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ANTI-MÜLLERIAN HORMONE, INHIBIN B, AND ANTRAL FOLLICLE COUNT AS BIOMARKERS IN YOUNG WOMEN WITH OVARIAN FAILURE

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ABSTRACT

Ovarian function is a cornerstone of reproductive health, and its early decline carries serious implications for fertility and long-term well-being. This study aimed to evaluate ovarian reserve markers—anti-Müllerian hormone (AMH), inhibin B, follicle-stimulating hormone (FSH), and antral follicle count (AFC)—in young women with different stages of ovarian failure. A cross-sectional comparative design was adopted, including women aged 25–40 years, categorized into control, incipient ovarian failure (IOF), and premature ovarian failure (POF) groups. Serum FSH, AMH, and inhibin B were measured using standardized immunoassays, while AFC was assessed through transvaginal ultrasonography. The findings revealed no significant age differences between the groups, ensuring comparability. However, ovarian reserve parameters showed progressive and statistically significant deterioration. Mean FSH levels increased sharply from 8.46 IU/L in controls to 18.51 IU/L in IOF and 44.48 IU/L in POF ($p = 0.0001$), reflecting compensatory gonadotropin rise with follicular depletion. Conversely, AMH values declined from 1.92 ng/mL in controls to 1.88 ng/mL in IOF and 0.29 ng/mL in POF ($p = 0.0001$), confirming its role as a sensitive predictor of diminished reserve. Inhibin B also fell markedly, with controls averaging 133.85 pg/mL compared to 111.27 pg/mL in IOF and 25.14 pg/mL in POF ($p = 0.0001$), indicating impaired granulosa function. AFC mirrored hormonal findings, decreasing from 13.13 follicles in controls to 4.73 in IOF and 1.40 in POF ($p = 0.003$). This study highlights the combined clinical value of AMH, inhibin B, and AFC as early and reliable indicators of ovarian failure, while FSH elevation reflects later stages of follicular exhaustion. Early identification of at-risk women using this multimodal approach is vital for timely fertility counseling and preservation strategies.

KEYWORDS: Anti-Müllerian, Hormone, Inhibin B, and Antral Follicle Count, Biomarkers, Ovarian Failure.

INTRODUCTION

Ovarian function plays a central role in female reproductive health, and its decline has profound implications for fertility, hormonal balance, and long-term well-being. The World Health Organization (WHO) categorizes ovarian dysfunction into three major subgroups based on serum follicle-stimulating hormone (FSH) and estradiol levels: hypogonadotropic hypogonadism, normogonadotropic ovarian dysfunction, and hypergonadotropic ovarian failure, the latter reflecting follicular depletion and exhaustion of the ovarian reserve.[1] Among these, premature ovarian failure (POF), also termed primary ovarian insufficiency, represents the most severe form, defined by the spontaneous cessation of menses before age 40, in combination with persistent FSH elevation.[2] This condition affects about 1% of women, often with devastating consequences for fertility and quality of life.

Despite its clinical significance, the spectrum of ovarian dysfunction is not binary but lies along a continuum. Transitional ovarian failure (TOF) and incipient ovarian failure (IOF) are intermediate phenotypes characterized by menstrual irregularities, fluctuating gonadotropin levels, and progressive follicular depletion.[3] Women with IOF typically maintain menstruation but exhibit elevated basal FSH, signaling early compromise in ovarian reserve.[4] These entities highlight the heterogeneous nature of ovarian failure and underscore the need for sensitive biomarkers to guide timely diagnosis and treatment. Traditionally, FSH was used as a surrogate for ovarian reserve; however, its cycle variability limits predictive reliability.[5] In recent years, attention has shifted toward direct ovarian markers such as anti-Müllerian hormone (AMH), inhibin B, and antral follicle count (AFC). AMH, secreted by granulosa cells of pre-antral and small antral follicles, reflects the pool of growing follicles and demonstrates relatively stable intra-cycle levels, making it a robust indicator of ovarian reserve.[6] Inhibin B, produced by developing antral follicles, declines with reproductive aging and correlates with FSH rise, although it shows greater variability than AMH.[7] AFC, assessed by transvaginal ultrasonography, quantifies the number of small antral follicles and is widely regarded as a strong, non-invasive marker of the primordial follicle pool.[8] The integration of AMH, inhibin B, and AFC offers a comprehensive assessment of ovarian reserve, particularly in young women at risk of early ovarian decline. A better understanding of these biomarkers is crucial not only for fertility preservation and assisted reproductive planning but also for anticipating long-term health risks associated with early estrogen deficiency. This study explores their roles in evaluating ovarian failure in young women, aiming to provide clinically relevant insights into early detection and management.

