The Professional Medical Journal
www.theprofesional.com

DOI: 10.17957/TPMJ/16.3495

ORIGINAL PROF-3495

IN VITRO FERTILIZATION;

IMPACT OF AMH AND FSH LEVELS ON IVF AND SUCCESS RATE A PROSPECTIVE ANALYTIC STUDY FROM LIFE CLINIC LAHORE PAKISTAN

arkhaja@gmail.com

Dr. Haroon Latif Khan[1], Prof. Dr. Yousaf Latif Khan[2], Mrs. Rameen Makhdoom[3], Dr. Abdul Rahman Khawaja[4]

1. MBBS, MCE (AYS) FECSM
Embryologist & CEO
Lahore Institute of Fertility and Endocrinology, (LIFE)
2. MBBS, FCPS,
Dip Endoscopy (FRANCE)
Consultant Endoscopic Surgeon & Gynecologist
HLH & LIFE
3. M.Sc Biostatistics
Assistant Biostatistician
Lahore Institute of Fertility and Endocrinology, (LIFE)
4. Biostatistician & Research Coordinator
Lahore Institute of Fertility and Endocrinology, (LIFE)

Correspondence Address:
Dr. Abdul Rahman Khawaja
Hameed Latif Hospital 14-Abu Bakr Block
New Garden Town, Lahore.
arkhaja@gmail.com

Article received on:
17/06/2016
Accepted for publication:
25/08/2016
Received after proof reading:
10/09/2016

ABSTRACT: Various ovarian reserve tests were developed to estimate the ovarian reserve and predict about the outcome in subfertile females undergoing evaluation for assisted reproduction. FSH and AMH levels are considered to be good ovarian reserve indicators along with antral follicle count. **Objectives:** To explore relationship of AMH and FSH in patients undergoing IVF with respect to ovarian reserve and outcome of the treatment. **Study Design:** Prospective cohort. **Study Period:** 1st January 2015 to 31st December 2015. **Place of study:** Lahore Institute of Fertility and Endocrinology, Hameed Latif Hospital, Lahore Material and **Methods:** In 346 IVF/ICSI patients after anthropometric measurements and transvaginal ultrasound antral follicle count were assessed in each ovary. For the hormone measurements blood samples were taken during the early follicular phase of menstrual cycle. Clinical pregnancy was also visualized through transvaginal ultrasound. **Results:** From the 346 IVF/ICSI patients 89 (25.79%) clinical pregnancies resulted. The mean age in pregnant group was 32.89 ± 2.99 years and in non-pregnant group was 33.62 ±4.36. Mean FSH and AMH in pregnant group was 6.38 ±2.38, 3.27 ±1.86 and in non- pregnant group was 7.54±3.76, 2.72 ± 1.82 respectively. Age and FSH are significantly associated with each other (p-vale = 0.000) and mostly patients had FSH below 9(mIU/mL). Age and AMH are significantly associated with each other (p-vale = 0.000) and mostly patients had AMH above 1.5 (ng/mL). **Conclusions:** Better pregnancy rate was associated with FSH below than 9 (mIU/mL) and AMH above 1.5 (ng/mL).

Key words: In Vitro Fertilization, Anti-Mullerian hormone, Ovarian Stimulation, Follicle count.

Article Citation: Khan HL, Khan YL, Makhdoom R, Khawaja AR. In Vitro Fertilization; Impact of AMH and FSH levels on IVF and success rate; a prospective analytic study from life clinic Lahore Pakistan. Professional Med J 2016;23(9):1138-1144. DOI: 10.17957/TPMJ/16.3495

INTRODUCTION

To become a parent is a wish of every couple irrespective of creed, clan or status but for a large minority this wish cannot be realized.[1] Last fifty years have seen huge strides in management of subfertility which has made it possible for childless sub fertile couples to consider assisted reproductive technology. Controlled hyperstimulation of the ovaries (COH), in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI) have evolved by the merger of scientific knowledge of biology, pharmacology, endocrinology and surgical technique. Knowledgeable manipulation of menstrual cycle through injectable drugs have made it possible to increase the number of mature oocytes and thus enhance the chances of becoming pregnant through IVF.[2]

Number of oocytes capable of fertilization leading to a successful and healthy pregnancy determines the capacity of the ovaries which is named as the ovarian reserve. It is a natural gift and is influenced by the age of the female. Human ovaries have abundant reserve of eggs which are supplied regularly throughout the reproductive years. Every month an oocyte is released by the ovaries with concomitant destruction of hundreds of other oocytes. Reduction in ovarian reserve starts in the early 30s, and maximizes in the mid-40s.[3]

Estimation of ovarian reserve is an important investigation that is performed before deciding for assisted reproduction in order to predict the response to IVF.[4] Recruitment and development of a number of ovarian follicles in response to external gonadotropins leading to retrieval of good

quality eggs available for IVF and subsequent supply of high quality embryos to be transferred to the uterus is the basis of successful assisted reproduction. When an insufficient response occurs treatment cycle is cancelled. It becomes easier for the fertility clinicians to deny the treatment if these poor responders are identified.[5]

Pharmacological manipulation of the menstrual cycle in IVF may have complications like ovarian hyperstimulation syndrome (OHSS), multiple pregnancies and surgical complications including ascites and even death; physiological and psychological stress and financial costs may be added burden. Assessment of prognostic factors predicting the success or failure of assisted reproductive procedure is imperative to save the patients from the above mentioned probable complications.[6]

Serum estimation of FSH (follicle stimulating hormone), E2 (oestradiol), AMH (anti-Müllerian hormone) and inhibin B is considered as noninvasive indirect evaluation of ovarian reserve.[3]

Assessment of vascularity and volume of the ovaries along with count of antral follicles by ultrasound may be another way of evaluation of the ovarian reserve.[7] The clomiphene citrate challenge test (CCCT), exogenous FSH ovarian reserve test (EFORT), and GnRH-agonist stimulation test (GAST) are provocative methods that have been used to assess ovarian reserve.[3]

Basal FSH (follicle stimulating hormone) levels estimated on day 3 of the menstrual cycles are a commonly used ORT (ovarian reserve test) to gauge the response of the ovaries to hyper stimulation. When population of the follicles is reduced levels of FSH rise. Diurnal variation and inter/ intra cycle variability has been seen in the levels of basal FSH.[8,9,10] Basal FSH levels generally lack sensitivity and specificity to predict a failure or negative outcome. In female who are regularly menstruating prediction of a poor response by serum FSH levels is possible only at very high values. so it is noteworthy serum FSH level has a limited value and that it is helpful in a minority of women only as screening test for poor response for counseling the women.[11,12]

When sub-fertile women are being counseled basal FSH level on day 3 can be used with other parameters when talking about a poor response but should not be used to exclude women who are menstruating regularly.[13]

AMH (Anti-Mullerianhormone) is produced in the ovaries only by granulosa cells. These cells are found in primordial follicles and small antral follicles.[14] Transition of primordial follicles to primary pre-anrtral follicles is marked by secretion of AMH which is biochemically a dimeric glycoprotein. When follicles take the shape of antral follicles (2-6 mm) the secretion of AMH stops. The number of small antral follicles is related to the population of primordial follicles.[15] There is a strong correlation between AMH levels and number of small antral follicles.[16] AMH is not detectable in the blood of post-menopausal women. AMH has certain characteristics which make it unique when ovarian reserve is being checked. There is no intra/ intercycle variability as well as diurnal variation.[17] It can predict poor response with 80–87% sensitivity and 64–93% specificity.[18,19] It can also predict a hyper response.[4]

So this study was planned with an aim to improve the understanding of relationship between AMH and FSH in patients undergoing IVF with respect to ovarian reserve and outcome of the treatment.

METHODS

Study Population

In this study all data were collected prospectively and recorded by authorized staff of the department from January 2015 to December 2015, IVF cycles performed at Lahore Institute of Fertility & Endocrinology with AMH concentrations.

At initial consultation all IVF patients gave an informed consent form for their data to be used for clinical research, statistical reports, and/or

educational purposes provided that their identity remains confidential. This study was approved by the institutional Ethics Committee.

Laboratory Analysis

Blood samples for the hormone measurements were taken during the early follicular phase of menstrual cycle (i.e., cycle days 3–5 of a spontaneous menstrual cycle or a withdrawal bleeding after 10mg of dydrogesteron for 10 days).

Clinical Investigation

Anthropometric measurements and transvaginal ultrasound scanning were performed in between 11-17 day of cycle (mid cycle). The number of follicles measuring 5–9mm in diameter or any dominant follicle (AFC) in each ovary was assessed by a single investigator (M.Š.A.) using a two-dimensional transvaginal probe 5–7MHz (Toshiba, Nemio, Japan). In this study pregnancy was defined as clinic pregnancy. Clinical pregnancy was defined as the visualization of a gestational sac with vaginal ultrasound in gestational week > 7.

Statistical Methods

Statistical analysis was performed using SPSS statistical software (version 15.0). Categorical variables were expressed as percentages and continuous variables were expressed as means ± SD. Comparison of variables was performed with the Chi-square test for categorical variables. All tests were 2 sided with P values <0.05 being considered statistically significant.

For the cross-tabulation analyses, the female patients were categorized by their age into four groups (<35, 35–37, 38–40 and >40 years) and serum AMH concentrations were divided into low, middle and high levels (<0.6, 0.6–1.5 and >1.5 ng/ml).

RESULTS

From the 346 IVF/ICSI patients 89 (25.7%) clinical pregnancies resulted. The mean age in pregnant group was 32.89 ± 2.99 years and in non-pregnant group was 33.62 ±4.36. Mean FSH and AMH in pregnant group was 6.38 ±2.38, 3.27 ±1.86 and in non- pregnant group was 12.44 ± 79.15, 2.72 ± 1.82 respectively. (Table-II)

Variables	Categories	Frequency	Percentages
Age	<35	234	67.6
	35-37	57	16.5
	38-40	37	10.7
	>40	18	5.2
FSH	<9	270	78
	9-11	51	14.7
	>11	25	7.2
AMH	< 0.6	14	4
	0.6-1.5	64	18.5
	> 1.5	268	77.5
Success	Positive	89	25.7
	Negative	257	74.3

Table-I. Descriptive statistics of age, serum FSH & AMH and success

	Pregnant (n=89)	Non pregnant(n=256)	p-value*
Age(years)	32.89 ± 2.99	33.62 ± 4.36	0.287
FSH(mIU/mL)	6.38 ± 2.38	7.54 ± 3.76	0.017
AMH(ng/mL)	3.27 ± 1.86	2.72 ± 1.82	0.057

Table-II. Mean and standard deviation of Age, serum FSH and AMH in pregnant and non-pregnant females

**p < 0.05 is considered statistically significant.*

AMH anti-Müllerian hormone; FSH follicle stimulating hormone;

Patients age was divided into four categories <35 years (n=234) 67.6 %, 35-37 (n=57) 16.5 %, 38-40 (n= 37) 10.7% and >40 years (n=18) 5.2 %. FSH was categorized into three categories < 9 (n=270) 78.0%, 9-11 (n= 51) 14.7 %, >11 (n=25) 7.2 %. Similarly AMH was divided into three categories <0.6 (n=14) 4.0%, 0.6 – 1.5 (n=64) 18.5 % and >1.5 (n=268) 77.5% (Table-I). Pregnancy rate and FSH are significantly associated with each other (p-vale = 0.017) and on the other hand, Pregnancy rate and AMH are significantly associated with each other (p-vale = 0.057) (Table-II).

Patients with age <35 had 29.9 % pregnancy rate, 35-37 had 22.8%, 38-40 had 13.5 % and >40 had 5.6%. Pregnancy rate with FSH and AMH had significant association. Patients with FSH, <9 had 29.3% pregnancy rate, 9-11 had 11.8% and >11 had 16.0 %. On the other hand patients with <0.6 AMH had 14.3%, 0.6 – 1.5 had 15.6 %and >1.5 had 28.7 % pregnancy rate (Table-III).

Age and FSH are significantly associated with each other (p-vale = 0.000) and our mostly patients had FSH below 9(mIU/mL) (Table-IV). Age and AMH are significantly associated with each other (p-vale = 0.000) and our mostly patients had AMH above 1.5 (ng/mL) (Table-V). So, it was concluded that FSH had better pregnancy rate in below than 9 (mIU/mL) and AMH had more than 1.5 (ng/mL).

	Categories	Pregnant (n=89)	Non pregnant (n=256)
AGE	<35	29.9% (70)	70.1% (164)
	35-37	22.8% (13)	77.2% (44)
	38-40	13.5%(5)	86.5% (32)
	>40	5.6% (1)	94.4% (17)
AMH	<0.6	14.3% (2)	85.7% (12)
	0.6-1.5	15.6% (10)	84.4% (54)
	>1.5	28.7% (77)	71.3% (191)
FSH	<9	29.3% (79)	70.7% (191)
	9-11	11.8% (6)	88.2% (45)
	>11	16.0% (4)	84.0% (21)

Table-III. Patient characteristics in pregnant and non-pregnant females

Age(years)	FSH (%, n)		
	<9 (n=270)	9-11 (n=51)	>11 (n=25)
<35	84.6 (198)	11.1 (26)	4.3 (10)
35-37	57.9 (33)	22.8 (13)	19.3 (11)
38-40	73.0 (27)	18.9 (7)	8.1 (3)
>40	66.7 (12)	27.8 (5)	5.6 (1)

Table-IV. Relationship of age with serum FSH

Age(years)	AMH (%, n)		
	< 0.6 (n=14)	0.6 -1.5 (n=64)	> 1.5 (n=268)
<35	3.0 (7)	12.0 (28)	85.0 (199)
35-37	5.3 (3)	29.8 (17)	64.9 (37)
38-40	5.4 (2)	35.1 (13)	59.5 (22)
>40	11.1(2)	33.3 (6)	55.6 (10)

Table-V. Relationship of age with serum AMH

DISCUSSION

Assisted reproduction consists of a series of processes which commence after diagnosis of a treatable cause and detailed assessment of the couple by the learned and experienced clinician. Controlled ovarian hyperstimulation (COH) is a vital step which is followed by in vitro fertilization (IVF) and Embryo transfer (ET).[20] COH is usually tailored according to the reproductive profile of the patient and this vital step may prove to be a failure. This is termed as a poor response.[21]This poor response actually pertains to be a poor ovarian response in which despite standard pharmacological protocol recruitment of sufficient number of follicles does not occur followed by cancellation of the cycle as the chances of a pregnancy are miniscule.[22]

To further elaborate the identification of a poor responder, the working group of (ESHRE) European Society of Human Reproduction and Embryology, declared that at least two of the three of the features mentioned below must be looked for, higher age of the female or another risk factor for the POR (Poor ovarian response), history of a previous POR and an abnormal test for ovarian reserve.[22]

Tests used to quantify the ovarian reserve prior to embark on the exasperating assisted reproductive techniques were thought to be logical and rewarding. Predictive value of day 3 basal FSH was found to be poor and it was proposed that test should not be performed routinely.[11] Female age; AFC and AMH estimation were considered as tests of choice to check the ovarian reserve. It was propounded by Broekmans that no ovarian reserve test is necessary and a first cycle poor response may be sufficient to predict success of ART treatment and whether to continue it or not.[23]

Collection of ovarian follicles and the oocytes contained by them form the ovarian reserve which gradually decrease with age and thereby lessen the reproductive potential of the female.[24] All of the follicles in both ovaries are established during the fetal life as primordial follicles.[25] When a female child is born the count of primordial follicles may be around one hundred thousand. With the start of menarche this count decreases to 0.3-0.5 million.[26,25]

Than Scott et al. published their research in 1989 and confirmed the findings of Muasher et al and thought about the utility of day 3 FSH levels in patients' counselling.[8] In the following 20 years many researchers presented their work about other parameters like AFC (the antral follicle count), Inhibin B and AMH (anti-mullerian hormone) and their predictability regarding ovarian responsiveness and ART outcome in terms of pregnancy.

In 2006 Hehenkamp et al., performed a study about levels of Anti-Müllerian hormone (AMH) throughout one menstrual cycle. The objective of the study was to find out consistence or fluctuation in the levels of AMH along with FSH, LH and E2. The results showed that only AMH showed little fluctuation and remained almost consistent throughout the menstrual cycle. It was concluded that AMH was a reliable marker for ovarian reserve which was not cycle dependent.[17]

AMH estimation may help to quantify the ovarian reserve and thus to titrate the COH, (controlled ovarian hyper stimulation).[24]

REFERENCES

1. Macaluso M, Wright-Schnapp TJ, Chandra A, Johnson R, Satterwhite CL, Pulver A, Berman SM, Wang RY, Farr SL, Pollack LA. **A public health focus on infertility prevention, detection, and management.** FertilSteril. 2010; 93(1):16.e1-10.

2. Feinberg EC, Bromer JG, Catherino WH. **The evolution of in vitro fertilization: integration of pharmacology, technology, and clinical care.** J PharmacolExpTher. 2005 Jun; 313(3):935-42. (Feinberg, Bromer and Catherino, 2005).

3. Sills ES, Alper MM, Walsh AP. **Ovarian reserve screening in infertility: practical applications and theoretical directions for research.** Eur J ObstetGynecolReprod Biol. 2009; 146(1):30-6.

4. Jirge PR. **Ovarian reserve tests.** J Hum Reprod Sci. 2011; 4(3):108-13.

5. Mol BW, Verhagen TE, Hendriks DJ, Collins JA, Coomarasamy A, Opmeer BC, Broekmans FJ. **Value of ovarian reserve testing before IVF: a clinical decision analysis.** Hum Reprod. 2006; 21(7):1816-23.

6. Chen YP, Wu WH, Wu HM, Chen CK, Wang HS, Huang HY. **Effects of anti-Müllerian hormone and follicle stimulating hormone levels on in vitro fertilization pregnancy rate.** Taiwan J Obstet Gynecol. 2014; 53(3):313-6.

7. Hrehorcak M, Nargund G. **"One-Stop" fertility assessment using advanced ultrasound technology.** Facts Views Vis Obgyn. 2011; 3(1):8-12.

8. Scott RT, Toner JP, Muasher SJ, Oehninger S, Robinson S, Rosenwaks Z. **Follicle-stimulating hormone levels on cycle day 3 are predictive of in vitro fertilization outcome.** FertilSteril. 1989; 51(4):651-4.

9. Scott RT Jr, Hofmann GE, Oehninger S, Muasher SJ. **Intercycle variability of day 3 follicle-stimulating hormone levels and its effect on stimulation quality in in vitro fertilization.** FertilSteril. 1990; 54(2):297-302.

10. Kwee J, Schats R, McDonnell J, Lambalk CB, Schoemaker J. **Intercycle variability of ovarian reserve tests: results of a prospective randomized study.** Hum Reprod. 2004; 19(3):590-5.

11. Bancsi LF, Broekmans FJ, Mol BW, Habbema JD, teVelde ER. **Performance of basal follicle-stimulating hormone in the prediction of poor ovarian response and failure to become pregnant after in vitro fertilization: a meta-analysis.** FertilSteril. 2003; 79(5):1091-100.

12. Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. **A systematic review of tests predicting ovarian reserve and IVF outcome.** Hum Reprod Update. 2006; 12(6):685-718.

13. Esposito MA, Coutifaris C, Barnhart KT. **A moderately elevated day 3 FSH concentration has limited predictive value, especially in younger women.** Hum Reprod. 2002; 17(1):118-23.

14. Durlinger AL, Visser JA, Themmen AP. **Regulation of ovarian function: the role of anti-Müllerian hormone.** Reproduction. 2002; 124(5):601-9.

15. vanRooij IA, Broekmans FJ, teVelde ER, Fauser BC, Bancsi LF, de Jong FH, Themmen AP. **Serum anti-Müllerian hormone levels: a novel measure of ovarian reserve.** Hum Reprod. 2002; 17(12):3065-71.

16. de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC. **Antimüllerian hormone serum levels: a putative marker for ovarian aging.** FertilSteril. 2002; 77(2):357-62.

17. Hehenkamp WJ, Looman CW, Themmen AP, de Jong FH, TeVelde ER, Broekmans FJ. Anti-Müllerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. J ClinEndocrinolMetab. 2006; 91(10):4057-63.

18. Muttukrishna S, McGarrigle H, Wakim R, Khadum I, Ranieri DM, Serhal P. **Antral follicle count, anti-mullerian hormone and inhibin B: predictors of ovarian response in assisted reproductive technology? BJOG.** 2005; 112(10):1384-90.

19. La Marca A, Giulini S, Tirelli A, Bertucci E, Marsella T, Xella S, Volpe A. **Anti-Müllerian hormone measurement on any day of the menstrual cycle strongly predicts ovarian response in assisted reproductive technology.** Hum Reprod. 2007; 22(3):766-71.

20. Venetis CA, Kolibianakis EM, Tarlatzi TB, Tarlatzis BC. **Evidence-based management of poor ovarian response.** Ann N Y Acad Sci. 2010; 1205:199-206.

21. Shanbhag S, Aucott L, Bhattacharya S, Hamilton MA, McTavish AR. **Interventions for 'poor responders' to controlled ovarian hyperstimulation (COH) in in-vitro fertilisation (IVF)**. Cochrane Database Syst Rev. 2007 ;(1):CD004379.

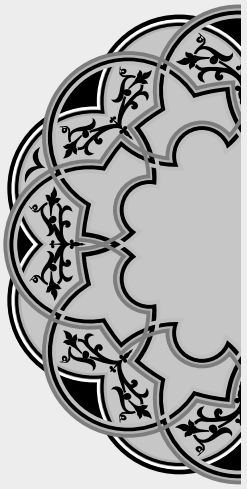
22. Badawy A, Wageah A, El Gharib M, Osman EE. **Prediction and diagnosis of poor ovarian response: the dilemma.** J ReprodInfertil. 2011; 12(4):241-8.

23. Broekmans FJ. **Testing for Ovarian Reserve in Assisted Reproduction programs: the current point of view.** Facts Views Vis Obgyn. 2009; 1(2):79-87.

24. Visser JA, de Jong FH, Laven JS, Themmen AP. **Anti-Müllerian hormone: a new marker for ovarian function.** Reproduction. 2006; 131(1):1-9.

25. teVelde ER, Scheffer GJ, Dorland M, Broekmans FJ, Fauser BC. **Developmental and endocrine aspects of normal ovarian aging.** Mol Cell Endocrinol. 1998; 145(1-2):67-73.

26. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. **Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause.** Hum Reprod. 1992; 7(10):1342-6.



"Perfection is not attainable,
but if we chase perfection we can catch
excellence."

Vince Lombardi

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Dr. Haroon Latif Khan	Main resource person conducted the procedures and generate the data	
2	Prof. Dr. Yousaf Latif Khan	Conceptualization & designing the study and interpretation of results	
3	Mrs. Rameen Makhdoom	Data extraction and data analysis	
4	Dr. Abdul Rahman Khawaja	Write up of introduction, discussion and conclusion	