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ROLE OF ANTI-MÜLLERIAN HORMONE (AMH) AND OTHER HORMONAL MARKERS IN THE BIOCHEMICAL ASSESSMENT OF FEMALE INFERTILITY

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ABSTRACT

INTRODUCTION:-Infertility is clinically defined as the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (World Health Organization [WHO], 2020). Female infertility is a multifactorial condition influenced by a broad spectrum of anatomical, genetic, immunological, and endocrine variables. The biochemical evaluation of female infertility typically encompasses a comprehensive analysis of ovulatory function, ovarian reserve, and the presence of endocrine disorders.

AIM: -To evaluate serum AMH levels and it's correlation with ovarian reserve & infertility status.

MATERIALS AND METHODS:-This study is a observational cross-sectional hospital-based study undertaken in the Department of Biochemistry & Obstetrics Gynecology, Index medical College & Hospital, Indore MP from July 2021 to June 2024. Total number of 200 patients, include Women with infertility aged 18 – 40 presenting in Department of Obstetrics & Gynecology (OB/GYN). Five milliliter of venous blood was collected, from each patient in plain tube. After centrifugation at 6000 rpm for 5 min. Biochemical Hormonal Parameters like serum LH, FSH, TSH, Prolactin and serum AMH to be assessed in the Department of the Biochemistry.

RESULT:-. In the present study, A total of 100 fertile patients & 100 infertile patients. On the basis of serum AMH level patients were divided into five groups and maximum patients (45%) belonged to group 3 with serum AMH level between 9-13ng/ml. AMH had an inverse relationship with BMI and obesity. Serum AMH level showed a significant positive correlation with LH: FSH ratio (p <0.001) while there was a significant negative correlation with FSH level (p = 0.011).

CONCLUSION: Female infertility is a challenge because of ever changing criteria and its definition as infertile women have variable clinical presentation. AMH level is consistently elevated in infertile patient as compared to controls. AMH is a useful biochemical marker for diagnosis of infertility. It has a greater sensitivity and specificity as compared to serum LH & LH: FSH ratio.

KEYWORDS: - Luteinizing hormone, Anti-Müllerian hormone, Follicle stimulating harmone.

INTRODUCTION

Infertility is clinically defined as the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (World Health Organization [WHO], 2020)[1]. Globally, this condition affects an estimated 10–15% of couples of reproductive age, making it a significant public health concern with profound psychosocial and economic implications.[2] Female infertility is a multifactorial condition influenced by a broad spectrum of anatomical, genetic, immunological, and endocrine variables. Among these, hormonal imbalances constitute one of the most common and diagnostically pivotal contributors.[3]

The biochemical evaluation of female infertility typically encompasses a comprehensive analysis of ovulatory function, ovarian reserve, and the presence of endocrine disorders. Hormonal profiling serves as a fundamental component of this assessment. Traditional hormonal biomarkers such as Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Estradiol (E2), Thyroid Stimulating Hormone (TSH), and Prolactin have long been employed to evaluate the functional status of the hypothalamic–pituitary–ovarian (HPO) axis[4]. These hormones are integral in regulating follicular development, ovulation, and endometrial receptivity, and their evaluation can provide important clues to underlying pathologies.

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The hormonal assessment of female fertility typically includes evaluation of ovulatory function, ovarian reserve, and the presence of any underlying endocrine disorders. Traditional markers such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), thyroid-stimulating hormone (TSH), and prolactin have been instrumental in evaluating the function of the hypothalamic–pituitary–ovarian (HPO) axis, which plays a pivotal role in the regulation of reproductive processes[5]. These markers help clinicians assess ovulatory dysfunction and other endocrine disturbances that may hinder conception.

In recent years, Anti-Müllerian Hormone (AMH) has emerged as a superior and more stable biomarker for assessing ovarian reserve and reproductive function. AMH is a dimeric glycoprotein belonging to the transforming growth factor-β (TGF-β) superfamily, primarily secreted by the granulosa cells of pre-antral and small antral follicles[6]. These follicles reflect the pool of recruitable oocytes, and hence, AMH levels are strongly correlated with the remaining quantitative ovarian reserve. One of the key advantages of AMH is its relative stability throughout the menstrual cycle and its independence from gonadotropin fluctuations and exogenous hormonal influences, making it a convenient and robust marker in clinical practice[7].

Keeping these factors in mind, the present study was planned to determine the evaluate the AMH and other hormonal markers in the biochemical assessment of female infertility.

MATERIALS AND METHODS
Study Design: Cross-sectional or case-control observational study
Place of Study: The study was conducted in the Department of Biochemistry, Index Medical College & Hospital, Indore, and Madhya Pradesh, India.
Duration of study:-July 2021 to June 2024
Sample size: Total number of patients included in the study is 200 with infertile patients.

Inclusion Criteria: - The following types of patients were included:
- Female patients with primary or secondary infertility

- Normal anatomical reproductive structures (confirmed by imaging)
Exclusion Criteria: The following types of patients were excluded:
- Women with known genetic, autoimmune, or infectious causes of infertility
- Recent hormonal therapy
Specimen collection:
Five milliliter of venous blood was collected, from each patient, in plain tube. After centrifugation at 6000 rpm for 5 min, serum was collected and stored at −20 °C for further serological analyses.
Sample processing:
Blood samples were collected from all study participants, and serum TSH was measured using a commercial chemiluminescence immunoassay CLIA or Enzyme-Linked Immunosorbent Assay (ELISA) are both sensitive and widely used methods for measuring hormone concentrations in serum. (Siemens centaur CP).

OBSERVATION AND RESULTS
During the July 2021 to June 2024 a total number of 200 patients were enrolled. 100 patient with fertile women's and 100 patients were infertility.

Table no. 1 Number of Fertile females in different age groups

AGE GROUP	NUMBER	PERCENTAGE
18-24	21	21%
25-29	29	29%
30-34	26	26%
35-40	24	24%
TOTAL	**100**	**100%**

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It was observed that maximum number of fertile women's were in the age group 25-29 (n=29) as shown in Table 1.

Table no. 2 Number of Infertile females in different age groups

AGE GROUP	NUMBER	PERCENTAGE
18-24	24	24%
25-29	27	27%
30-34	25	25%
35-40	24	24%
TOTAL	**100**	**100%**

It was observed that maximum number of infertile women's were in the age group 25-29 (n=27) as shown in Table 2.

Table-no. 3. Body mass index (BMI) of fertile Women.

BMI (KG/M2)	NO. OF PATIENTS	PERCENTAGE
Normal weight <18.5	36	36%
Overweight- 18.5 - 24-99	33	33%
Obese- 25-29.99	30	30%
Underweight- > 30	01	01%
TOTAL	**100**	**100%**

Among the fertile group, 36% of women had a normal BMI, and a fairly balanced number fell into overweight (33%) and obese (30%) categories. This shows a relatively healthier distribution overall.

Table- 4.Body mass index (BMI) of Infertile Women.

BMI (KG/M2)	NO. OF PATIENTS	PERCENTAGE
Normal weight <18.5	27	27%
Overweight- 18.5 - 24-99	25	25%
Obese- 25-29.99	47	47%
Underweight- > 30	01	01%
TOTAL	**100**	**100%**

In contrast, the infertile group exhibited a notable shift, with 47% falling into the obese category and only 27% in the normal range. This sharp rise in obesity among infertile women highlights a potential link between higher BMI and reproductive challenges. Only 1% in each group were underweight, making it a negligible factor in this sample.

Table 5 – Infertility type.

INFERTILITY	NO. OF PATIENTS	PERCENTAGE
Primary	59	59%
Secondary	41	41%
TOTAL	**100**	**100%**

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Out of the 100 women studied, 59% were diagnosed with primary infertility, while 41% experienced secondary infertility

Table 6 – Duration of Infertility

DURATION	NUMBER OF WOMEN	PERCENTAGE
1–2 years	42	42%
3–4 years	13	13%
5–6 years	45	45

A significant portion (45%) of the women reported infertility duration between 5–6 years,
42% had been facing infertility for 1–2 years, suggesting early-stage infertility,
Only 13% of the women had been infertile for 3–4 years

Table 7 – Biochemical Hormonal Parameters

Marker	Fertile (Mean)	Infertile (Mean)	Interpretation
AMH (ng/mL)	~3.1	~0.93	Significantly lower in infertile women, confirming AMH as a strong ovarian reserve marker.
FSH (IU/L)	~6.1	~11.1	Elevated FSH in infertile women suggests diminished ovarian reserve.
LH (IU/L)	~5.0	~8.4	Higher LH in infertile group, may point to ovulatory dysfunction or PCOS in some cases.
Estradiol (pg/mL)	~65.5	~87.3	Elevated E2 in infertile group could indicate early follicular recruitment or hormone imbalance.
TSH (µIU/mL)	~2.0	~2.3	Slightly elevated TSH in infertile women; borderline thyroid dysfunction may affect fertility.
Prolactin (ng/mL)	~13.5	~23.8	Much higher in infertile group, may indicate hyperprolactinemia affecting ovulation.
Testosterone (ng/dL)	~36.5	~68.4	Higher androgen levels in infertile women, consistent with PCOS-related infertility.
DHEAS (µg/dL)	~137.4	~253.6	Significantly elevated, further supporting PCOS or adrenal involvement

The comparative biochemical analysis reveals distinct hormonal patterns between fertile and infertile women. These variations hold significant clinical value in diagnosing, classifying, and managing infertility. Each parameter is interpreted in the context of reproductive endocrinology and pathophysiology.

DISCUSSION
This study aimed to evaluate serum AMH levels and correlate them with ovarian reserve and infertility status while also assessing a panel of reproductive and endocrine hormones (FSH, LH, TSH, Estradiol, Prolactin, Testosterone, and DHEAS) in a cohort of 200 women—100 fertile and 100 infertile. The objective was to determine whether these markers, individually and collectively, could explain the hormonal etiology behind infertility and support their use in clinical decision-making, particularly in assisted reproductive technologies (ART).

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The primary objective—evaluating serum AMH levels and their correlation with ovarian reserve—was strongly supported by our findings. Fertile women demonstrated a mean AMH level of approximately 3.15 ng/mL, while infertile women showed markedly lower levels averaging 0.93 ng/mL. The difference was statistically significant (p < 0.001, unpaired t-test), confirming AMH as a potent marker for ovarian reserve. The sharp decline in AMH levels among infertile women, especially those diagnosed with diminished ovarian reserve (DOR), demonstrates AMH's value in identifying poor responders and those at risk of early ovarian aging. It also reveals its reliability over age alone, as some younger women with infertility showed critically low AMH despite being under 35, signifying premature ovarian insufficiency. This finding is consistent with other studies. In a study by **Homberg R et al[8],** mean serum AMH was 10.86ng/ml as compared to 3.15ng/ml in control group. In a study by **Saxena U et al[9]**, mean serum AMH was found to be 4.32ng/ml in PCOS patients as compared to 2.32ng/ml in control group. In a study by **Yue CY et al[10]**, mean serum AMH was found to be 9.3 ng/ml in PCOS patient as compared to 5.7ng/ml in control group.
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Beyond AMH, the study analyzed six key hormones that influence female fertility:
FSH levels were significantly elevated in the infertile group (11.12 IU/L) compared to the fertile cohort (6.18 IU/L). This increase in FSH, particularly in early follicular phase readings, indicates decreased ovarian feedback—consistent with DOR and poor ovarian function. Elevated FSH is often seen in women who require higher doses of gonadotropins for ovarian stimulation and are less likely to respond adequately.LH values were notably higher in infertile women (8.45 IU/L vs. 5.07 IU/L in fertile women), particularly among those diagnosed with PCOS. A high LH/FSH ratio (>1.5), seen in many PCOS participants, corroborates the hypersecretion of LH due to hypothalamic-pituitary dysregulation, a hallmark of anovulatory PCOS. This finding supported previous studies **Sharara FI, et al[11]**, where elevated FSH was a late indicator of diminished ovarian reserve. Estradiol (E2) levels were significantly elevated in infertile women (87.3 pg/mL) compared to fertile women (65.5 pg/mL). Elevated baseline estradiol, especially when seen in conjunction with low AMH and high FSH, may reflect follicular recruitment disturbances or masking of poor reserve by residual estrogen activity. This finding supported to study **Homburg R, et al[8]**

TSH, while only marginally elevated (2.31 µIU/mL vs. 2.0 µIU/mL), showed that several infertile women hovered near the upper normal limit (2.5–4.0 µIU/mL). Subclinical hypothyroidism, even in its mild form, can impair ovulation, disrupt luteal function, and reduce implantation success, thus warranting early screening and management. This finding also related to study **EilertsenTBet al[12]**

Prolactin levels were significantly higher in the infertile group (23.8 ng/mL) versus the fertile group (13.5 ng/mL). Elevated prolactin impairs GnRHpulsatility and ovulation, leading to amenorrhea or luteal phase defects. This was especially relevant in patients with unexplained infertility and irregular cycles. These results also related to study **Natalia Pedachenko et al. [13]**
Testosterone and DHEAS levels were considerably higher in infertile women (68.4 ng/dL and 253.6 µg/dL, respectively), particularly among those with PCOS. Hyperandrogenemia contributes to follicular arrest and anovulation and plays a direct role in the hormonal imbalance seen in PCOS. This was evident in both clinical history and hormonal phenotype. This finding supported to study **Li-TeLin,et al[14]**

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