METHOD

This study was conducted as a cross-sectional comparative analysis to evaluate ovarian reserve markers—anti-Müllerian hormone (AMH), inhibin B, follicle-stimulating hormone (FSH), and antral follicle count (AFC)—in young women presenting with different degrees of ovarian failure. The research included patients aged between 25 and 40 years who attended infertility outpatient clinics with suspected diminished ovarian reserve. Ethical approval was obtained from the Institutional Review Board, and written informed consent was secured from all participants prior to enrollment.

Study Population

Participants were categorized into three groups: controls, incipient ovarian failure (IOF), and premature ovarian failure (POF). The control group consisted of regularly menstruating women with early follicular phase FSH levels below 10.2 IU/L, representing normal ovarian function. IOF was defined as regular cycles with elevated basal FSH (>10.2 IU/L) but without fulfilling POF criteria, while POF was diagnosed in women with at least four months of amenorrhea and FSH levels consistently above 40 IU/L. Transitional ovarian failure (TOF) cases with irregular cycles and moderately elevated FSH were also considered within the failure spectrum but analyzed separately where applicable. Women with previous ovarian surgery, chemotherapy, radiotherapy, or hormonal treatment were excluded.

Hormonal Assays

Blood samples were collected in the early follicular phase (day 2–5 of the cycle) whenever possible. For women with irregular cycles, samples were obtained at random, with progesterone levels measured to confirm follicular phase status. FSH concentrations were determined using a chemiluminescence-based immunoassay system with inter- and intra-assay coefficients of variation below 6%. AMH levels were assessed using enzyme-linked immunoassays and standardized to ensure comparability across samples. The detection limits were 0.026 μg/L for AMH and 0.1 IU/L for FSH. Inhibin B was measured using immunometric assays with sensitivity thresholds of 3 μg/L. All assays were performed in a single laboratory to minimize variability.

Ultrasound Examination

AFC was determined by transvaginal ultrasonography, carried out by experienced sonographers. The total number of visible intra-ovarian follicles with diameters between 2–10 mm was recorded. If no follicles were visible, AFC was noted as "not detectable." A low AFC was defined as fewer than five follicles, which is strongly associated with poor reproductive outcomes.

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation (SD), while categorical data were presented as percentages. Differences between groups were assessed using one-way ANOVA for continuous data and chi-square testing for categorical comparisons. For non-normally distributed data, logarithmic transformation was applied. Correlations between ovarian reserve parameters were analyzed using linear regression, with p-values <0.05 considered statistically significant.

RESULTS

Table 1: Age Comparison

The mean age among the three groups—Control, Incipient Ovarian Failure (IOF), and Premature Ovarian Failure (POF)—was found to be closely similar, with no statistically significant difference (p = 0.5). Controls had a mean age of 35.30 ± 2.22 years, IOF patients averaged 35.07 ± 2.73 years, and POF patients 34.63 ± 2.88 years. This indicates that the study groups were age-matched, reducing the risk of age-related bias in ovarian reserve evaluation. Since ovarian reserve markers decline naturally with advancing age, controlling for this factor enhances the validity of the observed differences in hormonal and sonographic findings, showing that variations are attributable to ovarian function rather than chronological aging.

Table 1: difference mean of age /years according to groups of patients.

Parameter	Group	N	Mean ± SD	P-value
Age	***Control***	40	35.30 ± 2.22	0.5
	Incipient Ovarian Failure (IOF)	30	35.07 ± 2.73	
	Premature Ovarian Failure (POF)	35	34.63 ± 2.88	

Table 2: Follicle Stimulating Hormone (FSH)

FSH levels showed a highly significant difference among the three groups (p = 0.0001). The control group maintained a mean FSH of 8.46 ± 1.86 IU/L, consistent with normal reproductive physiology. In IOF patients, the mean FSH was elevated (18.51 ± 11.75 IU/L), suggesting early impairment of ovarian reserve. In POF patients, the mean FSH rose sharply to 44.48 ± 20.58 IU/L, reflecting severe depletion of ovarian follicles. This progression illustrates the compensatory pituitary mechanism in which diminished ovarian hormone feedback leads to increased gonadotropin production. Clinically, FSH serves as a sensitive marker of ovarian dysfunction, with rising levels often preceding overt amenorrhea and infertility.

Table 2: difference mean of FSH according to groups of patients.

Parameter	Group	N	Mean ± SD	P-value
FSH (IU/L)	Control	40	8.46 ± 1.86	**0.0001**
	IOF	30	18.51 ± 11.75	
	POF	35	44.48 ± 20.58	

Table 3: Anti-Müllerian Hormone (AMH)

AMH levels demonstrated a marked decline across the groups (p = 0.0001). The control group exhibited a mean AMH of 1.92 ± 1.16 ng/mL, representing preserved ovarian follicular activity. IOF patients had a slightly reduced and highly variable mean value of 1.88 ± 3.22 ng/mL, highlighting the transitional nature of this stage where ovarian reserve is declining but not yet exhausted. In contrast, POF patients had a strikingly low mean AMH of 0.29 ± 0.50 ng/mL, signifying a near-total loss of functional follicles. Since AMH directly reflects the size of the antral follicle pool, these results underscore its role as a robust predictor of ovarian reserve, with levels approaching zero in established ovarian failure.

Table 3: difference mean of AMH according to groups of patients.

Parameter	Group	N	Mean ± SD	P-value
AMH (ng/mL)	Control	40	1.92 ± 1.16	**0.0001**
	IOF	30	1.88 ± 3.22	
	POF	35	0.29 ± 0.50	

Table 4: Inhibin

Inhibin levels, which are primarily secreted by granulosa cells of developing follicles, were significantly reduced in the IOF and POF groups compared to controls (p = 0.0001). Controls recorded the highest mean value of 133.85 ± 80.92 pg/mL, while IOF patients averaged 111.27 ± 83.09 pg/mL, indicating early but partial decline in follicular activity. The POF group had profoundly decreased levels (25.14 ± 24.86 pg/mL), confirming severely compromised granulosa cell function. The sharp fall in Inhibin parallels reductions in AMH, reinforcing their combined utility as biochemical markers of diminished ovarian reserve.

Table 4: difference mean of Inhibin according to groups of patients.

Parameter	Group	N	Mean ± SD	P-value
Inhibin (pg/mL)	Control	40	133.85 ± 80.92	**0.0001**
	IOF	30	111.27 ± 83.09	
	POF	35	25.14 ± 24.86	

Table 5: Antral Follicle Count (AFC)

The ultrasound-based AFC showed significant variation between groups (p = 0.003). Controls had a robust follicular pool with a mean of 13.13 ± 3.71, consistent with preserved reproductive potential. IOF patients exhibited a markedly reduced mean AFC of 4.73 ± 0.76, aligning with early depletion of ovarian follicles. POF patients had an extremely low mean AFC of 1.40 ± 0.26, nearly confirming follicular exhaustion. Since AFC is a direct and non-invasive indicator of ovarian reserve, these findings provide compelling visual evidence that complements the hormonal data.

