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The Correlation Between Female Age and Ovarian Reserve Biomarkers (FSH & AMH) and Its' Effect on The Response to Controlled Ovarian Hyper-stimulation (COS) and Pregnancy Rate Following Intracytoplasm sperm injection (ICSI)

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ABSTRACT

Several methods are developing to assess the biological and functional age of women ovary. Since ovarian reserve (OR) may change over time, limited ovarian reserve needs further tests to be confirmed. The likelihood of sub-fertility treatment success is highly dependent on the women's age. With advanced age, the likelihood of having a child decreases significantly making those women in high need of assisted conception.

The study aimed to assess the association between female age, ovarian reserve biomarkers (follicular stimulating hormone and anti-mullerian hormone), response to controlled ovarian stimulation (COS) represented by the dose of gonadotropin stimulation, number and quality of retrieved oocytes, cycle cancellation rate and pregnancy rate in those who undergo ICSI.

One hundred twenty six couples complained of sub-fertility collected from Al-Kafeel Fertility and IVF Center/ Holy Kerbala/ Iraq throughout a period between 2016-2019. These couples have been evaluated and subjected to COS/ICSI and divided into two groups below and above 35 years old. Assessment of cycle day 2 FSH and AMH, response to COS, cycle cancellation rate, and the pregnancy rate has been performed, and the results compared between both.

The study showed that baseline serum level of FSH and AMH did not differ between women older and younger than 35 years old (in younger women, mean serum FSH 6.6±4.0 and AMH 2.4±1.8 vs 7.7±4.3 and 2.2±2.8 respectively in older ones, p-value=0.15). Women older than 35 years old needed significantly higher doses of gonadotropin stimulation(mean total dose of r-FSH 2179.4±1222.2 vs 1987.2±947.6, p-value=0.036 and mean total dose of HMG 3122.3±1456.8 vs 2468.7±1454.8, p-value=0.02), produced significantly lower number of oocytes (6.09±4.7 vs 9.8±6.4,p-value=0.0001), lower number of mature oocytes (4.4±3.8 vs 6.7±5.1,p-value=0.005), higher rate of cycle cancellation(17.7% vs 4.6%) and insignificantly lower pregnancy rate(23.5% vs 37.7%, p-value=0.107) following ICSI in comparison with those younger than 35 years old.

Age is a significant determinant factor that affects the ovarian reserve, can lead to ovarian aging, and lower the women's fertility rate. There is a weak correlation between age, FSH, and AMH levels. Older women exhibit a low response to ovarian stimulation with a higher cancellation rate, produce a small number of oocytes with low quality following COS despite their higher need for large doses of gonadotropin stimulation. The pregnancy rate is affected by advanced women's age following ICSI.

Keywords: Female age, Ovarian reserve, FSH, AMH and ICSI

Introduction

As a woman gets older, her ovarian storage of oocytes gradually declines with time until depletion at menopause. Although we except the ovary to age in a certain way, there are times when it does not behave as predicted. Thus, screening for ovarian reserve (OR) is a fundamental part of the initial evaluation for sub-fertility patients of any age (Lee *et al.*, 2009).

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The term OR refers to women's current supply of oocytes and is closely associated with reproductive function; the greater the number of oocytes, the better the chance for conception. Conversely, low OR significantly reduces a patients' chances of having a child. The primary value of OR markers is to provide a guide in selecting an appropriate protocol or initial dose of gonadotropins for controlled ovarian stimulation (COS) in IVF cycles or both (Maheshwari *et al*., 2006).

In addition to being makers for the ovarian response, an efficient indicator of pregnancy outcome before COS would be an enormous help during counseling, especially for expensive treatments, such as IVF and ICSI. As a result, markers for OR and ovarian aging before COS are frequently using to predict the pregnancy potential of IVF cycles (Broekmans *et al*., 2006). However, OR and ovarian aging biomarkers before COS fail to predict pregnancy outcome efficiently (Bancsi *et al*., 2002).

The early follicular phase serum FSH level of 6.7-15 iu/l with or without antral follicular counts (AFC) of 5-7 represent the most common biochemical and ultrasound markers for ovaries aging in clinical practice, respectively (Muttukrishna *et al*., 2005).

Anti-Müllerian hormone (AMH) is a member of the transforming growth factor (TGF-β) super-family, which is produced by granulosa cells within the pre-antral and small antral follicles (2-6 mm) in the ovaries of female humans (Durlinger *et al*., 2001). An accepted lower limited value is 0.5-1.2 ng/dl, while values below 0.5 ng/ml represent an insufficient number of follicles, and these between 1.2 up to 4.0 ng/ml are considered the best representation of sufficient number, good quality oocytes (van Rooij, 2002). At any day of the menstrual cycle, the serum AMH level has the best ability in predicting the quantity and quality of stored oocytes, response to COS compared to other markers of ovarian reserve (Weenen *et al*., 2004). The predictive value of AMH on the pregnancy rate has recently derived the attention of the reproductive physician. However, conflicts regarding the correlation between the AMH level, ovarian reserve, and pregnancy outcome still reported in the literature (Lekamge *et al*., 2007).

