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Effect of maternal age on the ovarian reserve markers, and pregnancy outcome in a sample of Kurdish women in Erbil city

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Abstract

Background and objective: The ovary has a biological age that does not always correspond to the chronological age; this may be of great importance for the evaluation of women reproductive outcome. This study aimed to determine the effect of maternal age on the pregnancy rate, and the ovarian reserve markers (antimullerian hormone, follicular stimulating hormone, and antral follicular count).

Methods: A cross-sectional study was conducted in the in vitro fertilization center at the Maternity Teaching Hospital located in Erbil city, from January 1, 2015, to January 31, 2016. A convenience sample of 300 infertile women of different age groups was included in the study. Transvaginal ultrasound was conducted to determine antral follicular count, and blood test was done for determination of serum antimullerian hormone and follicular stimulating hormone. The study sample was divided into four age groups. Chi square test, ANOVA test, Pearson correlation, and logistic regression were used to determine the associations.

Results: The biochemical pregnancy rate of the study population was 37% and the clinical pregnancy rate was 32%. The most common type of infertility among the studied sample was primary infertility (74%). Results showed a strong inverse significant correlation between antimullerian hormone and antral follicular count with age, but there was no statistically significant association between maternal age and serum follicular stimulating hormone.

Conclusion: Maternal age is a significant factor that can affect ovarian reserve and causes ovarian aging. The pregnancy rate decreases with advanced maternal age.

Keywords: Antimullerian hormone; Antralfollicularcount; Female age; Ovarian reserve.

Introduction

Ovarian reserve is defined as the ability of the ovary to produce oocyte capable of fertilization and pregnancy. The ovary has a biological age that does not always correspond to the chronological age; this may be of great importance for the evaluation of women reproductive outcome.[1] Woman's age is the single most important factor that determines spontaneous and treatment-related conception, with a gradual decline in fertility especially after the age of 35 years.[2] Although female fertility decline with age, it is difficult to predict the pace of reproductive decline. Women of the same age can have very different responses to ovarian stimulation and have different reproductive outcomes.[3] Ovarian aging is a major determinant factor of pregnancy achievement and it is related to other issues of women's health.[4] Basal serum follicular stimulating hormone (FSH) has been used in in vitro fertilization (IVF) cycles to predict ovarian reserve (OR) and pregnancy rates, but they have limited use since they have a low predictive value.[5,6] AFC has been demonstrated to be a reliable marker of OR , since it correlates significantly with the age-related follicle

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decline, and with ovarian response to IVF stimulation cycles. Decreased antral follicle numbers of basal ultrasound is a sign of ovarian aging, which is a feature observed before an increase in FSH levels,[7] but it doesn't predict failure to conceive.[6] Antimullerian hormone (AMH) production diminishes, as the follicles become FSH dependent.[8] However, its potential to predict embryo quality; implantation and pregnancy rates are still controversial.[9] Although the level of AMH is a good predictor of oocyte quantity, it may not provide information about egg quality. Thus, young women with low AMH levels may have a reduced number of oocyte but appropriate oocyte quality.[10] AMH and antral follicular count (AFC) biomarkers seem to be good predictors of ovarian aging although none has been confirmed as conclusive evidence to predict pregnancy achievement in an assisted reproductive technique (ART) setting. The debate remains which of the two predictors is the most suitable in ART as well as non- ART settings.[11] This study aimed to determine the effect of maternal age on the pregnancy rate, and the ovarian reserve markers (AMH, FSH, and AFC).

