ORIGINAL ARTICLE

Association of ovarian reserve with age, BMI and serum FSH level in subfertile women

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Abstract

Objective: To examine the association of age, body mass index, serum follicle stimulating hormone levels and antral follicular count with fertility outcomes in terms of conception and delivery.

Methods: The prospective cohort study was conducted at Baqai Institute of Reproductive and Developmental Sciences, Karachi, from January to December 2012.and comprised women undergoing the first cycle of in-vitro fertilisation who were categorised into groups A and B depending upon age. Group A consisted of women below 35 years of age, while Group B had women over 35 years of age. Age body mass index (BMI), basal level of serum follicle stimulating hormone and antral follicular count were determined in all subjects. Intracytoplasmic sperm injection was dispensed, and all subjects were followed up prospectively and their cycle outcomes were recorded. SPSS 20 was used for statistical analysis.

Results: Of the 148 women in the study, 98(66%) were in Group A and 50(44%) in Group B. In Group A, 15(38.5%) women with body mass index <25 and 24(61.5%) with >25with normal follicle stimulating hormone levels showed positive outcome. Moreover, 20(51.3%) women with normal ovarian reserve and 19(48.7%) with higher reserves showed positive pregnancy test and outcome. Group B showed <7 follicular count on ultrasonic examination, <5 eggs were retrieved in 36(72%) women and all of them (100%) had negative outcome.

Conclusion: Sub-fertile women at age 35 years and above and body mass index over 33 had reduced fertility potential compared to younger women, and ultimately had lower success rate of fertility treatment, including in-vitro fertilisation.

Keywords: Assisted reproduction technique, Ovarian reserve, Body mass index, Antralfollicular count. (JPMA 66: 409; 2016)

Introduction

Ovarian reserve is an estimate of the primordial follicle pool in the ovaries and is indicative of the reproductive age of a woman as opposed to chronological age. It helps in assessing a woman's reproductive potential in predicting possible response in assisted conception techniques, as it correlates with functional status of ovaries and quality of oocyte.[1]

Ovarian reserve consists of two separate components, both of which determine a woman's chance of conceiving a child with in-vitro fertilisation (IVF). The first component is the number of extra follicles that are available to undergo recruitment with treatment using fertility medications. The second component is actual health of follicles and the quality of eggs.[2] Those women who respond well to fertility treatment are described as having normal ovarian reserve, while those with poor response are described as having diminished ovarian reserve. The concept of ovarian reserve testing therefore represents a means by which a physician attempts to evaluate a woman's reproductive potential both in terms of number of follicles and their health.[3]

The need to test ovarian reserve arises when women present with more than 30 years of age, unexplained infertility, recurrent miscarriages, prior ovarian surgery, family history of premature ovarian failure and poor response to assisted reproduction techniques (ARTs) etc.[4] Early ovarian ageing currently affects 10% of the general population and may well be an important cause of infertility. As such, identification of individuals with shorter reproductive lifespan is imperative as ovulation induction is one of the greatest achievements of reproductive endocrinology.[5]

Various methods have been proposed and are currently in vogue for the assessment of ovarian reserve. The commonly used methods include estimation of biochemical markers such as serum follicle stimulating hormone (FSH), estradiol, inhibin and antimullerian hormone levels, as well as ultrasonic measurement of ovarian volume and antral follicle count (AFC) per ovary.[6] Antral follicles are small resting follicles 2-8mm in diameter that can be seen, measured and counted by transvaginal ultrasound (TVU). The count predicts the number of mature follicles stimulated when injectable

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medications are used for IVF.

Age has been considered the single most important factor influencing natural oocyte endowment and reproductive outcomes. Fertility potential reaches its peak in early 20s, declines significantly after age 30, reaching zero at mid-40s. Literature search revealed that poor ovarian response to gonadotrophins is a significant problem if age is more than 35 years; moreover cutoff age value between successful and unsuccessful outcomes is proved to be 35 years by many studies.[3,7-10] Age has also been used to predict excessive response to treatment by a meta analysis of 57 studies reporting databases.[11]

Ovarian aging is a multifactorial trait, governed by several factors, including genetic, lifestyle, medical and idiopathic etc. Poor ovarian reserve is indicated by low number and poor quality of oocytes and embryos, resulting in poor implantation and high miscarriage rates.[12] AFC count and anti-mullerian hormone levels seem to be most reliable predictors of ovarian aging.[13] Ovarian aging patterns differ between races and appear to affect fertility treatment outcomes as African donors demonstrate large oocyte yields than Caucasians and Asians. This variation has been recently associated with specific ovarian genotype and subgenotype of FMR1 gene.[14]

The adverse effects of increase in body mass index (BMI) on fertility has also been reported in many studies, as overweight and obese women had a statistically significant low AFC compared to normal-weight women.[15] On the contrary, another study reported that AFC did not differ by body size but anti-mullerian hormone levels were lower in obese women compared to normal-weight women.[10]

High FSH levels strongly predict poor IVF response in older women compared to younger women. A study reported that elevated basal day 3 FSH level is strongly correlated with diminished ovarian reserve in women aged more than 35 years and is associated with poor pregnancy rate (6% versus 42%) after induction of ovulation.[16] The combined use of age and basal FSH significantly improves the predictive power for these parameters, but age is a better predictor of pregnancy potential for women undergoing IVF.[17]

TVU assessment of ovarian volume and AFC is a practical and cost-effective tool for ovarian reserve testing. Moreover, it is an easy-to-perform and non-invasive method and predicts ovarian response more accurately compared to basal FSH level.[18]

Ovarian aging is a serious issue and should be considered before referring a patient to ART as none of the stimulation protocols or ART interventions has been shown to be convincingly beneficial to women with aging ovaries.[13] Unfortunately, lack of knowledge about the availability of ARTs leads to late referral of patients to IVF centres in our population with exceeding age and diminishing ovarian reserve.

The current study was planned to find out the relationship and interaction between indicators of ovarian reserve and IVF treatment outcomes in terms of conception and delivery of normal child.

Subjects and Methods

The prospective cohort study was conducted from January to December 2012 at Baqai Institute of Reproductive Sciences (BIRDS), Karachi, and comprised healthy women enrolled for first cycle of IVF.

The subjects were categorised in groups A and B on the basis of age. Group A comprised women below 35 years of age, whereas Group B had subjects over 35 years of age.

The sample size was calculated by reviewing fertility outcomes according to age in previous studies[19-23] and applying following formula with 95% confidence interval (CI) and 5%chance of error:

$$n = \frac{(Z_{\alpha/2} + Z_{1-\beta})^2 \times pq}{e^2}$$

After approval from the Research and Ethical committee of Baqai University and written consent by the subjects, baseline serum FSH, Luteinizing hormone (LH) and estradiol levels were determined prior to the enrolment of patients. Age, height and body weight of every participant was recorded and BMI was calculated. The BMI was categorised as normal up to the value of 25, overweight between 25-27 and obese over 27. A general examination was done to rule out any general illness.

Subjects with more than 40 years of age and those suffering from any chronic illness or previous history of ovarian surgery, pelvic adhesions, polycystic ovary etc. were excluded.

For the determination of serum FSH level in early follicular phase (1-3 day of menstrual cycle), 2 cc blood was drawn and serum FSH was determined with commercially available kit on enzyme-linked immunosorbent assay (ELISA).

For the measurement of AFC, a TVU examination on day 2 to 5 of menstrual cycle was done.

Using the Long protocol, in the 1st cycle pituitary down-regulation with gonadotropin-releasing hormone (GnRH)

analogues in the form of nasal spray was started 1-5 times a day on day 21.Inj. FSH was given daily for 5 days subcutaneous or intragluteal (dose adjusted according to age, FSH and ovarian reserve). Ultrasound-guided follicular tracking was done on days 6, 8, 10 and 12, and dose of gonadotrophins was adjusted. LH was added daily when size of follicle was more than 1.4cm. Human chorionic gonadotrophins (HCG) 10,000 iu was given when follicle size exceeded 1.8 cm. Ultrasound-directed follicular aspiration (UDFA) was done under anaesthesia and the collected ova were incubated in specific media.