Recently, reports suggested that OR tests are of limited value in predicting ongoing pregnancy in couples with mild male infertility and unexplained infertility and proposed that their efficiency in predicting pregnancy outcome is better for couples with advanced women age and with exclusive female sub-fertility (Rooij *et al*., 2006).

It helps clarify the effectiveness of OR markers for specific groups of patients who seek IVF/ICSI treatment in clinical practice (Hansen *et al*., 2008). The current study was designed to assess the predictive value of age on the biomarkers of ovarian reserve and the outcome of ICSI cycles.

Material and Methods

One hundred twenty-six couples complained of sub-fertility collected from Al-Kafeel fertility and IVF center/Holy Kerbala/Iraq throughout 2016-2019. An initial evaluation performed by history, cause, type, duration of sub-fertility, examination, and investigations was done age, cycle day 2 FSH, LH & AMH, the trans-vaginal ultra sound (US) for number of AFCs and endometrial thickness and male partners seminal fluid analysis. They seek ICSI treatment either due to male factor (oligo-, astheno-, terato- or azo-spermia) according to normal semen parameters by WHO, 2010 or female factor (tubal obstruction, mild PCOS) and unexplained infertility. All couples were included in the ICSI program. The females were subjected to COS via pituitary down-regulation using either gonadotropin-releasing hormone (GnRH) antagonist (Cetroleix 0.25 mg*1dose) or agonist (Decapeptyle 0.1 mg*1). Then a controlled ovarian hyper stimulation using either by recombinant FSH (r-FSH); (Follitrope 75 iu-2doses S.C) or purified HMG (Merional 75iu*2 I.M) for 7-14 days, which was conducted under a close supervision employing serial trans-vaginal ultrasound (TVUS) and serum E2 level. Ovulation trigger has performed using human chorionic gonadotropin (hCG) injection (Pregnyl 5000 iu*2) when the total number of the follicles 8-14 and their size are more than 17-mm. Oocytes pickup has performed under general anesthesia and TVUS. The fresh ejaculated semen sample has concomitantly prepared by centrifugation and swim-up from the pellet. Microscopic assessment of oocytes' maturity (MII, MI, and GV) conducted after denudation the oocytes. Mature oocytes are those that resumed their first meiotic division (MI) and reached the second meiotic division (MII) and appropriate for injection by expelling the 1st polar body (Irit & Nava D, 2018; Denny & David (2018).

The females divided into two groups below and equal to 35 years old n=64 and above n=62. Correlation between age and FSH & AMH, assessment of response to COS by calculating the total dose of gonadotropins used, counting the number of retrieved oocytes, their quality and

Table 1. The Mean Duration (Mean ± Sd) and Type (Total Number) of Sub-Fertility In Both Groups

Parameter	≤35 ys., mean±SD	>35ys.,mean±SD	p-value
Duration of sub-fertility	7.6 ±4.3	8.1± 5.5	0.58
	Type of sub-fertility		
primary	56 (87.5%)	38 (61.3%)	p-value
secondary	8 (12,5)	24 (38.7)	0.001
Total	64 (100%)	62 (100%)	

Table 2. Biochemical Markers of Ovarian Reserve In Both Groups

Parameters	≤35 ys., mean±SD	>35ys., mean±SD	p-value
FSH iu/l	6.6 ±4.0	7.7 ±4.3	0.15
AMH ng/ml	2.4± 1.8	2.4± 2.8	0.15
LH iu/l	5.8± 4.3	5.1 ±2.8	0.27

pregnancy rate has performed, and the results compared between both. Calculation of pregnancy rate was done by dividing the number of females with +ve pregnancy test by the number of females who two-three good quality embryos were transferred *100%. The study is a cohort; the patients followed up retrospectively. The hormones level measured on the 2nd day of the menstrual cycle by the ELIZA test. Data analysis was done by SPSS, V.24. The comparison between the results of both groups was by using either independent sample t-test (for continuous data; mean ± SD) or Fisher's Exact test/ Chi-square test (for categorical data; percentage/number) at a significant p-value ≤0.05

Results

The first table shows the duration of infertility represented by years and type of infertility. There is no significant difference between these parameters.

Table 2 demonstrates cycle day two hormones; FSH, AMH & LH in both groups. There was no significant difference between them in both groups.

Table 3 Shows the response to COS, the total dose of gonadotropin (Gn) and HMG, the total number of retrieved oocytes, and their maturity in both groups.

There was a significant variation between the total number of oocytes and their maturity being higher in the females of age equal and less than 35 years old.

Table 4 illustrates the type of induction protocols that have been used in both groups (short GnRH agonist, long GnRH agonist and GnRH antagonist). A significant difference with most of women in both groups where the GnRH antagonist protocol was used.

Table 5 shows the cause of sub-fertility in both groups, whether due to male (oligo-, astheno-, trato- or azo-spermia or female (tubal, mild PCOS or unexplained). There was no significant difference in both groups.

