levels and PCOS in our study.

As far as duration of infertility is concerned some studies found a negative association between the serum AMH level and time to pregnancy in fertile women but large variation in fecundity within similar AMH concentrations precluded any establishment of cut-offs.[18] Though AMH and primordial follicles decrease with age after sexual maturation no correlation has been evident in literature between duration of infertility and low AMH.[19] Our present study did not find any correlation between AMH and duration of infertility.

Anti-mullerian hormone and lifestyle

Exercise and body mass index

The effect of overweight on granulosa cells, therefore, seems to be different from the one on theca cells, which is acting mainly through hyperinsulinism. It does not seem to act through a negative effect on the FNPO, as both variables were independently significant in our multivariate model. In agreement, Su *et al*., 2008 reported that, with equal FNPO, a significant negative correlation was observed between BMI and serum AMH.[20] The negative effect of BMI on serum AMH level might only be the consequence of its modified clearance, owing to a sequestration effect or different circulating molecular forms but these hypotheses have not been clarified so far. Conversely, the assumption of a defect in AMH production by the granulosa cells is supported by the fact that obese women present more anovulatory cycles than non-obese women.[21] This shows the negative effect of obesity on the functions of granulosa cells, but the mechanisms are still not fully understood. Obesity also affects the adipocytes' functions, resulting in changes in the serum levels of adipokines, and in particular a decrease in adiponectin, that may directly affect the ovarian functions.[22] Another adipokine, omentin-1, was also found more abundant in the sera of obese women, and this increase seemed to be inversely correlated to the decrease of serum AMH.[23] Finally, obesity is responsible for an increase in serum leptin levels.[22] In rodents, leptin supplementation was responsible for decreased follicular growth and decreased ovulation rate.[24] *In vitro* cultures of luteinized granulosa cells from infertile women without PCOS, leptin suppressed AMH expression.[25] Although these results need to be confirmed, they suggest that obesity affects serum AMH levels through an exaggerated secretion of products from the adipose tissue, which is more important in obese women with PCOS than in the general obese population.

There have been many studies that have found a correlation between Obesity and Low AMH. Su *et al*. observed that while antral follicle count did not differ by body size, AMH was lower in obese compared to normal weight late reproductive age women.[20] These data suggest that lower AMH levels in obese, late reproductive age women result from physiologic processes other than decreased ovarian reserve. Amsiejiene *et al*. studied 1134 treatment cycles found that women with lower BMI conceived more easily than otherwise.[26] Bernardi *et al*. tried to find the relationship between obesity and AMH in reproductive aged African American women. They found that women with BMI >25 kg/m^2 had AMH concentrations 23.7% lower than those with a lower BMI.[27] Moy *et al*. conversely found in 350 women that elevated BMI correlates negatively with AMH in Caucasian women but not in African-American, Hispanic, or Asian women.[28] Yet another study found that there was a trend towards an association with lower AMH levels for underweight (BMI <18.5Kg/m^2) and obese (BMI >kg/m^2).[29]

Diet

Modifiable exposure such as diet may affect reproductive function in women yet few studies have examined association between dietary factors and markers of ovarian reserve.

In a sister study cohort on 296 women in late premenopausal (35–45 years) AMH concentrations were positively correlated with total carbohydrate intake and inversely related with total fat intake. In analysis of dietary fat subtypes, modest inverse association with AMH were observed for intake both Monounsaturated fatty acids (MUFAs) and Polyunsaturated fatty acids (PUFAs). Other dietary factors, such as ω^{-3} and ω^{-6} fatty acids, protein intake and glycemic index were not strongly related to AMH concentration.[30] High dietary fat intake has been speculated to compromise the ovarian reserves by its potential direct effect on ovarian morphology and function. This association has been potentially mediated by increased inflammation as reported by Skaznik-Wikiel *et al*.[31] Others have also found that a high fat diet may increase follicular atresia.[32-34] In yet another study dietary intake of dairy foods such as milk, fermented dairy, dairy carbohydrate, and dairy fat and dairy proteins may delay the age of menopause. They also found a similar association in women consuming vegetarian food.[35] Whereas in a recent study fast foods and saturated fats were significantly associated with lower AMH and modifying their intake may be an important strategy for increasing the ovarian reserves.[36]

A prospective study following 259 women for 2 menstrual cycle found no significant association between dietary factor (micronutrients, sucrose, starch, total sugar and fibre) consumed and the AMH levels.[37] Thus, some studies suggest that maintaining a diet rich in fruits and vegetables, high quality carbohydrates both plants based or seafood sources of proteins and fat by their anti-inflammatory effect may help in decreasing the decline in AMH levels in women of reproductive age group. However, we did not find any significant correlation between vegetarian or non-vegetarian diet as well as in between snacking on AMH levels.

However, there have been other studies which have reported no correlation between low AMH levels and Obesity. Sahmay *et al*. observed 250 premenopausal participants to evaluate correlation between AMH and Obesity. They did not find any correlation between low AMH and obesity.[38] Pereira *et al*. reviewed the data of 995 women who were undergoing fertility work up and did not find any association between Obesity and AMH levels.[39]

Polycystic ovarian syndrome, BMI, exercise and AMH levels may be inter connected. Dewailly *et al*. studied 691 with PCOS aged <35 years to study the effect between BMI and AMH. They found that metabolic status had no effect on AMH in normal women; a significant, albeit weak, negative correlation was found between serum and BMI in women with PCOS. They concluded that it is unnecessary to establish different serum AMH standards in obese and non-obese women in clinical practice.[17] Luo *et al*. conducted a study where they wanted to find a correlation between body fat percentage (BFP) and BMI. They argued that BMI may not be an accurate estimate of metabolic indices as it includes both fat and muscle. AMH levels were negatively associated with BFP in the PCOS group However, BFP was not associated with AMH in the non-PCOS group.[40] Thomson *et al*. found that in overweight and obese women with PCOS and reproductive dysfunction, a 20-week weight loss intervention on a calorie restricted diet resulted in improvements in reproductive function but no change in AMH levels.[41] Moran *et al*. reported contrarily the same. They examined the effect of exercise on AMH and menstrual and ovulatory function in women with and without PCOS. Overweight women with and without PCOS of comparable age, weight and BMI undertook a 12-week intensified endurance exercise training program (1 h 3 times/week) with no structured energy restriction Exercise decreased BMI, total and android fat mass and improved insulin sensitivity for all women. women without PCOS had no change in AMH while women with PCOS had a decrease in AMH.[42]

Though sedentary life usually has a negative effect on reproductive function and moderate levels of activity is beneficial, excessive physical exercise has been associated with poor menstrual and reproductive outcomes. Many have tried to find a correlation between AMH and excessive physical activity. AMH levels were found to be lower in women athletes when compared with sedentary women. Longer periods of sports and more frequent weekly trainings affect AMH level negatively.[43]

The studies therefore indicate that moderate activity may perhaps be beneficial to overall reproductive performance and AMH. This does not translate when severe degrees of physical activity are taken into account. On the contrary, that seems to have an opposite effect. Effect of physical activity and BMI seems to have different effect in different age groups, ethnicity and metabolic conditions, i.e., PCOS and non PCOS.

These studies however have some shortcomings. Many studies investigating the relationship between serum AMH and BMI are biased by the small numbers of patients, different ethnic groups, poorly defined phenotypes, various assays for serum AMH or an inappropriate statistical approach, neglecting, in particular, the confounding effect of age. Oldfield *et al*. evaluated 13 studies reporting AMH and BMI. They commented that these studies remain limited by

small sample sizes, cross-sectional designs, and lack of representation across the entire adiposity spectrum. Ultimately, the degree to which obesity may negatively impact AMH levels, and possibly ovarian reserve, in otherwise healthy women with regular menstrual cycles should be deemed uncertain at this time. This conclusion is prudent considering that the biological basis for an impact of obesity on AMH production is unknown.[44]

In our study we did not find any correlation between activity, BMI and AMH. Since age and BMI are continuous variables, we did not subcategorize them further, albeit we lose information. Thus, larger study sample in future may help us find more information regarding AMH and categories of age and PCOS and non PCOS with regards to BMI and activity. Another limitation is that the level of activity was self-reported and was not objectivized.

Smoking

As far as effect of smoking on AMH is concerned, the present study could not find any correlation between low AMH and smoking. We could find both contradictory and supporting studies in literature. A cross-sectional study by Dólleman *et al*. suggested that current smoking is associated with lower AMH irrespective of smoking dose. At the same time however they found that effect of smoking on AMH is reversible.[7] Barriere *et al*. in a retrospective study compared serum AMH and ovarian response in non-smoking and active smoking women. AMH was found to be significantly lower in smokers than non-smokers.[45]

On the contrary, a cross-sectional study by Oladipupo, suggested no significant correlation of smoking with ovarian reserve. They observed that non-significant results from previous studies in literature which found a correlation between smoking and low ovarian reserves are based on self-reports and not accounting for duration and quantity of smoking. They also proposed that results from previous studies are difficult to reconcile because of differences in definition of exposure status and adjustment factors across studies.[46]

Many other retrospective studies in literature failed to find any correlation between low ovarian reserve and smoking.[47-49] However, larger homogenous data is needed to reach to a conclusion.

Sleep

To our knowledge, AMH has not been extensively studied in the context of sleep disorders. The majority of evidence for the association between sleep disturbance and diminished ovarian reserves has been within the area of shift work. Bisanti *et al*. conducted a multi centric study in Europe to study the effect of shift work on reproductive outcomes. They found a correlation between shift work and delayed fecundity but not menstrual cycle pattern. They did not study any ovarian reserve marker.[50] Labyak *et al*. found that in nurses doing shift work those having more sleep disturbances had more menstrual dysfunction too.[51] Mahoney commented that in women, perturbations in daily rhythms, as occurs in shift work, jet lag, and sleep deprivation is associated with an increased menstrual cycle irregularity, increased risk of miscarriage and difficulty in conceiving. They however, did not study its direct correlation to ovarian reserve markers.[52] The mechanisms underlying this association are likely to be multifactorial and complex. Interestingly, woman with diminished ovarian reserves were found to be 30 times more likely to have disturbed sleep, while controlling for race, BMI and vasomotor symptoms.[53] In addition to genetics, circadian disruption may impact reproductive outcome through interference with hypothalamic pituitary adrenal axis, insulin resistance, oxidative stress and systemic inflammation.

Lyttle *et al*. found that sleep disturbance did correlate with depression scores for infertile patients.[54] However, no correlations were found between sleep disturbance and AMH, peak estradiol, total medication received, number of oocytes retrieved, and number of oocytes frozen. AMH hormone shows a subtle but not clinically significant circadian rhythm pattern, whereas increased luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin levels were observed in shift workers during their daytime sleep compared to night time sleep and was unchanged on night's off.[55,56] Merklinger-Gruchala *et al*., 2008, theorized that increased light exposure during sleep suppresses the melatonin levels which in turn could increase estrogen levels which is detrimental to reproductive health. Melatonin could also enhance the effect of FSH and LH during the follicular phase but not the AMH levels. Hence the relationship between sleep and AMH levels is not well established.[57] Similarly, in our study we found no correlation between the AMH levels and sleep disturbances.

Cell phones

Mobile phones are low-powered radiofrequency (RF) transmitters, operating at frequencies between 450 and 2700 MHz with peak powers in the range of 0.1–2 watts. It has a universal presence. Arjmandi *et al*. commented that recent studies indicated that exposure of human skin cells to light emitted from electronic devices, even for exposures as short as 1 h, may cause reactive oxygen species generation, apoptosis, and necrosis. It could be

argued that such effects may be transmitted to other body cells, like the ovaries.[58]

Kesari *et al*. conducted a review focusing on radiation deriving from cell phones, laptops, Wi-Fi and microwave ovens and exploring the effect of exposure to RF radiations on the male fertility pattern. They concluded that RF-electromagnetic fields (RF-EMF) have deleterious effects on sperm parameters (like sperm count, morphology, motility), affects the role of kinases in cellular metabolism and the endocrine system, and produces genotoxicity, genomic instability and oxidative stress.[59]

Most of the studies on harmful effects on cell phones have been done on animal models. The few human studies have explored the oncological side effects of cell phone on women.[60] We could not find any study on the effects of cell phone on female ovarian reserve.

One factor that causes impaired oocyte maturation is wireless fidelity (Wi-Fi) radiation which has a RF field of 2.45 GHz. Today all smart phones use Wi-Fi. Nurbayatin *et al*. suggested that the radiation from Wi-Fi may increase the activity of free radical cells through the fenton reaction pathways that cause infertility because of the disrupted oocyte development.[61] On the contrary Saygin *et al*. found that long exposure EMR (2.45 GHz Wifi) increased the AMH levels. They proposed that this could be due to hyperstimulation of granulosa cells due to the long exposure. The addition of Vitamin C had a protective effect.[62]

Goldhaber *et al*. reported that pregnant women who were exposed to video display terminals more than 20 h/week in their first trimester seemed to be having a higher chance of miscarriage. They suggested larger robust trials.[63] We could find no follow up studies on this matter.

Calis *et al*. conducted research in which pregnant albino rats were divided into two groups, Group 1 received no RF radiation and Group 2 received it. At 42 days, bilateral oophorectomy was performed on all female offsprings. Follicle count was measured and immunohistochemical staining was done. Group 2 had significantly lower mean number of primordial, secondary follicles, and a higher atresia score.[64]

Concerning their effect on the female genital system, different studies on mice have shown that EMFs are able to prevent the formation of antral follicle, to inhibit ovulation and to reduce the total number of corpora lutea and given their capacity to extend the lifetime of free radicals they favor cell apoptosis by increasing the oxidative stress resulting in DNA damage.[65] Combined with the fact that an elevated number of macrophages has also been found in rats' growing follicles and corpora lutea, some authors postulate that EMFs exposure could accelerate apoptosis in ovarian cortical tissue responsible for oocytes degeneration.[66]

Bakacak *et al*. studied the direct effect of EMF exposure close to the ovaries. The study group consisted of rats exposed to an EMF in the abdominal region for 15 min/d for 15 days. After the treatment period of 15 days, the ovaries of the rats were extracted, and sections of ovarian tissue were taken for histological evaluation. study group had significantly fewer follicles.[67]

Non ionizing radiation from cell phones are here to stay. Till robust studies confirm their harmful effects, if any, certain modifications can be adapted to minimize the damage. Changing the spectral output of LED-based smartphones' flashes can be introduced as an effective method for reducing the adverse health effects associated with exposure to blue light.[58] Ovarian reserve of offspring diminished with RF exposure during pregnancy. Omega-3 supplementation during pregnancy may reduce the potential premature ovarian failure in female offsprings.[64] Vitamim C appears to have a protective effect.

A further criticism emerging from the literature is the difficulty to understand whether EMF-induced fertility abnormalities are caused by direct gonadal damage or by disruption of the hypothalamic-pituitary-gonadal axis.[68] Indeed, most studies rely on total body exposure of small animals within cages. In this regard, the application of EMF-emitting devices to abdominal regions[67] or the use of large animal models may help to elucidate the mechanisms underlying Extremely low frequency electromagnetic field (ELF-EMF) and RF biological effects.

Bernabò *et al*. reviewed 104 research articles on the effect of NI radiation on fertility and found huge heterogeneity in the studies. They concluded that important efforts must be undertaken to adopt more standardized models and to improve the research quality and the information exchange within the scientific community, with the aim of improving the reliability and usefulness of the results of research regarding the effect of NI-EMFs on fertility.[69]

The limitations of our study were that we did not stratify regarding the kind of cell phones, whether it was being used hands off, the total time the cell phone was close to the body, night time presence of cell phones and more objective measurement of the duration (we relied on self-reported time). This is however the first human

study to study the effect of cell phone use on AMH levels. Cell phones are universal and here to stay. We need to find out whether their use will lead to reduction in female fertility. We urgently need more studies to confirm or refute our findings so that we can counsel our patients well.

Laptop use

Laptops emit both heat and electromagnetic radiations. Specific absorption ratio (SAR) is a standard unit or rate at which RF-EMF energy is imparted to an element or mass to measure the penetration of energy within human tissues.[59] The amount of SAR absorbed by human tissue depends on many factors such as the frequency, intensity, polarization and duration of exposure and most importantly the position of devices while used[70] A higher radiation absorption rate could be observed while talking on phone, keeping phone near head or in pants pocket, using laptop computer on lap connected with Wi-Fi and frequently use of microwave ovens. The use of laptops and Fertility has been mainly associated and studies in male infertility. From currently available studies it is clear that RF-EMF have deleterious effects on sperm parameters (like sperm count, morphology, motility), affects the role of kinases in cellular metabolism and the endocrine system, and produces genotoxicity, genomic instability and oxidative stress.[59]

Laptop overuse is however is known to be associated with erythema ab igne. Continuous exposure to infrared radiation initially causes transient erythema progressing to pigmentation and keratosis.[71] Bellieni *et al*. in 2012 warned that we need to be observing the effects carefully.[72] Though no mention of female fertility and AMH levels were made, concern regarding future cancer risks and exposure to fetus was expressed. In the LTCs analyzed, EMF values (range 1.8–6 μT) are within International Commission on Non-Ionizing Radiation Protection guidelines, but are considerably higher than the values recommended by recent guidelines for computer monitors magnetic field emissions, MPR II (Swedish Board for Technical Accreditation) and TCO (Swedish Confederation of Professional Employees), Laptops cannot function without WiFi. Repeated Wi-Fi studies show that Wi-Fi causes oxidative stress, sperm/testicular damage, neuropsychiatric effects including EEG changes, apoptosis, cellular DNA damage, endocrine changes, and calcium overload. Most of the studies reviewed were conducted on rodents.[73]

Santini *et al*.(2018) in their review article on the effect of RF-EMF on female fertility, did find a correlation between depletion of antral follicles and its exposure in rodents. No human studies were reported. They concluded that EMF related bio medical research is poorly investigated. There is lack of uniformity in experimental design including using different models and variable sources exposure sources and protocols. There is also lack of control of temperature during EMF exposure.[68]

However, role of Laptop use in women has not been widely studied. As the world is emerging towards more and more online use, the time spent on laptop by women is rapidly increasing. It is therefore important to know whether the over use of laptop leads to reduction in women fertility. To the best of our knowledge this is the first study which is trying to see any association between laptop use and its effect on ovarian reserve. If any such correlation is found, women should then, limit exposure or give gaps when excess use is anticipated. However, if no such correlation is found, women may not unnecessarily be stressed about their device usage habits.

We retrospectively studied correlation of AMH levels with laptop use in 440 women. We divided the hours of exposure into <2, 2–5 and >5 h. After doing robust statistical analysis we did not find any correlation between duration of Laptop use and AMH levels. Our finding could well be because the current electromagnetic radiation released by the laptops may be below the critical zone which leads to reduced frequency induced or free radical induced damage to ovarian cells. Further standardized studies need to be done to confirm this finding as this will have major implications in the coming generations for the coming generation. The limitations of our study were that this was a retrospective study, the distance of the laptop from the pelvic area was not measured and other sources of RF-EMF (cell phones) was not accounted for. However, this is the first study of its kind and many more need to be done to assure women and the future generation the safety of Laptops and its effect on ovarian reserve.

We acknowledge the help of Dr Priyanka Verma for data collection and filling of questionnaire. We acknowledge the help of Indian fertility society, New Delhi for ethical clearance.

Limitations

The limitations of our study were that this was a retrospective study and many of the parameters were self-reported. Specifics like distance of the laptop from the pelvic area was not measured and other sources of RF-EMF (cell phones) was not accounted for. We also did not stratify regarding the kind of cell phones, whether it was being used hands off, the total time the cell phone was close to the body, night time presence of cell phones and more objective measurement of the duration (we relied on self-reported time). Quality of

sleep was also a subjective assessment of the individual person.

Conclusion

To the best of our study, ours is one of largest study on the effect of reproductive and lifestyle factors on AMH levels. We found a direct association of low AMH with increasing age, short cycles, amenorrhea and women with family history of premature menopause. We found a direct correlation of high AMH and women with PCOS and those whose partners had OATS or azoospermia. There was no correlation with smoking, sleep, diet, BMI, cell phone or laptop use in our study. Environmental factors may affect ovarian reserve and we felt there was a dearth of human studies in this area. To the best of our knowledge this is the first human study on the effect of AMH on laptop and cell phone use. We urgently need more studies to confirm or refute our findings so that we can counsel our patients well.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Data availability statement

Data can be shared as per requirement.

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Annexure

Annexure 1

Proforma

Date:

Name:	Age:
UHID:	Telephone No:
E-Mail id:	Address:

Age at Menarche

Infertility Duration:	>2 years, 2-5 years, >5 years
Menstrual Cycle:	Regular/Irregular/Short/Amenorrhea
Male Factor:	Normal/Subnormal/OATS/Azoospermia
Tubal Status:	Not Known/Open/One blocked/Both blocked
Other Known Factors:	PCOS/Fibroid/Endometriosis/Others
Height:	BMI:
Weight:	
Smoking:	Yes/No
If yes, No. of Cigarettes/Bidis per day:	<2, 2-5, >5
Any other form of Tabacco Consumption:	Pan/Gutka
Passive Smoking at home:	Yes/No
Alcohol:	Yes/No
If Yes:	Daily/Weekly/Occasionally
Sleep:	Good/Disturbed
Diet:	Veg/Non-Veg
Home Cooked/Outside	
Snacking between meals:	No/Once/More than once

Any medications apart from fertility medications

Work:	Please Specify
Hours on mobile phone per day:	<2/2-5/>5
Hours of sitting per day:	<2/2-5/>5
Hours on laptop per day:	<2/2-5/>5
AMH last 3 months:	<1/1-2/2-4/>4
H/O Premature menopause in family:	Yes/No