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ORIGINAL RESEARCH

Role of Hormones in Unexplained Infertility

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ABSTRACT

Unexplained infertility is a term applied to an infertile couple whose standard infertility investigations and workup are normal. The aim of the study is to assess the role of hormones in women with unexplained infertility. The female reproductive system is regulated by a balanced hormonal interaction between the hypothalamus, anterior pituitary, and ovaries. Follicle stimulating hormone (FSH) and luteinizing hormone (LH) are important for ovulation and stimulation of secretion of estradiol and progesterone from the ovaries. Anti-Müllerian hormone (AMH) is an important marker to predict the ovarian reserve. The primary function of the ovary is the production of a mature and viable oocyte capable of fertilization, embryo development, and implantation. Fifty women diagnosed with unexplained infertility were enrolled as cases. These were age matched with 50 healthy fertile women volunteers. Body mass index (BMI) was found to be significantly higher in women with unexplained infertility. Serum FSH, LH, and estradiol were significantly higher in cases. LH:FSH ratio and serum AMH were significantly lower in cases as compared to controls. To conclude, serum AMH, FSH, and LH:FSH ratio indicated poor ovarian reserve in women with unexplained infertility.

Keywords: Hormones, Role, Unexplained infertility.

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INTRODUCTION

Infertility is the failure to achieve a successful pregnancy after 12 months or more of unprotected intercourse. Evaluation for infertility is initiated after 1 year of trying to conceive. However, in couples with advanced female age (> 35 years), a diagnostic evaluation is carried out after an inability to conceive for 6 months. Approximately 10 to 20% of couples who are unable to conceive have unexplained infertility.[1] Unexplained infertility is a term applied to an infertile couple whose standard investigations (semen analysis, tubal patency, and laboratory assessment of ovulation) are normal.

Hormone interaction between the hypothalamus, anterior pituitary gland, and ovaries regulates the female reproductive system. Follicle stimulation hormone (FSH) and luteinizing hormone (LH) are two pituitary glycoproteins that are essential for normal gonadal function.[2] Follicle stimulating hormone plays an important role in oogenesis. It triggers the maturation of follicles (e.g., the proliferation of granulosa cells) and synthesis of the androgen-converting enzyme aromatase. Furthermore, it plays a central role in the recruitment of the dominant follicle.[3] Luteinizing hormone and FSH promote ovulation and stimulate secretion of the sex hormones estradiol and progesterone from the ovaries.

The ovary at birth contains a finite number of oocytes for folliculogenesis. This definite number of available oocytes is termed as the ovarian reserve. It is important to determine the ovarian reserve for the assessment and treatment of infertility.[4] One of the markers to predict the ovarian reserve is serum anti-Müllerian hormone (AMH). Anti-Müllerian hormone is expressed in the growing preantral or small antral follicles in the ovary and it is a reflection of the recruited ovarian follicular pool. The advantages of AMH are its little inter- and intracycle variability. Serum AMH levels decrease steadily with age and are almost undetectable after menopause.[5]

A delicate balance of various hormones involved in regulating the reproductive organs enables and maintains fertility. These hormones control changes like release of egg from ovary (ovulation) and thickening of the uterine wall lining (endometrium). Unexplained infertility may result if this balance is disturbed. Therefore, hormones play an important role in the etiopathogenesis of unexplained infertility.

MATERIALS AND METHODS

The study is a hospital-based observational case–control study conducted in the Department of Biochemistry in collaboration with the Department of Obstetrics and Gynecology, Lady Hardinge Medical College, New Delhi. The study was undertaken after approval from the institutional ethical committee. Fifty women with a diagnosis of unexplained infertility were enrolled as cases. These were age matched with 50 healthy fertile volunteers who were enrolled as controls.

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Selection Criteria for Control Group

The control group included age-matched healthy fertile women.

Selection Criteria for Cases

Inclusion Criteria

Women diagnosed with unexplained infertility:

- In age group between 21 and 35 years
- With normal regular menstrual cycles and documented ovulation
- With patent fallopian tubes as documented with hysterosalphingography (HSG) or diagnostic HSG and laparoscopic chromopertubation, and
- Whose partners have normal semen analysis.