Table 6 represents pregnancy rate comparison between both groups. It was insignificantly less in females older than 35 years old, 23.5% vs. 37.7%. During the calculation of pregnancy rate, 11 out of 62 women older than 35 years old and 3 out of 64 women younger and equal to 35 years old were excluded due to failure of producing any oocytes following COS, so cycle cancellation rate in older women is higher 17.7% vs. 4.6% in younger ones.

Discussion

The reproductive function of women often declines as women get older before other organ systems. By the age of forty, about half of the women become sub-fertile while the other half exhibit a marked decreased in fecundity when compared to younger age groups. Women's age provides the best predictor of oocyte quality both in vitro and in vivo. In women, the chance of conception decreases significantly as their age increases with an increased risk of miscarriage and embryo aneuploidy (Eldar-Geva *et al*., 2005).

Cycle day two hormones (FSH and AMH) serve as biochemical markers of woman age. Both (FSH and AMH) are good indicators of the number of antral follicles in the ovaries(ovarian reserve), and their levels usually reflect the quantity and quality of oocytes within ovaries. Once the number of oocytes gets decreases, the level of FSH and AMH are affected (AMH is decreased as it is no longer

Table 3. The Response To Cos, The Total Dose of Gonadotropin and Hmg, The Total Number of Retrieved Oocytes and Their Maturity In Both Groups

Parameters	≤35 ys, mean±SD	>35 ys, mean±SD	p-value
Total dose of Gn	1987.2±947.6	2179.4±1222.2	0.36
Total dose of HMG	2468.7±1454.8	3122.3±1456.8	0.02
Total no. of oocytes	9.8±6.4	6.09±4.7	0.0001
Total no. of MII	6.7± 5.1	4.4 ±3.8	0.005
Total no. of MI	1.4 ±1.6	0.9± 1.1	0.004
Total no. of GV	1.3± 1.7	0.7± 1.1	0.02

Table 4. The Type of Induction Protocols Used In Both Groups

Parameters	≤35 ys	>35 ys.	p-value
Short agonist	16 (25%)	30 (48.4%)	0.009
Long agonist	3 (4.7%)	0 (0%)	
Antagonist	45 (70.3%)	32 (51.6%)	
Total	64 (100%)	62 (100%)	

Table 5. The Cause of Sub-Fertility In Both Groups

Parameters	≤35 ys	>35 ys	p-value
Female cause	31 (48.4%)	23 (37.1%)	0.30
Male cause	33 (51.6%)	39 (62.9%)	
Total	64 (100%)	62 (100%)	

Table 6. Pregnancy Rate Comparison Between Both Groups

Parameters	≤35 ys	>35 ys	p-value
Pregnant	23 (37.7%)	12 (23.5%)	0.107
Non-pregnant	38 (62.3%)	39 (76.5)	
Total	61 (100%)	51 (100%)	

secreted from the granulosa cells of the antral follicles, while FSH is increased due to depletion of ovarian follicles, decreased estrogen production and positive feedback to anterior pituitary to produce FSH) indicating aged ovaries (Nelson, 2013). However, ovarian age is not always associated with woman chronological age (Durlinger *et al.*, 2001& van Rooij, 2002).

As showed in table 2, the current study revealed no significant difference in the serum levels of FSH and AMH in women who are 35 years old or less compared to those who are older than 35 years old. These results indicate that FSH and AMH are weak predictors of the aged ovary in women, and advanced age is not necessarily associate with high FSH and low AMH levels (Hussein *et al.*, 2018). However, some studies are in disagreement with our finding, suggesting that baseline FSH & AMH are significantly correlated with women's age(Lee *et al.*, 2009, Eldar-Geva *et al.*, 2005, de Vet *et al.*, 2002). Recent data by American Society for Reprodutive Medicine,2020. concluded that hormonal markers of ovarian reserve had been shown as good predictors of oocyte yield but poor, independent predictors of reproductive potential and cannot be used as a test of female fertility or a dependable marker of IVF success independently from age(Penzias *et al.*, 2020).

Older women who were stimulated with higher doses of FSH and HMG revealed a lower response to COS by producing a small number of oocytes that strongly affect the maturity of oocytes (table 3). This usually due to poor OR, and older women produced a small number of mature oocytes when compared with younger women. Several studies are in agreement with these findings and suggested that women older than 35 years old produce a lower number of oocytes and usually of poor quality. Thus, higher doses of Gn are required for

stimulation (Karim, S. 2018, Balasch, 2010, Yan *et al*., 2012).

The pregnancy rate appeared to be less in old women compared to young ones (< 35 years) with a high rate of cycle cancellation, as demonstrated in table 6. This is usually due to a small number of antral follicles in the ovaries of old women. These results seem to be consistent with other results obtained by several researchers suggesting that advanced women's age is strongly and negatively affect pregnancy rate, embryos chromosomal status, their implantation potential, ultimately ICSI outcome(Lee *et al*., 2009, Yan *et al*., 2012, Thum *et al*., 2008, Liu *et al*., 2011, Gnoth *et al*., 2011).

From the current and previous studies data, we can conclude that ovarian reserve represents the number of remaining oocytes in the female ovaries (so its quantitative not a qualitative measure). