RELATION OF ANTRAL FOLLICULAR COUNT AND FOLLICULAR STIMULATING HORMONE WITH ANTI-MULLERIAN HORMONE IN DETERMINING OVARIAN RESERVE IN INFERTILE WOMEN PRESENTING IN A TERTIARY HEALTH CENTRE OF NEPAL

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ABSTRACT

Introduction

Infertility is a disorder of the reproductive system defined by failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Globally, about 10-15% married couples are sub-fertile. 20-40% of female factors for infertility include ovarian cause. Ovarian reserve refers to the size of resting primordial follicle in the ovaries which determines the capacity of the ovary to provide egg cells for fertilization resulting in a successful pregnancy. Ovarian reserve is one of the most reliable investigations to determine ovarian regression, which regresses with age. Follicular Stimulating Hormone (FSH), Estradiol (E2), Antral Follicular Count (AFC) and Anti-Mullerian Hormone (AMH) are investigations to determine ovarian reserve.

Objective

To determine the correlation of antral follicular count and follicular stimulating hormone with anti-mullerian hormone in determining ovarian reserve among infertile women attending infertility clinic of Tribhuvan University Teaching Hospital (TUTH).

Methodology

This was a prospective, hospital based study conducted among 78 patients attending infertility clinic in the Department of Obstetrics and Gynecology at TUTH, Kathmandu from 14th April 2017 to 13th April 2018. After taking history and examination, routine infertility investigation was sent. On the Day 2or Day 3of the cycle, serum FSH and AMH were sent. On the 3-5 day of the cycle, trans-vaginal scan was done for antral follicular count. Follicular monitoring was done every alternate day from day 9 of natural cycle till the follicle became 18 to 22 mm. If no follicle developed even after day 20 of menstruation, monitoring was stopped and development of follicle or failure was noted. This monitoring was done in single cycle. Early follicular phase serum FSH, AMH and AFC were correlated in relation to follicular development.

Result

Among 78 patients included, mean age was 27.6 ±3.14 years. The mean FSH, AMH and total AFC were 6.46 ± 1.90 IU/ml, 5.01 ± 3.46 ng/ml and 15.88 ±5.21 respectively. A strong positive linear correlation between AFC and AMH ($p<0.001$, $r=0.811$) and weakly negative correlation between FSH and AMH ($p=0.182$, $r= -0.153$) was found.

Conclusion

Serum AFC level showed a strong positive correlation with AMH and could be used as an alternative to AMH to assess ovarian reserve.

KEYWORDS

Antral Follicular Count, Anti-Mullerian Hormone, Follicle Stimulating Hormone,Ovarian Reserve.

INTRODUCTION

Infertility is a disorder of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more despite of regular unprotected sexual intercourse.[1] Among the couples without any detectable abnormality, 57% conceive within 3 months, 72% conceive within 6 months, 85% conceive within 1 year and 93% conceive within 2 years.[2] Globally, about 10-15% married couples are sub-fertile. 20-40% of female factors for infertility include ovarian cause.[3]

The number of oocytes peaks in the fetus by 20 weeks of gestation which is 6-7 million.[4] At birth, only 1 to 2 million oocytes remain in the ovaries, and at puberty, only 300,000 oocytes are available for ovulation. Of these, only 400 to 500 eggs are ultimately released during ovulation. By the time of menopause, the ovary will be composed primarily of dense stromal tissue with only rare interspersed oocytes remaining. Therefore, more than 99.9% of follicles undergo atresia through apoptosis. With each ovarian cycle, a number of follicles begin to develop, but only one reaches full maturity and other degenerate and become atretic. So, with age ovaries become exhausted.

Ovarian reserve refers to the size of non-growing, or resting, primordial follicle population in the ovaries which determines the capacity of the ovary to provide egg cells that are capable of fertilization resulting in a healthy and successful pregnancy. Ovarian reserve is one of the most reliable investigations to determine ovarian regression.[4,5] It determines the number of growing follicles and reproductive potential of their oocytes. Diminished ovarian reserve can refer to diminished oocyte quality, quantity, or reproductive potential. Ovarian reserve assessment includes age, estradiol (E2), and follicular stimulating hormone (FSH), antral follicle count (AFC), anti-mullerian hormone (AMH), serum inhibin B, ovarian volume and stromal blood flow.

Follicle-stimulating hormone (FSH) is a gonadotropin, a glycoprotein polypeptide hormone synthesized and secreted by the gonadotropic cells of the anterior pituitary gland, and regulates the development, growth, pubertal maturation, and reproductive processes of the body. FSH and luteinizing hormone (LH) work together in the reproductive system. The antral follicle count (AFC) is the number of antral follicles, present in both ovaries which can be determined by transvaginal ultrasonography during early follicular phase of cycle. anti-müllerian hormone (AMH) is a member of the transforming growth factor-beta (TGF-ß) family and is expressed by the small (<8 mm) pre-antral and early antral follicles. The AMH level reflects the size of the primordial follicle pool, and may be the best biochemical marker of ovarian function across an array of clinical situations.

Traditionally, FSH and E2 were used to determine ovarian reserve. However, FSH is derranged only when ovarian function is largely decreased. So, AFC was considered as the first choice.[6,7] However, due to high inter-observer variability, anatomical variations and unavailability of two-dimensional (2D) ultrasonography, nowadays, AMH was used to determine ovarian reserve.[8] Higher AMH level is associated with greater number of retrieved oocytes.[9,10] But it is expensive and not easily available everywhere in Nepal.

Though AMH is the gold standard for determining ovarian reserve, it is an expensive investigation not being easily available in all types of health service centers in Nepal. Despite being relatively cheaper and accessible investigations, serum FSH and AFC are equally reliable as compared to serum AMH in determining ovarian reserve in infertile women. So, this study was performed to correlate the relation of antral follicular count and follicular stimulating hormone with anti-mullerian hormone in determining ovarian reserve in infertile women presenting in tertiary health center in Nepal.

METHODOLOGY

A prospective hospital based study was conducted among 78 patients who visited Infertility Clinic of the Department of Obstetrics and Gynecology, Tribhuvan University Teaching Hospital, Kathmandu from 14th April 2017 to 13th April 2018. The patients under chemotherapy and radiotherapy, having endocrine disorders (abnormal thyroid function test, prolactin & testosterone), having suboptimal visualization of the ovary by trans-vaginal ultrasonography, having any ovarian, tubal or uterine structural abnormalities and drop outs on follow up were excluded from the study. Ethical approval from Institutional Review Board of Maharajgung Medical Campus Tribhuvan University Teaching Hospital was obtained. After taking informed consent detailed history was taken and examination of patient was done. Routine infertility investigations (Hemoglobin, blood group and Rh compatibility, Erythrocyte Sedimentation Rate, Total Count, Differential Count, Venereal Disease Research Laboratory test, Human Immunodeficiency Virus test, Hepatitis B Surface Antigen test, Blood Sugar, Mantoux test if required, husband's semen analysis) were sent. For the entire female partners on the 2nd or 3rd day of the cycle, serum FSH and AMH were sent for analysis. On the day 3-5 of the cycle, trans-vaginal scan was done for determining number of antralfollicles; for determining complete shedding of endometrium, and to rule out endometrial polyp and ovarian cysts. The endovaginal sonogram in B mode, with volume probe of 7.5 megahertz was used by single operator. Follicular monitoring was done every alternate day from day 9 of natural cycle till the follicle becomes 18 to 22 mm. If no follicle developed even after 20 days of menstruation monitoring was stopped and development of follicle or failure was noted. This monitoring was done in a single cycle.

Early follicular phase serum FSH, AMH, AFC and age were correlated in relation to follicular development. Data analysis was done using Statistical Package for Social Science 22 (SPSS 22) and correlation of data was tested by Pearson Correlation.

RESULTS

Among 78 patients included, most of them were 25-29 years of age (43.5%) and the mean age was 27.6 ±3.14 years.50% had AMH value more than 4 ng/ml. 39.7% had 1.5-4 ng/ml and 6.5% had 1-1.4 ng/ml. Only 3.8% women had 0.5-1 ng/ml of serum level of AMH which reflected the unfavorable AMH value for fertility prognosis (Figure 2).

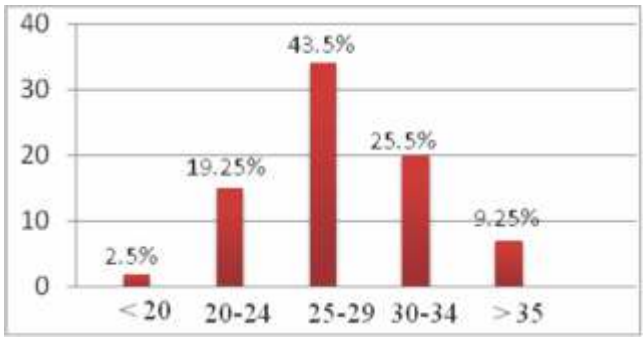

Figure 1: Age group of patients attending infertility OPD (n=78)

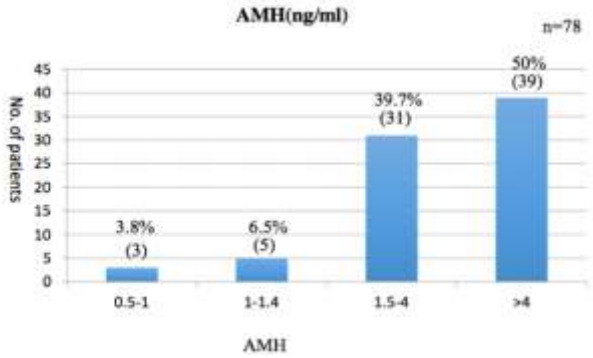

Figure 2: Serum AMH level in patients attending infertility clinic (n=78)

97.5% of the patients had FSH value between 1.9-11.2 IU/ml which denotes the normal range of FSH in females of reproductive age group. 87.2% had FSH level of 3.0 – 8.9 IU/ml, 7.7% of patients had FSH 9- 11.2 IU/ml, 2.6% had FSH level of > 11.2 IU/ml and 2.6% had FSH level of 1.99- 2.99. However, none of the patient enrolled had FSH level of < 1.98 (Figure 3).

Around 67% percent of patients had normal AFC count i.e. 10-18 follicles. 10.5% were poor responders with < 7 follicles whereas 23% of them were hyper responders with > 20 follicles.

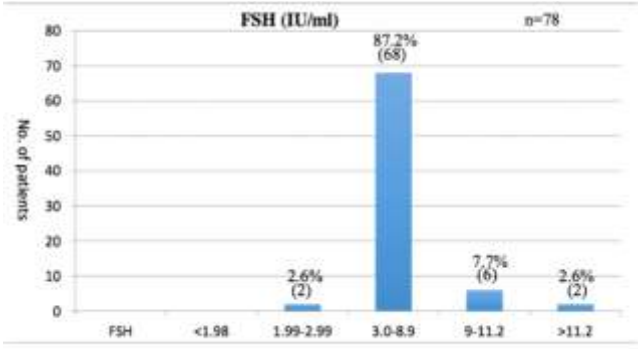

Figure 3: FSH value in patient attending infertility clinic (n=78)

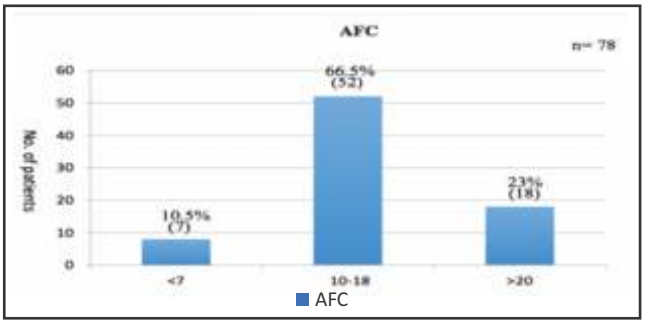

Figure 4: AFC value in patients attending infertility clinic (n=78)

No correlationwas found between age and FSH level (p = 0.064) (Table 1). Weak negative correlation was found between age and AMH(p=0.005 and r= - 0.313) (Figure 5) and age and AFC (p = 0.044). As age increased there was decrease in level of AMH and the result was statistically significant.

