Original Article

Factors Associated with Poor Ovarian Reserve in Young Infertile Women: A Hospital-based Cohort Study

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Abstract

Background: In practice, we encounter many young infertile women with poor ovarian reserve though ovarian reserve starts to decline after 35 years of age. One of the established risk factors for poor ovarian reserve in young women is endometriosis. There are other conditions that are reported to be associated which require further research. **Aims:** We aimed to study the prevalence of poor ovarian reserve and to find out the associated factors in women who are <35 years of age. **Settings and Design:** This was a prospective observational cohort study conducted in a tertiary care setting. **Materials and Methods:** Women aged more than 21 years and <35 years without Polycystic Ovarian Syndrome (PCOS) or ovarian dysgenesis with normal male factor were included after ethical approval. The sample size was 166 and serum anti-Mullerian hormone (AMH) was estimated by immunoenzymatic assay and expressed in ng/ml. AMH ≤0.99 ng/ml was considered poor ovarian reserve. Apart from established risk factors, the proposed risk factors studied were age 31–35 years, presence of medical disorders, gynaecological pathology and history of repeated ovulation induction (OI). **Statistical Analysis Used:** Data were analysed by SPSS version 25. Chi-square test and Fisher's exact test were used to compare the variables between normal ovarian reserve and poor ovarian reserve. Risk estimation was done by logistic regression and was expressed in odds ratio (OR). **Results:** Poor ovarian reserve was diagnosed in 40% of this cohort, and 62% were between 31 and 35 years. After adjusting for age >30 years, women with endometrioma, hypothyroidism and prior history of ≥3 cycles of OI were found to be having poor ovarian reserve (OR was 5.7, 2.5 and 2.3, respectively). **Conclusion:** Poor ovarian reserve was present in 40% of young women, and significantly associated factors were hypothyroidism and history of repeated multiple OI. This could be a confounder for other underlying mechanisms driving early exhaustion of ovarian reserve in certain young women. Hence, along with established risk factors, these women should undergo AMH testing irrespective of age.

Keywords: *Age, anti-Mullerian hormone, antral follicle count, diabetes, hypothyroidism, obesity, poor ovarian reserve*

Introduction

Poor ovarian reserve is an important cause of infertility and it leads to a poor ovarian response (POR) and it negatively correlates with pregnancy rates. Poor ovarian reserve describes women of reproductive age whose response to ovarian stimulation or fecundity is reduced compared with women of comparable age.[1] It is essential to know the reserve to predict the response

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prior to subjecting women to controlled ovarian stimulation.

It is an established fact that ovarian reserve decreases as the age increases. Anti-Mullerian hormone (AMH) is a test of choice for ovarian reserve, and it is recommended for women of 35 years of age or more.[2] In practice, many women <35 years of age are found to have low ovarian reserve. Identifying the risk factors in young women will help us to treat them before initiation of ovarian stimulation protocol to achieve pregnancy. Hence, this study was conducted to explore risk factors associated with poor ovarian reserve in young infertile women.

Materials and Methods

This was a prospective observational cohort study conducted between April 2019 and April 2021 in a tertiary care institute catering to infertility services. The study included infertile women aged more than 21 <35 years with normal male factor. Women with PCOS and ovarian dysgenesis were excluded. Women with PCOS were excluded as they have high or normal AMH.

Sampling population

Women attending an infertility and assisted reproductive technique (ART) clinic of the Department of Obstetrics and Gynecology, WCH (Women and Children Hospital), JIPMER, Puducherry, from April 2019 to April 2021 were enrolled in this study.

Sample size calculation

The sample size was calculated with 95% confidence level, power of 80% and absolute precision of 5% using OpenEpi software version 3.0 (https://www.openepi.com). Based on 10% prevalence of poor ovarian reserve amongst infertile women, the sample size was 139. With 20% dropout rate, the total sample size was 166.

After ethical approval (No. JIP/IEC/2019/199), the subjects were explained regarding the study and informed consent was taken. The study adhered to the principles of the Helsinki Declaration (2013). A detailed past, present and treatment history was taken and clinical and ultrasonographic examination was done and recorded on the pro forma. Five millilitres of venous blood was collected on day 2 or 3 of menstrual cycle and was sent for hormone estimation (follicle-stimulating hormone [FSH], luteinising hormone [LH], prolactin, testosterone, AMH, estradiol and thyroid-stimulating hormone [TSH]) to the Department of Biochemistry. Access chemiluminescence procedure was used for hormone estimation. The principle of access AMH assay is a simultaneous one-step immunoenzymatic ('sandwich') assay, and it is expressed in ng/ml. A 75-g glucose tolerance test was done as per the patients' convenience after 8 h of fasting. Those with unexplained infertility were subjected to endometrial biopsy, and the latter was sent for histopathological examination and GeneXpert to detect endometrial tuberculosis (TB).

We classified the causes of poor ovarian reserve as established and proposed risk factors. Amongst the established risk factors, a history of ovarian surgery like cystectomy, genital TB, chemotherapy, radiotherapy and the presence of endometrioma were considered. We planned to study the association of the following proposed risk factors, namely obesity (body mass index [BMI] >29.9 kg/m^2), hypothyroidism (TSH >4 mIU/ml), history of repeated cycles (≥3) of ovulation induction (OI), diabetes mellitus (DM) (fasting plasma glucose >126 mg/dl or 2-h plasma glucose >200 mg/dl), impaired glucose tolerance (IGT) (2-h plasma glucose is 140–200 mg/dl), myomectomy and salpingectomy.

The outcome of the study was poor ovarian reserve as determined by serum AMH. We diagnosed poor ovarian reserve when the AMH was below 1 ng/ml. Classification of poor ovarian reserve into low and severely low was adopted from the study by Umarsingh *et al*.[3]

Statistical analysis

The data were analysed using SPSS version 25 (IBM, Corp, Chicago, USA) and STATA (STATA Corp LLC, TEXAS, USA).

The continuous variables (age, AMH, FSH, LH, estradiol, testosterone, prolactin and TSH) were summarised as mean and standard deviation. Unpaired *t*-test was used for continuous variables, and $P < 0.05$ was considered statistically significant.

Categorical variables (BMI, presence of endometrioma, history of chronic pelvic inflammatory disease, hypothyroidism, diabetes, history of menstrual abnormalities, history of cystectomy or ovariectomy, history of myomectomy or salpingectomy, chemotherapy and history of OI) were summarised as proportions. Chi-square test and Fisher's exact test were used to compare the variables between normal ovarian reserve and poor ovarian reserve. Logistic regression analysis was performed to find the association between risk factors and poor ovarian reserve.

Results

Women with poor ovarian reserve constituted 40% (66/166). The majority (55%) of women came from upper-middle-class families, 26% belonged to upper-lower-class families, 10% belonged to upper-class

families and 9% belonged to lower-middle-class families. In both the groups of ovarian reserve, majority of women belonged to upper-middle-class families.

Clinical profile is shown in Table 1. Significantly more number of women in the age group of 31–35 years had poor ovarian reserve. BMI was normal in majority of women in both the groups, but 33.3% of women with poor ovarian reserve had overweight when compared to 23% with normal ovarian reserve. Menstrual cycles were regular in majority of both the groups, but irregular cycles were more common in women with poor ovarian reserve. Most of the women in this cohort were primary infertility and all women with poor ovarian reserve gave history of prior OI and 68% with normal ovarian reserve underwent OI. There is a significant difference amongst those who underwent ovarian stimulation of more than three cycles when compared to those who underwent <3 cycles.

Table 2 shows hormonal profile. FSH >10 IU/l and TSH >4 mIU/l were recorded significantly high in women with poor reserve compared to those with normal reserve. The associated medical, gynaecological conditions are shown in Table 3. The association of medical disorders was high in women with poor ovarian reserve (70%) when compared to normal ovarian reserve (53%), and the most common medical disorders were hypothyroidism, IGT and Type II DM. Gynaecological disorders were diagnosed in 86% of women with poor ovarian reserve and 45% of women with normal ovarian reserve. The most common disorders associated were endometriosis and fibroid uterus. Significantly more women with hypothyroidism and those with endometriosis had poor ovarian reserve.