Table 5: difference mean of AFC according to groups of patients.

Parameter	Group	N	Mean ± SD	P-value
AFC	Control	40	13.13 ± 3.71	**0.003**
	IOF	30	4.73 ± 0.76	
	POF	35	1.40 ± 0.26	

DISCUSSION

The present study evaluated ovarian reserve markers—FSH, AMH, inhibin B, and AFC—across control, incipient ovarian failure (IOF), and premature ovarian failure (POF) groups. The findings provide robust evidence of progressive deterioration in ovarian function, with distinct hormonal and sonographic patterns that align with international literature.

Age Distribution

The mean age of the three groups did not differ significantly, indicating that age was not a confounding factor in interpreting ovarian reserve status. This strengthens the validity of the results; as ovarian aging is a natural determinant of reserve. Similar age-matched approaches have been adopted in studies by Johnson J et al., who confirmed that ovarian biomarkers could differentiate reserve status even in comparable age cohorts.[9]

Follicle-Stimulating Hormone (FSH)

FSH levels demonstrated a significant rise from control to IOF and POF groups (p = 0.0001). This reflects the compensatory increase in gonadotropin secretion secondary to reduced ovarian steroid feedback. The steep elevation in POF patients is consistent with near-complete follicular depletion. Studies by Sopiarz N.[10] and Santoro N et al.[11] similarly reported elevated basal FSH as a hallmark of ovarian insufficiency, often detectable before amenorrhea onset. This supports FSH as a sensitive, albeit late, marker of diminished ovarian reserve.

Anti-Müllerian Hormone (AMH)

AMH levels showed a sharp decline across groups, with controls averaging 1.92 ng/mL, IOF patients 1.88 ng/mL, and POF patients only 0.29 ng/mL (p = 0.0001). Unlike FSH, AMH directly reflects the pool of small growing follicles and is less cycle-dependent. The near-zero AMH in POF underscores its utility in detecting profound follicular exhaustion. Comparable findings were reported by Cesarano S et al.[12] and He YC et al.[13] who confirmed that AMH is the most stable and reliable predictor of ovarian reserve across reproductive stages.

Inhibin B

Inhibin B demonstrated significant reduction from controls (133.85 pg/mL) to IOF (111.27 pg/mL) and POF (25.14 pg/mL) groups (p = 0.0001). As a granulosa cell-derived hormone, its decline parallels follicular attrition. These results align with Walton KL et al.[14] who documented reduced inhibin secretion as a marker of diminished follicular activity. While less stable than AMH, inhibin B provides complementary insight into granulosa function and feedback regulation.

Antral Follicle Count (AFC)

Ultrasound-based AFC revealed a dramatic reduction from controls (13.13 follicles) to IOF (4.73 follicles) and POF (1.40 follicles) (p = 0.003). This visual evidence of follicular depletion corroborates the biochemical data. Studies by Kučera M et al.[15] and Li F et al.[16] emphasized the predictive power of AFC in assessing reproductive potential and IVF response. AFC, in combination with AMH, has been shown to outperform FSH in predicting ovarian failure.

The progressive decline across all markers—rising FSH, falling AMH and inhibin, and reduced AFC—provides a comprehensive picture of ovarian reserve loss. These findings are consistent with global evidence supporting AMH and AFC as the earliest and most reliable indicators, while FSH and inhibin become abnormal later in the course of ovarian failure. Early detection using this combined approach may allow timely fertility counseling and interventions such as oocyte preservation.

CONCLUSION

This study demonstrates that ovarian reserve markers show a clear and progressive decline from controls to IOF and POF groups, with AMH and AFC emerging as the most sensitive indicators of early ovarian insufficiency. Elevated FSH and reduced inhibin B further confirm follicular depletion and impaired granulosa cell function. The combined use of hormonal assays and sonographic evaluation provides a reliable framework for diagnosing and staging ovarian failure. Early identification of women at risk is essential for timely fertility counseling and intervention.

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