Intra-cytoplasmic sperm injection (ICSI) was dispensed in good-quality oocytes with morphologically normal sperms and were further incubated for fertilisation. Embryo transfer was done after 2-3 days, decided on the basis of number and quality of eggs. Serum Beta-HCG was performed to confirm pregnancy on day 12-14 of the embryo transfer.

Using the Short protocol, stimulation and down-regulation of pituitary was done on day 2 of menstrual cycle in which ICSI were planned. The rest was the same as in the Long Protocol from day 2 till egg pick-up.

Data was analysed using SPSS 20. Frequency and percentage were calculated for categorical variables like age and BMI. Chi-square test was applied to determine the significance between the categorical variables. P<0.05 was taken as significant and 95% CI was used to see effect size. Logistic regression was also performed to ascertain the predictive power of ovarian reserve and FSH levels on the likelihood of fertility outcomes.

Results

Of the 148 women in the study, 98(66%) were in Group A and 50(44%) in Group B. The overall BMI was <25 in 47(32%) subjects, and >25 in 101(68%).

In Group A, fertility outcome in terms of conception and delivery was positive in 15(38.5%) females with BMI <25 and in 24(61.5%) women with BMI >25; while it was negative in 20(33.9%) with BMI <25 and 39(66.1%) with BMI >25.The serum FSH level was normal (<8) in 27(36%) subjects with BMI <25 and in 48(64%) with BMI >25; whereas higher levels were recorded in 8(34.8%) subjects with BMI <25 and in 15(65.2%)with >25 BMI.

Poor ovarian reserve of less than 10 follicles was not found in any women in Group A with positive outcome; normal reserve between 10 to 15 follicles was present in 20(51.3%) and more than 15 follicles were found in 19(48.7%) women with positive outcome. It was observed that 25(42.4%) women with normal ovarian reserve and 33(55.9%) with higher ovarian reserve in Group A did not conceive. Normal ovarian reserve was associated with normal FSH level in 34(45.3%) subjects, while 40(53.3%) with higher reserve had normal FSH levels (Table-1).

In Group A, less than 10 follicles were retrieved in 15(15.3%) women, while more than 10 follicles were achieved in 83(84.6%). Less than 5 eggs were fertilised in 34(34.6%) subjects, while more than 5 eggs were fertilised in 64(65.3%). Pregnancy test was positive in 39(39.7%) cases and negative in 59(60%) cases.

In Group B, less than 7 follicles were retrieved in all 50(100%) patients; no egg was retrieved in 14(28%);1-5 eggs in 36(72%); and just two women were able to conceive but aborted at 8th week, so the outcome was

Table-1: Association of ovarian reserve with fertility outcome and serum FSH level in women <35 years (Group A: n=98).

Characteristics		Ovarian Reserve			
		Poor Reserve Frequency (%)	Normal Frequency (%)	Chances of Hyperstimulation Frequency (%)	P-value
Fertility Outcome	Positive	0(0)	20(51.3)	19(48.7)	0.523
	Negative	1(1.7)	25(42.4)	33(55.9)	
Serum FSH level	Normal (< 8)	1(1.3)	34(45.3)	40(53.3)	0.846
	Abnormal	0 (0)	11 (47.8)	12 (52.2)	

FSH: Follicle stimulating hormone.

Table-2: Characteristics of women with respect to BMI, Ovarian reserve and FSH level in women >35 years (Group B: n=50).

Characteristics		Frequency	Percentage
BMI Groups	< 25	23	46.0
	25-27	10	20.0
	> 27	17	34.0
Ovarian Reserve	< 10	50	100.0
Serum FSH level	Normal	10	20.0
	Abnormal	40	80.0

BMI: Body mass index
FSH: Follicle stimulating hormone.

Table-3: Logistic regression for predicting ovarian reserves and FSH level with fertility outcomes.

Variable	Odds ratio	95% CI
Ovarian reserves		
Poor Reserve	1a	-
Normal	1.156	0.239 - 3.146
PCO and Hyperstimulation	0.636	0.215 - 1.955
FSH		
Abnormal	1a	-
Normal	1.306	0.555 - 3.072

CI : Confidence Interval, 1a : Reference category.
FSH: Follicle stimulating hormone.

negative in all of them (Table-2).

The result of logistic regression model explained 5.2%variance in fertility. Normal ovarian reserve (odds ratio [OR]: 1.156; 95%CI: 0.239, 3.146) and chances of polycystic ovarian syndrome (PCOS) and hyperstimulation of ovary (OR: 0.636, 95%CI: 0.215, 1.955) were more likely to influence fertility outcomes than poor ovarian reserve. Normal level of FSH (OR: 1.306; 95%CI: 0.555, 3.072) were more likely to influence fertility outcome than abnormal levels (Table-3).

Discussion

The study investigated the association of increasing age, BMI and follicular count on serum FSH level and fertility outcomes in 148 women undergoing IVF. Data revealed that advanced maternal age was associated with reduced fertility and adverse pregnancy outcomes. The decay in oocyte quality contributed to gradual decline in fertility as age advanced.

Among Group A subjects aged less than 35 years and with normal or more than normal ovarian reserve, 39 out of 98 women showed positive pregnancy outcome and delivered at term; whereas in Group B with women above 35 years of age, 48 women showed negative pregnancy outcome, 2 conceived but aborted at 8th week. This is consistent with a study which reported that declining cohort of antral follicles with age resulted in gradual elevation of FSH level, a decrease in anti-mullerian hormone level and a poor response to ovarian stimulation.[24]

Increased BMI of patients entering IVF have been documented to have a negative impact on final outcome and certainly reduces the success of the process resulting in reduced clinical pregnancy. Interaction of BMI and age showed a strong and significant impact on the outcome of IVF, seen through the achievement of clinical pregnancy.[25]

When categorised according to BMI, our study revealed that 15(38.5%) women with BMI<25 had successful outcomes; while 24(61.5%) with BMI >25 showed positive outcome. Thus, BMI was not found to be associated with fertility outcome in our study up to age 35.

Overweight and obese women with diminished ovarian reserve (DOR), as defined by high day 3 serum FSH levels, have been reported to have lower number of oocytes retrieved compared with non-obese women with DOR. This is confirmed by another study in which women with elevated BMI and DOR had lower number of oocytes retrieved compared with normal BMI and DOR.[26,27]

In group A, serum FSH level was normal (<8) in 27(36%) women having BMI <25 and in 48(64%) with BMI >25; whereas the level was higher than normal in 8(34%) and 15(65.2%) women with BMI <25 and >25 respectively. When FSH levels were compared with BMI, no statistically significant association was found.

When FSH levels were compared with ovarian reserve, normal FSH levels were significantly associated with normal or higher ovarian reserve compared to poor reserve. Abnormally high FSH levels were found in 11(47.8%) cases with normal and 12(52.2%) cases with higher than normal ovarian reserve. Both increasing age and higher basal FSH were associated with reduced number of oocytes collected, oocytes fertilised and embryos transferred.

After stimulation in Group A, less than 10 eggs were retrieved in 17(17.3%) and >10 in 81(82.6%) cases. In 35(35.7%) women <5 eggs and in 53(64.3%) women >5 eggs were fertilised after ICSI. Embryo transfer was done in all women and resulted in positive outcome in 39.

Among Group B subjects, 100% had ovarian reserve of <7 follicles, 54% women had BMI >25, 44% women had Serum FSH level <8 while 56% had more than 8. After stimulation, no eggs were retrieved in 14(28%) cases, while <5 eggs were obtained in 36(72%) cases. No egg was fertilised in 10% and less than 5 eggs were fertilised in 90% cases. In this group, just 2 women were able to conceive but aborted at 8th week, whereas rest of them had negative outcome. This finding is confirmed by a study which suggested that ART should not be attempted in women over 35 years of age as none of the stimulation protocol intervention has been shown to be convincingly beneficial to ovary-aging women.[13]

Thus, age up to 35 years was not found to be associated with serum FSH level and fertility outcome. Similar

observation was made with BMI. However, ovarian reserve was associated both with FSH level and fertility outcome below age 35 years, and above this age it was significantly associated with decline in fertility outcome.

Conclusion

The number of antral follicles and normal FSH levels reflected the ovarian pool and indirectly the reproductive age. Thus, early referral of infertile couples to IVF centres is imperative, especially in a society where neither donor eggs are accepted for IVF, nor is the concept of surrogate mother applicable.

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