Tables 4a and b represent the logistic regression analysis (univariate and multivariate analysis, respectively). It was observed that in women with endometrioma, the odds of developing poor ovarian reserve were 5.78 times higher than in women without endometrioma with a 95% confidence interval (CI) (2.75–12.15) and $P < 0.001$. When multivariate analysis was performed using logistic regression, the following observations were made:

Women with endometrioma had higher odds of having poor ovarian reserve after adjusting for age >30 years, prior OI history of ≥3 cycles and hypothyroidism (odds ratio [OR]: 5.78, 95% CI: 2.75–12.15). Women with prior history of OI ≥3 cycles had higher odds of developing poor ovarian reserve after adjusting for age >30 years, endometrioma and hypothyroidism (OR: 2.5, 95% CI: 1.29–4.85). Women with hypothyroidism

Table 1: Clinical profile and ovarian reserve

Parameter studied	Total (*n*=166), *n* (%)	Poor ovarian reserve (*n*=66), *n* (%)	Normal ovarian reserve (*n*=100), *n* (%)	*P*
Age (years)				
21–25	20 (12.04)	3 (4.5)	17 (17)	0.024*
26–30	59 (35.5)	22 (33.3)	37 (37)	
31–35	87 (52.4)	41 (62.1)	46 (46)	
BMI (m^2/kg)				
<18.5	5 (3)	2 (3)	3 (3)	0.737*
18.5–24.9	109 (65.6)	41 (62.1)	68 (58)	
25–29.9	43 (25.9)	20 (33.3)	23 (23)	
≥30 (obese)	9 (5.42)	6 (9)	3 (3)	
Menstrual cycles				
Regular	149 (89.7)	54 (81.8)	95 (95)	0.008
Irregular	17 (10.2)	12 (18.2)	5 (5)	
Type of infertility				
Primary	130 (78.3)	54 (81.8)	76 (76)	0.373†
Secondary	36 (21.6)	12 (18.2)	24 (24)	
History of OI	134 (80.7)	66 (100)	68 (68)	
OI (oral ovulogens) only (cycles)				
≤3	15/134 (11.9)	5 (7.6)	10/68 (14.7)	0.145
>3	55 (41)	30 (45.5)	25 (36.8)	
Gonadotropins for OI (>3 cycles of OI + IUI 3 cycles)	47 (35)	21 (31.8)	26 (38.2)	
COH for ART	17 (12.6)	10 (15.5)	7 (10.3)	
Total cycles of OI ≥3	119 (88.8)	61 (92.4)	58 (85.3)	0.01
Total cycles of OI <3	15 (11.19)	5 (7.6)	10 (7.6)	

*Fisher's exact test, †Chi-square test. BMI=Body mass index, OI=Ovulation induction, IUI=Intrauterine insemination, ART=Assisted reproductive technique, COH=Controlled ovarian hyperstimulation

Table 2: Hormonal profile and ovarian reserve

Hormones	Total subjects (*n*=166), *n* (%)	Women with poor ovarian reserve (*n*=66), *n* (%)	Women with normal ovarian reserve (*n*=100), *n* (%)	*P*
FSH (IU/L)				
Normal (FSH <10)	112 (67.4)	29 (43.9)	83 (87)	<0.001†
High (≥10)	54 (32.5)	37 (56)	17 (17)	
Prolactin (ng/mL)				
Normal (0–25)	149 (89.7)	60 (90.9)	89 (89)	0.194†
High (>25)	16 (9.6)	6 (9)	11 (11)	
Testosterone (ng/mL)				
Normal (14–76)	149 (89.7)	60 (90.9)	89 (89)	0.777*
High (>76)	5 (3)	1	4	
Low (<14)	12 (7.2)	5	7	
TSH (mIU/mL)				
Normal (0.4–4)	134 (80.7)	48 (72.7)	86 (86)	0.034*
Hypothyroid (>4)	32 (19.2)	18 (27.3)	14 (14)	

*Fisher's exact test, †Chi-square test. TSH=Thyroid-stimulating hormone, FSH=Follicle-stimulating hormone

Table 3: Associated conditions and ovarian reserve

Associated conditions	Women with poor ovarian reserve (total *n*=66), *n* (%)	Women with normal ovarian reserve (total *n*=100), *n* (%)	*P*
Medical disorders			
Diabetes mellitus	9 (10.4)	9 (9)	0.580*
IGT	17 (25.7)	24 (24)	
Hypothyroidism	18 (27.3)	14 (14)	0.034†
TB	2	3	1.000*
Autoimmune disease	0	3	0.277*
Total medical disorders	46 (69.7)	53 (53)	
Gynaecological disorders			
Endometrioma	32 (48.5)	14 (14)	<0.001†
Chronic PID	-	9 (9)	0.347†
Fibroid uterus	11 (16.7)	12 (12)	0.394†
Adenomyosis	1	4	0.649*
Asherman's syndrome	0	1	
Uterine anomalies	4	5	0.742*
Total gynaecological disorders	57 (86.4)	45 (45)	
History of surgery			
Unilateral oophorectomy	1	2	1.000†
Cystectomy	5	6	0.755†
Myomectomy	3	5	1.000*
Salpingectomy	3	5	1.000*
Surgery for malignancies	1	1	
Total surgeries	12 (18.2)	17 (17)	
Chemotherapy	3	0	

*Fisher's exact test, †Chi-square test. PID=Pelvic inflammatory disease, IGT=Impaired glucose tolerance, TB=Tuberculosis

were at higher risk for developing poor ovarian reserve after adjusting for age >30 years, prior history of OI ≥3 cycles (OR: 2.3, 95% CI: 1.05–5.04).

Discussion

The established factors for poor ovarian reserve in young women are endometrioma, exposure to radiotherapy and chemotherapy, etc., Endocrine disorders such as hypothyroidism, DM obesity, gynaecological disorders and infectious disorders are also reported to be associated with low ovarian reserve but not proven as aetiological factors. In this study, we made an attempt to find out the association of the above-proposed factors for poor ovarian reserve.

One of the important factors determining the ovarian response for ovarian stimulation is the women's ovarian reserve of the follicular pool. AMH and antral follicle count (AFC) are the most commonly used markers of

ovarian reserve, of which AMH is the recommended test. ACOG recommends ovarian reserve testing for women with risk factors such as age of more than 35 years, who have history of smoking, who underwent endometriosis, oophorectomy, cystectomy and gonadotoxic therapy and those suffering from autoimmune diseases such as Hashimoto's thyroiditis and Systemic Lupus Erythematosus (SLE).[4] These factors are well established to be associated with poor ovarian reserve. Literature search revealed that there are other factors in young women such as age 30–35 years,[5,6] obesity,[7,8] DM,[9] hypothyroidism[10,11] and history of multiple cycles of OI,[12] and these factors needed further study.

Age

Studies have shown that even women aged below 35 years can have an accelerated decline in their ovarian reserve. In a study by Zhang *et al*., the mean age for women with a poor ovarian reserve was 29.5 years.[5] Pereira *et al*., in their study, found that approximately 45% of women were with poor ovarian reserve, and their mean age was 32 years.[6] The mean age of women with poor ovarian reserve was 32 years in the study by Fatima.[13] In the present study, out of 166 women, 109 were 30–34 years old after excluding PCOS. Amongst them, 48 (44%) women had a poor ovarian reserve, and the mean age was 31 years.

Table 4a: Univariate analysis of risk factors and poor ovarian reserve

Risk factors	OR (95% CI)	*P*
Established risk factors		
Endometrioma	5.78 (2.75–12.15)	<0.001
Cystectomy	1.28 (0.38–4.39)	0.069
Chronic PID	1.148 (0.319–4.134)	0.833
Proposed risk factors		
Age >30 years and <35 years	2.01 (1.03–3.93)	0.041
Obesity	0.75 (0.18–3.09)	0.686
Prior history of OI of ≥3 cycles	2.5 (1.29–4.85)	0.007
Hypothyroidism	2.303 (1.053–5.038)	0.037
Diabetes	1.675 (0.614–4.569)	0.314
IGT	1.186 (0.569–2.47)	0.648
Myomectomy	1.264 (0.202–7.917)	0.802
Salpingectomy	1.554 (0.204–11.804)	0.670

OR=Odds ratio, CI=Confidence interval, IGT=Impaired glucose tolerance, PID=Pelvic inflammatory disease, OI=Ovulation induction

Obesity

In the literature, there are conflicting results about the effect of BMI on ovarian reserve markers. Previous studies have suggested that obesity might cause apoptosis of granulosa cells and hence the decline in the AMH levels. Roth *et al*. studied 20 young women and found no significant difference in the mean AMH levels between the two groups of women.[7] In the following year, Woloszynek *et al*. observed similar results between the two groups comprising 76 women.[14] However, in a larger cohort of 575 women, Steiner *et al*. noted a significant difference in the mean AMH levels (P = 0.0001) between the two groups of women.[8] No significant association was found between obesity and poor ovarian reserve in the present study (OR: 0.75, 95% CI: 0.18–3.09, P = 0.686). Similar observations were noted in a survey by Gorkem *et al*., where no significant differences were found in the BMI amongst the two groups (poor and normal ovarian reserve). They concluded that obesity does not influence the markers of ovarian reserve.[15] In contrast, a systematic review and meta-analysis conducted by Moslehi *et al*., where they analysed articles up to 2016 on the impact of obesity on AMH, concluded that in infertile women without PCOS, AMH was significantly lower in obese in comparison to non-obese women and that AMH was inversely correlated with BMI.[16]

Hypothyroidism

TSH acts synergistically with FSH in stimulating granulosa cell proliferation. We found 32 (19%) women who had hypothyroidism. Amongst them, 18 women (27.3%) were found to have a poor ovarian reserve and 14 (14%) were found to have a normal ovarian reserve. This difference in observation was noted to be statistically significant, with P = 0.034. Furthermore, most of them had subclinical hypothyroidism (TSH between 4 and 10 mIU/ml). The relationship between hypothyroidism and a poor ovarian reserve has been studied in large cohorts in the past. A study conducted by Michalakis *et al*. showed that 18% of women with subclinical hypothyroidism had poor ovarian reserve as defined by FSH >14 IU/L or AFC <5 or previous poor response to ovarian stimulation, although it was

Table 4b: Multivariate analysis of risk factors and poor ovarian reserve

Risk factors	Un AOR	*P*	AOR	*P*
Age >30 years and <35 years	2.01 (1.03–3.93)	0.041	1.747 (0.834–3.663)	0.139
Endometrioma	5.78 (2.75–12.15)	<0.001	5.869 (2.723–12.653)	<0.001
Prior history of OI of >3 cycles	2.5 (1.29–4.85)	0.007	2.481 (1.201–5.123)	0.014
Hypothyroidism	2.303 (1.05–5.04)	0.037	2.516 (1.048–6.038)	0.039

AOR=Adjusted odds ratio, OI=Ovulation induction

not statistically significant.[17] In a study conducted by Polyzos *et al*. comprising 4894 women, there were no significant differences found in the prevalence of subclinical or overt hypothyroidism amongst both poor and normal ovarian reserve groups.[10] The present study shows that hypothyroidism is a risk factor for developing poor ovarian reserve, but a casual inference cannot be drawn due to the small numbers. Similarly, a significant association was found in the study conducted by Rao *et al*.[11] This observation was not supported by Kucukler *et al*. who did not find any correlation between TSH and AMH levels in women of reproductive age group.[18] More studies are needed to ascertain the effects of hypothyroidism on ovarian reserve.

Endometrioma

In the present study, 46% of women were affected with endometrioma. Amongst these women, 32 (48.5%) had a poor ovarian reserve. Endometrioma was a strong risk factor for developing poor ovarian reserve (OR: 5.87, 95% CI: 2.72–12.65). Kasapoglu *et al*., in their study, observed that the percentage of AMH decline was significantly more in women with endometrioma and that the decline was more in women with bilateral endometriomas.[19] Romanski *et al*. studied the effect of the presence of endometriosis on AMH levels. They observed that AMH levels were lower in women with endometrioma irrespective of prior cystectomy (OR: 2.39, 95% CI: 1.31–4.36) in women with both endometriosis and history of previous ovarian surgery (OR: 2.67, 95% CI: 1.41–5.08) in women with endometriosis only.[20]

Ovulation induction

In the present study, amongst the women who underwent ≥3 cycles of OI, 92.4% had poor ovarian reserve. They were at a significantly higher risk of developing poor ovarian reserve than women who never underwent or had <3 cycles of OI (OR: 2.5, 95% CI: 1.29–4.85). Ahmed Ebbiary *et al* studied the impact of repeated cycles of ovulation induction on Ovarian reserve by assessing the consistency of ovarian response to various ovulation stimulation protocols. They concluded that in the women who had undergone 3–6 cycles of OI, the ovarian response to stimulation was similar compared to the control group.[21] These findings need to be interpreted with caution as OI for three or more cycles is a common practice and the mechanism underlying a high incidence of poor ovarian reserve seen in those receiving repeated OI could be an intrinsic one which is driving an early exhaustion of follicles in such individuals. Alternatively, it could be an environmental factor as yet not understood. Repeated OI is more likely to be a confounder for poor ovarian reserve than a causative factor.

In a prospective investigational cohort study which investigated the effect of ovarian reserve with three cycles of increasing doses of clomiphene citrate amongst 50 infertile women, there was a decrease in ovarian reserve which is not statistically significant. This was because 70% of women in this study were PCOS and they have not received any gonadotropins for OI.[22] This may be the reason for observing a non-significant decrease in AMH across three cycles. There are not many studies on this aspect, and a study published in 2010 reported that there was no significant decline in ovarian reserve up to three cycles of ovarian hyperstimulation; however, they state that age is the detrimental factor.[12] The intercycle variability of AMH is a matter of debate, and serum levels of AMH during proliferative phase and secretory phase vary biologically. A prospective study involving women with normal menstrual cycles used three models to find out these variations, and they found higher circulating AMH levels during the follicular phase of the menstrual cycles, as compared to the luteal phase.[23] The reasons for these observed differences are unclear, but Kelsey *et al*. postulated that measured serum AMH levels reflect the recruited follicle pool rather than the overall number of primordial follicles resident in both ovaries.[24]

Ovarian reserve is the sum total of primordial and pre-antral follicles in the ovarian cortex. These constitute a majority of follicles in the ovaries. Small antral follicles <2 mm (not visible on ultrasound) have FSH dependence for growth, but this is a tonic FSH: a basal level without its cyclicity, which sufficiently drives their growth to the next stage: mid-antral follicle. Small antral follicles are not immediately available for second-phase recruitment, selection, dominance or ovulation. On the other hand, mid-antral follicles between 2 and 10 mm constitute the dynamic reserve of ovaries. These follicles are responsive to the cyclical endogenous FSH rise and fall and are available for second-phase recruitment, selection, dominance and ovulation.

AMH is secreted from pre-antral and small antral follicles. Of all the ovarian reserve markers, AMH has minimal intercycle and intracycle variability and has high sensitivity (44%–97%) and specificity (41%–100%) for predicting POR.[25] As follicles gain FSH dependence, their AMH secretion declines. In addition, the proportion of antral follicles to primordial and pre-antral follicles is very small. Therefore, any proposed influence of stimulation on ovarian reserve is only likely to effect a very small proportion of follicles that is mid-sized antral follicles. Exogenous FSH (via ovarian stimulation) only acts upon a cohort of mid-sized antral follicles (2–10 mm) that have reached a stage in their

development where they can either ovulate or undergo apoptosis. Ovarian response is defined as the actual oocyte yield after ovarian stimulation. POR is identified by a reduction in follicular response to maximal stimulation during the *in vitro* fertilisation procedure, resulting in a reduced number of retrieved oocytes, and Bologna criteria cannot be used for all women and different authors adopted different criteria to ascertain a woman to have POR.[26] Although we used the criteria described by Cohen *et al*. for poor ovarian reserve, there is conflict in literature regarding the terminology of decreased ovarian reserve (DOR), POR and pre-mature ovarian insufficiency.[27]

The recruitment of primordial follicles into the growth phase is tightly regulated to avoid a pre-mature depletion of the follicular pool. A key player of this process is AMH, a member of transforming growth factor-β family expressed in granulosa cells after the formation of primordial follicles up to the antral follicle stage. Three genes (WNT4, RSPO1 and FOXL2) are essential for ovarian determination, differentiation and/or maintenance in the stage of gonadal differentiation. Balance between pro-apoptotic factors (e.g. B-cell lymphoma 2 [BCL2]-associated Xprotein) and anti-apoptotic factors (e.g. BCL2) is essential, and this determines the follicular pool at birth.[28]

Even if there was no stimulation, the process of apoptosis would afflict these antral follicles. These follicles naturally wither with declining FSH in the mid-proliferative phase, unless selected for ovulation. In either case whether naturally picked up or exogenously stimulated, their life after beginning their journey under influence of late luteal phase FSH rise is no longer than 15 days. This is the situation in when monofollicular stimulation protocols are practiced. However, in ART cycles where controlled hyperstimulation is the norm, more number of follicles are recruited from the follicular pool causing follicular exhaustion.

It is important to understand that lower reserve may be the cause of multiple stimulations and not its effect. That women with low reserve seen in their 30s may be born with a smaller pool of follicles, or do not have intrinsic mechanisms to prevent rapid daily follicular loss or have unexplored or unknown factors for accelerated decline. The influences are mainly genetic or epigenetic: governed by intrauterine conditions. Environmental factors may have a role to play, but these are factors that are directly gametotoxic, and these are not well understood in this group of women who were recruited. Many other factors such as intrauterine and post-natal nutrition of the offspring, intrauterine exposure to androgens, exposure to environmental pollution, endocrine disruptors and socioeconomic factors influence the ovarian reserve of women, and these were not explored in this study. However, the 40% of women with poor ovarian reserve belonged to upper lower socioeconomic status. Socioeconomic status may have influence ovarian reserve through diet and environmental factors.[29] To conclude the effect of repeated OI, it can be stated that repeated ovarian stimulation is associated with low ovarian reserve but may not be a causative factor.

The study of SART CORS in US population reported that the trend of DOR is increasing in women of ≤40 years of age. They concluded that DOR and POR are not interchangeable, and if a patient is diagnosed with DOR based on ovarian reserve testing, it does not signify that she will have POR during her stimulation. However, DOR when properly assigned should signify that a patient is at increased risk of POR and may indicate a higher starting dose of gonadotropins than for similar-aged patients.[30]

One important limitation of our study is that there were few women in specific categories (myomectomy, salpingectomy, chemotherapy, etc.). Larger sample size could probably help in the intended analyses. Details of records of surgery of women with endometrioma could not be obtained, and genetic causes of ovarian reserve in young women are not explored.

Conclusion

Poor ovarian reserve was present in 39.7% of infertile women of <35 years of age. Amongst the established risk factors, endometrioma *per se* is a strong risk factor for developing poor ovarian reserve. Amongst the proposed risk factors, hypothyroidism and a history of three or more cycles of OI are associated with poor ovarian reserve.

However, it is reasonable to conclude that women younger than 35 years should be offered ovarian reserve testing by AMH to unmask hidden POR, if conception is not achieved with 1–2 cycles of OI or other endocrine disorders such as hypothyroidism are diagnosed. This will encourage judicious planning of further management and appropriate counselling of couples.

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Conflicts of interest

There are no conflicts of interest.

Data availability statement

Data is available with the investigators in excel sheet as anonymised.

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