Methods

A cross-sectional study was conducted on a convenience sample of 300 infertile women attending the outpatient clinic at the Maternity Teaching Hospital in Erbil city, Kurdistan region, Iraq from January 1, 2015, to January 31, 2016. The study was approved by the Scientific Council of Obstetrics and Gynecology Specialization, Iraqi Board for Medical Specializations. Informed consent was obtained from the entire participants who were included in the study. Women with a history of infertility (primary or secondary, of different ages) were included in the study. Infertility was diagnosed being unable to achieve a successful pregnancy after 12 months of unprotected intercourse in participants aged less than 35 years or after six months of unprotected intercourse regarding women aged over 35 years.[3] Primary infertility was defined as those who have never conceived in the past and who have regular unprotected intercourse for at least one year. Secondary infertility was defined as those who have conceived in the past and who have regular unprotected intercourse for at least one year.[12] Biochemical pregnancy was defined as a pregnancy diagnosed only by the detection of Human Chorionic Gonadotrophin (HCG) hormone in serum. Clinical pregnancy was defined as the evidence of the pregnancy by ultrasound parameters (ultrasound visualization of a gestational sac, embryonic pole with a heartbeat).[13] The inclusion criteria included women of any age complaining from unexplained primary or secondary infertility, having of body mass index (BMI) less than 30 Kg/m^2 and accept to participate in the study. The exclusion criteria included polycystic ovarian syndrome, endocrinological disorders (such as diabetes mellitus, hyperprolactinemia, thyroid diseases, congenital adrenal hyperplasia, Cushing syndrome, adrenal insufficiency), history of oophorectomy, history of ovarian cystectomy, history of chemotherapy exposure, history of radiotherapy exposure, history of ovarian trauma, pelvic inflammatory disease or any pelvic adhesions, history of endometriosis, male factor infertility, chromosomal abnormalities that can cause premature ovarian failure (like Turners syndrome, Down syndrome), and positive serological markers of (HBV, HCV, HIV). The diagnostic approach was clearly and thoroughly explained to both partners when the couples agreed to be fully investigated; they were interviewed separately as well as together, to record all relevant information. For each woman a complete history was recorded then a general physical, as well as the systemic examination was performed including the pelvic examination. Ultrasonic examination (for AFC detection) and hormonal assay were done in the early follicular phase.

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The male factor was judged by semen analysis and detailed history. Weight (in kg) was measured using the same outpatient balance scale for all the women. Height (in cm) was measured on a vertical scale with rigid-adjustable arm piece with the women standing erect and without shoes. Calculation of body mass index was done using the formula (BMI= weight in kilograms/ height in square meters).[14] The integrity of uterus and ovaries for all participants were assessed by transvaginal ultrasound. Ovarian ultrasound scanning was performed to evaluate the number and sizes of antral follicles. All the scans were performed by a single operator on a GE p3 equipped with a 6.5 MHz endovaginal probe (probe destination 8CS/ E8C). The antral follicle count measurement was performed on days 3-5 of the menstrual cycle. All echo-free structures in the ovaries with a mean diameter (of two dimensions) between 2-10 mm were counted as antral follicles. Measurements were taken separately for the right and left ovaries. AFC for each woman was determined by summing total AFC for both ovaries. Hormonal tests were analyzed in the same laboratory (Laboratory department of Fertility Center, Maternity Teaching Hospital). Circulating levels of serum FSH was measured with electro-chemiluminescence immunoassays machine using the E170 kit (Elecys 2010, Modular Analytics E170, cobas e 601, Roche Diagnostic, Germany). The measuring range for FSH was 0.1 to 200 mIU/mL. The normal range of FSH in follicular phase was 3.5- 12.5 mIU/mL. AMH levels were assayed using an ultrasensitive enzyme-linked immunosorbent assay (ELISA) (AMH Gen II ELISA, Beckman Coulter, Inc, 250 S. Kraemer Blvd, Brea, CA 92821 U.S.A.). The lowest detection rate limit and intra-assay and inter-assay coefficients of variation for AMH were 0.03 ng/mL, 3.4, and 7.7 %, respectively. The unit of measurement used for AMH was ng/ml (1 ng/mL = 7.14 pmol/l). Total B-hCG hormone was measured by an electro chemiluminescense immunoassay. The intra- and inter- assay coefficients of variation were 1.7 and 4.5% measuring range was 0.100- 10000 mIU/mL (defined by the lower detection limit and the maximum of the master curve). Measuring level more than 5mIU/ml was regarded as a confirmed chemical pregnancy

Statistical analysis:

Data were analyzed using the statistical package for the social sciences (version 19). Student t-test for two independent samples was used to compare means of two independent groups. One way analysis of variance (ANOVA) was used to compare means of the four groups. A post hoc test (LSD) was used to show significant differences between each two groups (out of the four mentioned groups). Pearson correlation was used to measure the correlation between two numerical variables. Logistic regression analysis was used to show the association between clinical, and chemical pregnancies with several covariates. A *P* value of ≤0.05 was considered as statistically significant.

Results

Three hundred infertile women were included in the study. The mean age (± SD) of the studied sample was 33.36 ± 4.5 years, ranging from 25 to 45 years. The median was 33 years. Table 1 shows that 41% of the sample were in the age group 30-34 years, and only 8.3% were 40 years and over. The majority (74%) were of primary infertility. Table 2 shows that the more the age, the less the rate of chemical and clinical pregnancies (*P* = 0.005 and 0.007 respectively). The biochemical pregnancy rate of the study population was 37% and the clinical pregnancy rate was 32%. Table 3 shows that there was a significant decrease of the means of AMH and AFC with increasing age (*P* <0.001). ANOVA showed no significant differences in the means of FSH of different age groups (*P* = 0.079).