Table 1: Correlation between Age and FSH (n=78)

		Age	FSH
Age	Pearson Correlation	1	.211
	Sig. (2-tailed)		.064
	N	78	78
FSH	Pearson Correlation	.211	1
	Sig. (2-tailed)	.064	
	N	78	78

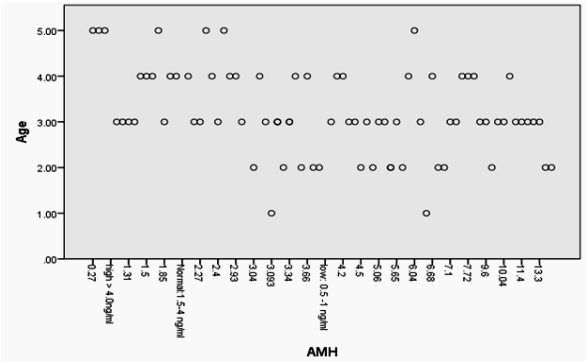

Figure 5: Correlation between Age and AMH (n=78)

There was a linear relationship between AFC and AMH (Figure 6) with a statistically significant strong positive correlation (p= <0.001, r= .811) (Figure 2). This represents thatan increase in AMH results in increase in AFC and vice versa. Thus concluding that AFC can be used as an alternative to AMH in determining ovarian reserve.

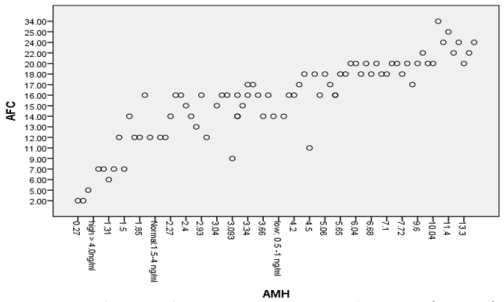

Figure 6: Correlation between AFC and AMH (n=78)

Also, a weak negative correlation was found between FSH and AMH (Figure 7). However, the data was not statistically significant (p = 0.182). So, with this result, it could be concluded that FSH cannot be used as a beneficial alternative to AMH for determining the ovarian reserve.

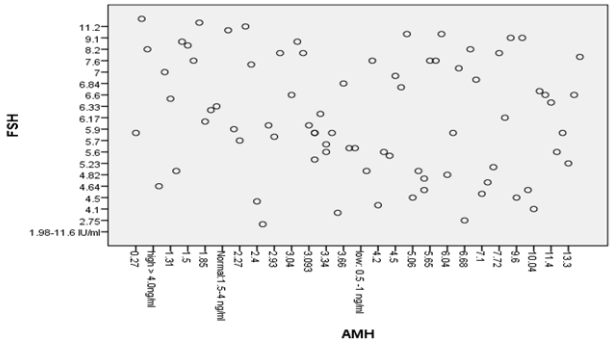

Figure 7: Correlation between FSH and AMH (n=78)

DISCUSSION

Prevalence of infertility is 3.5-16.7% in developed and 6.9-9.3% in developing countries. It is estimated that overall median prevalence of infertility is 9% worldwide.[11] The main causes of infertility is diminished ovarian reserve secondary to aging.[12-14] Attempts for becoming pregnant at earlier ages is considered as the only and the best effective treatment for diminished ovarian reserve.[15,16]

Serum follicle stimulating hormone (FSH), antral follicle count (AFC) and serum anti-Mullerian hormone (AMH) concentration can be used as predictors of ovarian response to gonadotropin stimulation during IVF treatment.[17-20] The age-related decrease in female fertility can be most likely attributable to deterioration in quantity and quality of oocytes. As a result of diminished ovarian reserve, the women's ability to conceive naturally is limited after the age of 40.[21,22] However, relationship between age and ovarian reserve is highly variable.

There are not enough studies performed, assessing the predictive value of these markers in different age groups since some ovarian reserve markers may have different accuracy in different ages. The aim of the current study is to correlate antral follicular count and follicular stimulating hormone with anti-mullerian hormone for determination of ovarian reserve among infertile women attending infertility clinic of TUTH.

In the present study, total patients enrolled were 78 with the mean age of the patients being 27.6 ±3.14 years. Mean FSH was 6.46 ±1.90 IU/ml. There was no any correlation between age and FSH (r=0.211,p=0.64). Similarly,in the study done by Goksede et al. among 141 infertile patients, mean age was 29.18±5.54 years and there was no any correlation between Age and FSH.[23] However, Barbakadze et al. (112 patients) and Battikhi et al.(61 patients) showed positive correlation between age and FSH (r=0.38, p<0.0001) and (r= 0.28, p<0.001) respectively.[24,25] However, in contrary to this study, Bala et al. (75 infertile patients) showed the mean age of infertile females to be 35.39 ± 2.46 years and with increasing age, FSH increased.[26]

AMH is an anti-mullerian hormone secreted by the pre-antral and antral follicle. Mean AMH was 5.01 +/- 3.46 in this study. Here, as the age increases AMH value decreases showing negative correlation of age with AMH (p=0.005, r=-0.313). This could be because of the decreasing pre-antral and antral follicular count in advancing age groups. Similarly, Barbakadze et al. (112 infertile patients) found significantly elevated negative correlation between age and AMH level (r=-0.67, p=0.0001).[24] Bala et al. (75 infertile patients) and Battikhi et al. (61 infertile patients) (r=-0.51,p=0.001) also showed, AMH levels in serum as an indication of a decline in the follicular reserve of the ovaries, which decreases as age advances.[25,26]

The antral follicle count (AFC) is the number of antral follicles, present in both ovaries. It can be determined by trans-vaginal ultrasonography during early follicular phase of cycle. Mean AFC was 15.88 +/- 5.21. There was a negative correlation between Age and AFC (r=- 0.313, p=0.005) meaning, with increasing age there is decrease in AFC. This could be because with increasing age there is regression of follicular pool which has constant number of oocytes. Barbakadze et al. and Himabindu et al. also found negative correlation between age and AFC (r=-0.55,p=0.001) and (r=-0.31, p<0.01) respectively.[24,27]Goksede et al.(141 infertile patients) also showed negative correlation between age and AFC (r= - 0.40, p<0.001) as in this study.[23]

Antral follicle count is typically defined as the number of follicles measuring 2 to 10 mm in greatest diameter. It has been shown that the number of antral follicles in the ovaries is proportionally related to the size of the primordial follicle pool from which the follicles are recruited. Additionally, antral follicles are responsive to FSH and may be considered a predictor of ovarian response to gonadotropins.[28,29] Present study showed that with increasing AFC, AMH value increases. This showed a positive correlation between AFC and AMH (p<0.001, r =0.811) as AMH is also a product of granulosa cells of the pre antral and small antral follicles and AMH is only present in the ovary until menopause after which follicles decline.[30] Similarly, study done by Goksede et. al. showed that serum AMH levels were tightly correlated (r=0.467, p<0.0001) with the number of the early antral follicle count.[23] Gada R et al.(correlation coefficient; R = 0.72) and Bala et al. (r = 0.641, P < 0.001) also showed a positive correlation between AMH and AFC.[26,31] Moreover, Klenov V, Jungheim E. demonstrated AMH was a sensitive and specific predictor of women with high AFC values similar to our study.[32] However, Nelson S, Klein B, Arce J. found AMH to be more strongly correlated with oocyte yield than AFC.[33]

FSH is a gonadotropin, a glycoprotein polypeptide hormone. FSH is synthesized and secreted by the gonadotropic cells of the anterior pituitary gland, and regulates the development, growth, pubertal maturation, and reproductive processes of the body. Weak negative correlation was found between FSH and AMH. However, the relation was not statistically significant (p=0.182, r= -0.153). This could be because FSH is affected by menstrual cycle, however AMH is not. Bala et al.also

showed, although AMH and FSH are not directly dependent, there was a significant inverse relationship between serum AMH and FSH concentration (r = −0.448, P < 0.001) which was taken on day 3 of menstrual cycle.[26] They showed that as AMH increases, FSH decreases which was similar to our study where FSH and AMH showed weak negative correlation. However, Barbakadze et al.(112 infertile patients) demonstrated in their study statistically significant negative correlation between FSH and AMH (r= -0.55, p<0.001).[24] Similar to Barbakadze et al, Battikhi et al.(61 infertile patients) also showed significant negative correlation (r = -0.33, p<0.001) between FSH and AMH.[24,25]

Out of the total patients included in the study 8 patients had conceived. Among those who conceived all had normal AFC and FSH but their AMH level were on higher side. This demonstrated that women who had high AMH level have better conception, however, this result is to be verified with further larger studies.

CONCLUSION

There is a strong positive correlation between serum AFC and AMH levels. However, weak negative correlation was demonstrated between FSH and AMH. Antral Follicular Count is an equally reliable method as anti-mullerian Hormone to see the ovarian reserve. However, other large scale studies should be performed to establish this correlation and determine its value.

LIMITATIONOF THE STUDY

1. Inter-observer variation.
2. Participants lost to follow up.
3. Smaller sample size.

CONFLICT OF INTEREST

We declare no conflict of interest.

FINANCIAL DISCLOSURE

We have no financial disclosure to make.

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