Exclusion Criteria

- Women with irregular menstrual cycles
- Women with known endocrinopathies.

Sample Collection and Analysis

Morning fasting 6 mL venous blood samples was collected from the study subjects during second to fifth day of menstrual cycle. Serum was separated from the sample and analyzed for routine investigations. Serum for AMH was stored at –20°C for further analysis. Routine investigations were carried out on Beckman coulter AU480 clinical chemistry analyzer with a standard reagent and kit. Anti-Müllerian hormone levels were estimated using Ultra-sensitive AMH ELISA Kit by ANSH LABS.

Statistical Analysis

All statistical data was coded, entered, and analyzed using the free available version of Statistical Package for the Social Sciences (SPSS) version 20. The data were checked for normality by applying the Shapiro–Wilk test of normality and was expressed as mean ± SEM. The difference between the groups was evaluated using the Student's t-test. The frequency of genotype between the groups was compared using Fisher's exact test. A two-tailed p value of ≤ 0.05* was considered as significant.

RESULTS

The BMI was significantly higher in cases as compared to controls.

- Serum FSH, LH, and estrogen were found to be significantly higher in cases as compared to controls with a p value < 0.05
- Serum LH:FSH ratio and AMH was found to be significantly lower in cases as compared to controls.
- Serum prolactin and progesterone were found to be significantly higher in cases as compared to cases with p-value of < 0.05.
- Testosterone was found to be significantly lower in cases as compared to the controls.

DISCUSSION

In this study, we compared the demographic and anthropometric parameters between the cases and controls. It was found that the body mass index (BMI) was higher in cases. The mean value of BMI was 22.79 ± 0.16 and 21.60 ± 0.27 in cases and controls respectively as shown in Table 1. The difference was significant, with p-value < 0.001. In a study conducted by Savadkouhi et al on the effect of BMI on the success rate of In vitro fertilization (IVF) for couples with different causes of infertility suggested that overweight women with BMI > 27 kg/m^2 had a 33% less chance of having a successful pregnancy in their first IVF cycle, and the unfavorable effect was largest among women with unexplained infertility.[6]

On comparison of the hormones in the cases and controls, it was found that the serum FSH was significantly higher in cases (8.29 ± 0.37) as compared to controls (5.79 ± 0.21), with p-value of < 0.001 (Table 2). Elevated basal FSH levels is suggestive of decreased ovarian reserve such that the fertility potential is lowered in infertile women with elevated basal FSH levels.[7] Leach et al[8] demonstrated higher basal FSH levels in unexplained infertility compared with fertility-proven women, a finding suggesting a subtle diminution of ovarian reserve as one of the etiologic factors in unexplained infertility. A more recent study shows that higher basal FSH levels in women with ovarian failure are associated with larger amplitudes of FSH pulses and greater responses of FSH to Gonadotropin Releasing Hormone (GnRH).[7] Though, in the present study, FSH has emerged as a good marker of ovarian reserve, this finding was contradicted by Islam et al,[9] who found that FSH is not a good predictor of ovarian reserve.

Table 1: Demographic and anthropometric parameters in cases and controls

	Cases (n = 50) Mean ± SEM	*Control (n = 50) Mean ± SEM*	*p-value*
Age (years)	28.04 ± 0.26	28.3 ± 0.30	0.487
BMI (kg/m^2)	22.79 ± 0.16	21.60 ± 0.27	< 0.001*

*indicates significant *p*-value

Table 2: Markers of ovarian reserve

Parameter	*Cases (n = 50) Mean ± SEM*	*Control (n = 50) Mean ± SEM*	*p-value*
FSH (IU/L)	8.29 ± 0.37	5.79 ± 0.210	< 0.001*
LH (IU/L)	7.04 ± 0.43	5.37 ± 0.196	0.001*
LH/FSH ratio	0.84 ± 0.051	0.99 ± 0.052	0.049*
Estradiol (pg/mL)	77.86 ± 3.07	66.42 ± 2.58	0.005*
AMH (ng/mL)	2.55 ± 0.26	3.76 ± 0.203	< 0.001*

*indicates significant *p*-value

The serum LH in the present study was found to be higher in cases as compared to control, with a p-value of 0.001 (Table 2). But on comparing the mean of FSH and LH in cases, it was found that serum FSH had higher mean value. The LH-to-FSH ratio was also found to be significantly lower in cases as compared to controls, with p-value of 0.049 (Table 2). Liu et al[10] and Seckin et al[11] have shown that the day 3 LH-to-FSH ratio can be used as a predictor of the ovarian reserve. The serum FSH increases early as compared to LH, which rises at a later stage, thus leading to decreased basal LH-to-FSH ratio, which might be a sign of diminished ovarian reserve even with normal basal FSH. The LH-to-FSH ratio decreases as the ovarian reserve declines; therefore, the value of LH-to-FSH ratio could play a significant role in determining the appropriate status of ovarian reserve.[5] The LH-to-FSH ratio is commonly used in the assessment of polycystic ovary syndrome, although its sufficient utility has not been proven in this clinical scenario. However, the ratio has been little investigated as a marker of fertility or ovarian reserve. Recently, the evaluation of LH-to-FSH ratio in the field of infertility showed the highest correlation of LH-to-FSH ratio with clinical pregnancy over other measures of ovarian reserve.[10] Another study reported that a high FSH-to-LH ratio may be used as an early biomarker of poor ovarian response.[12]

In the present study, estradiol was found to be significantly higher in cases than controls with a p-value of 0.005 (Table 2). Blacker et al[13] and Leach et al[8] in their studies on the hormonal profile of women with unexplained infertility have shown the serum estradiol levels to be elevated in women with unexplained infertility, suggesting altered folliculogenesis. Earlier studies have suggested that increased early follicular-phase FSH and estradiol values correlate with poor ovarian reserve and reduced fertility potential in controlled ovarian hyperstimulation treatment cycles.[14,15] Elevated FSH has been suggested to induce enhanced estradiol production by granulosa cells.[15]

Anti-Müllerian hormone is emerging as the hormone of choice for assessing ovarian reserve. It has been suggested that AMH is the single best predictor of poor response for assisted reproductive therapy.[16] The fact that AMH is secreted without dependence on other hormones, particularly the gonadotropins, and that AMH is expressed at a constant level, independent of cycle day, makes AMH a marker of direct measurement of ovarian reserve.[17] Lekamge et al[18] conducted a study in which serum AMH concentration and antral follicle count (AFC) were used to predict ovarian reserve prior to IVF treatment. The patients with lower serum AMH and AFC produced a significantly ($p < 0.001$) lower number of oocytes compared with patients with higher serum AMH/AFC. This finding correlates with the present study's result in which serum AMH was significantly lower in women with unexplained infertility as compared to controls with p-value of < 0.001 (Table 2).

Table 3: Comparison of other hormonal test between cases and control

Parameter	*Cases (n = 5) Mean ± SEM*	*Control (n = 50) Mean ± SEM*	*p-value*
TSH (μIU/mL)	2.89 ± 0.26	2.46 ± 0.175	0.165
T3 (pg/mL)	2.69 ± 0.076	3.22 ± 0.053	0.141
T4 (mg/dL)	1.04 ± 0.042	0.899 ± 0.022	0.510
Prolactin (ng/mL)	13.62 ± 0.71	11.33 ± 0.45	0.007*
Testosterone (ng/dL)	17.18 ± 1.37	26.12 ± 1.19	< 0.001*
Progesterone (ng/mL)	0.74 ± 0.125	0.48 ± 0.028	0.046*

*indicates significant *p*-value

Serum prolactin and progesterone were found to be significantly higher in cases as compared to controls, with p-value of < 0.05 and testosterone was found to be significantly lower in cases as compared to controls (Table 3). It has been stated by Blacker et al[13] and Leach et al[8] in their studies that women with unexplained infertility have subtle hormonal anomalies during the luteal phase when compared with fertile controls, which supports our findings.

CONCLUSION

This study describes the role of hormones in unexplained infertility, indicating that the AMH, FSH, and LH:FSH ratio are predictors of ovarian reserve in women with unexplained infertility. We conclude that hormones play a pivotal role in unexplained infertility so much so that the AMH levels can be used to predict the IVF outcome and reduce the incidence of cycle cancellations.

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