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Table 1: Distribution of the sample by age and type of infertility.

Variables	Categories	No.	%
Age (years)	25-29	75	25.0
	30-34	125	41.7
	35-39	75	25.0
	≥ 40	25	8.3
Type of infertility	Primary	222	74.0
	Secondary	78	26.0
Total		300	100.0

Table 2: Rates of chemical and clinical pregnancies by age.

Age (years)	N	Chemical pregnancy		Clinical pregnancy	
		No.	%	No.	%
25-29	75	38	50.7	34	45.3
30-34	125	47	37.6	40	32
35-39	75	22	29.3	19	25.3
≥ 40	25	4	16	3	12
Total	300	111	37	96	32
		p = 0.005		**p = 0.007**	

Table 3: Means of FSH, AMH, and AFC by age.

Variables	Age (years)	N	Mean	SD	p (ANOVA)	LSD (groups)	*P* (LSD)
FSH	A) 25-29	75	6.19	1.05	.079	A X B	.010
	B) 30-34	125	6.85	2.18		A X C	.087
	C) 35-39	75	6.68	1.33		A X D	.223
	D) ≥ 40	25	6.68	1.70		B X C	.500
	Total	300	6.63	1.73		B X D	.659
						C X D	.993
AMH	A) 25-29	75	3.30	.51	< 0.001	A X B	< 0.001
	B) 30-34	125	2.82	.62		A X C	< 0.001
	C) 35-39	75	1.45	.58		A X D	< 0.001
	D) ≥ 40	25	.96	.62		B X C	< 0.001
	Total	300	2.44	1.01		B X D	< 0.001
						C X D	< 0.001
AFC	A) 25-29	75	22.24	1.46	< 0.001	A X B	< 0.001
	B) 30-34	125	18.75	2.99		A X C	< 0.001
	C) 35-39	75	12.85	3.56		A X D	< 0.001
	D) ≥ 40	25	7.20	1.73		B X C	< 0.001
	Total	300	17.19	5.30		B X D	< 0.001
						C X D	< 0.001

FSH: Follicular Stimulating Hormone(mUI/ml), AMH(ng/ml): Antimullerianhormone, AFC: Antral Follicular Count

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Table 4 shows that the mean age of women with successful pregnancy was significantly less than the mean age of women with pregnancy failure (*P* <0.001), while the means of AMH and AFC were significantly higher among those with successful pregnancies compared with means of those with failed pregnancies. Figure 1 shows no significant correlation between FSH and age,

Table 4: Mean age, FSH, AMH, and AFC of patients with pregnancy outcome.

		Means ± SD			
		Age(y)	**FSH**	**AMH**	**AFC**
(Chemical pregnancy)	Success	32.1±3.9	6.5±1.7	2.7±0.9	18.1±4.7
	Failure	34.1±4.6	6.7±1.7	2.3±1	16.6±5.5
***P* value**		< 0.001	0.331	< 0.001	0.016
(Clinical pregnancy)	Success	32±4	6.5±1.7	2.7±0.9	18.2±4.7
	Failure	34±4.6	6.7±1.7	2.3±1	16.7±5.5
***P* value**		< 0.001	0.347	0.001	0.021

FSH: Follicular Stimulating Hormone(mIU/ml), AMH(ng/ml): Antimullerianhormone, AFC: Antral Follicular Count

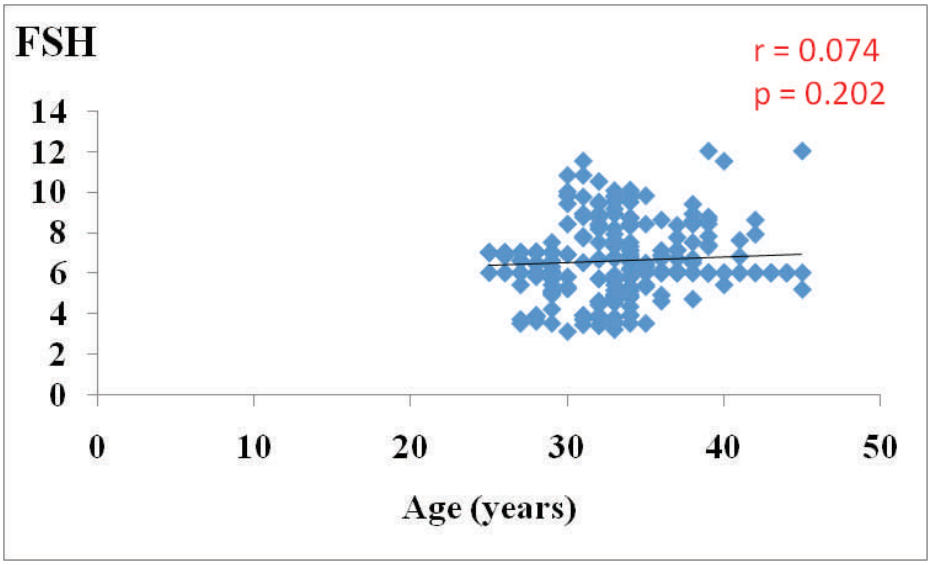

Figure 1: Correlation between FSH (Follicular Stimulating Hormone) and age.

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whereas Figure 2 and 3 show a strong inverse significant correlation between AMH and AFC with age (*P* <0.001). Table 5 shows that chance for successful clinical pregnancies is more among those with secondary infertility compared with those with primary infertility (OR = 1.92, *P* = 0.022).

Table 5: Logistic regression analysis between successful clinical pregnancies as a dependent variable, with infertility type, age, AMH, and AFC as independent variables.

Variables	B	*P* value	OR	95% C.I. for OR	
				Lower	Upper
Secondary infertility	.657	.022	1.929	1.102	3.377
Primary infertility (reference)					
Age(year)	-.131	.025	.877	.782	.984
AMH(ng/ml)	.293	.163	1.341	.888	2.024
AFC	-.073	.108	.929	.850	1.016
Constant	3.932	.141	51.022		

AMH(ng/ml): Antimullerian hormone , AFC:Antral Follicular Count.

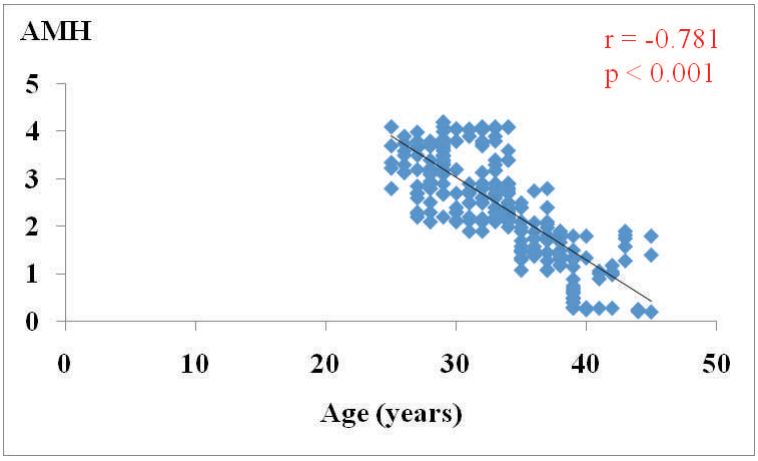

Figure 2: Correlation between AMH: Antimullerianhormone and age.

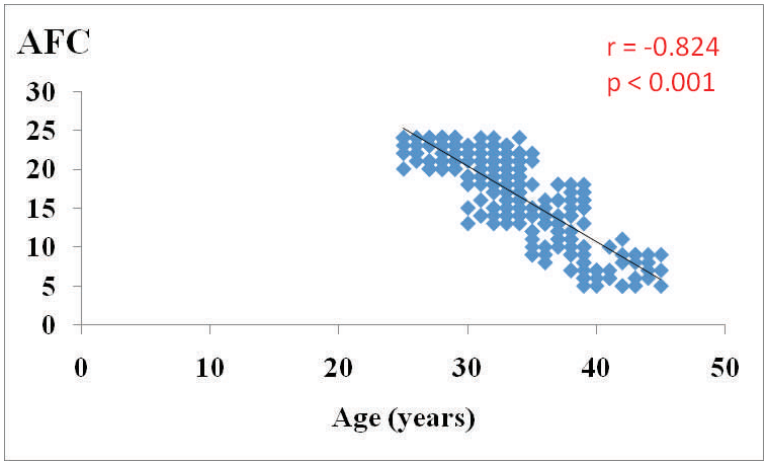

Figure 3: Correlation between AFC (Antral follicular count) and age.

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Table 5 and 6 show that age is inversely related to successful clinical and chemical pregnancies (OR = 0.877, *P* = 0.025; OR = 0.893, *P* = 0.043, respectively). The more the age (irrespective of other factors), the less chance for clinical and chemical pregnancies.

Discussion

Women experience an age related decline in reproductive function before other organ systems begin to fail. By the age of forty, half of the women are infertile. The other half exhibits markedly decreased fecundity compared to younger age groups. Maternal age provides the best predictor of oocyte quality and development capacity in vitro and in vivo. As women become older, their chance of conception decreases while the risk of miscarriage and aneuploidy in the conceptus increases.[15] The mean age of the women seeking infertility services in the current study was 33.3 ± 4.50 years, which is similar to that reported in Alexandria, Egypt [16]and that reported in the UK where the average age of women was 31 years.[17] However, the mean age of women in this study was higher than what was reported in neighboring countries, and the Eastern Asian countries. In Kuwait, the mean age was 29.9± 5.4 years,[18] while it was 29 ± 6.0 years in Iran and 25.9 ± 4.18 years in India.[19,20] The causes may be the lower educational attainment, cost issues and most importantly the delay in establishing of fertility and ART centers in our country because of the wars and civil strife for decades with minimal infrastructure and organizations. A considerably high frequency of primary infertility (74%) was observed in the current study. This finding confirms other regional and national studies in this aspect. A study in Duhok, Iraq on 250 infertile couples revealed that 77.2% had primary and 22.8% had secondary infertility.[21] A meta-analysis study in Iran reported that 78.4% of couples had primary and 21.6% had secondary fertility problems.[1] In Kuwait, 63% of the infertile women had primary infertility and 37% had secondary infertility,[18] and in Egypt the rates of primary and secondary infertility were 70.7% and 29.3% respectively among infertile couples.[22] The most comprehensive study of infertility was that of the WHO on 5800 infertile couples seeking help at 33 medical centers in 22 developed and developing countries, which revealed that the majority of infertile couples around the world suffer from primary infertility. However, in sub-Saharan Africa, the percentage of primary infertility was 37.1% and secondary infertility was 62.9%.[23] In many cases in the developing countries, secondary infertility in women result from untreated pelvic inflammatory disease, a sequel of STDs or other reproductive tract infections.[24] These data implicate that in Muslim countries such as Iraq, Iran, Kuwait and Egypt the pattern of infertility is different and the primary type is more common. It could be due to religious related factors, cultural factors and sexual

Table 6: Logistic regression analysis between successful chemical pregnancies as a dependent variable, with infertility type, age, AMH, and AFC as independent variables.

Variables	B	*P* value	OR	95% C.I. for OR	
				Lower	Upper
Secondary infertility	.391	.161	1.478	.856	2.553
Primary infertility (reference)					
Age	-.114	.043	.893	.800	.996
AMH(ng/ml)	.278	.168	1.321	.890	1.961
AFC	-.060	.165	.942	.865	1.025
Constant	3.469	.177	32.115		

behavior. The correlation coefficient (r) between age and AMH in the current study was (-0.781). The reported correlations between age and AMH are dependent on the population studied; the value varied between (r= 0.30 to 0.66).[25] Similarly, Rosen in 2012 reported a correlation value of 0.46; they do report a quantitative loss across different age groups. This age related decrease in AMH levels agrees with the results from previously published studies where a negative correlation between age and serum AMH levels has been reported.[26] AFC declines progressively over time and its correlation with reproductive aging is nowadays well established.[27] A negative moderate correlation between AFC with age was noticed, this is attributed to age related follicle loss in the ovaries.[28] Elevated day three menstrual cycle of serum FSH level correlate well with those in late perimenopause and menopause, and milder elevations have been considered the hallmark of ovarian aging.[29] Earlier studies suggested that serum FSH levels significantly rise starting in the fifth decade of life.[30]A study conducted by Scheffer revealed a positive weak correlation between serum FSH level and age (r= 0.25, *P* <0.05); while our study showed non-significant correlation.[31] There is a considerable amount of variation in the correlation with any marker of ovarian reserve that cannot be explained by age alone; that is why only a moderate or weak correlation between the age and the ovarian reserve markers was demonstrated. Even with the best markers, AFC and AMH, it has been suggested that more than 70% of the variation in women of reproductive age is left unexplained by age. This finding illustrated that age might be the sole determinant of OR.[32] It is noted that the number of the growing antral follicles is correlated to the number of primordial follicles.[33] Most of these markers of ovarian reserve, other than serum FSH, are the direct product of growing antral follicles. If there is a disturbance in the number of growing follicles, measures of the ovarian reserve may not be reliable.[34] Moreover, with increasing age the prevalence of large follicles increase, which may also have a negative association with serum AMH.[35]

Conclusion

Age is a major determinant that affects the ovarian reserve and causes ovarian aging; age decreases fertility rate. There is an inverse correlation between age and AMH and AFC levels and a weak correlation between age and FSH.

Competing interests

The authors declare that they have no competing interests.

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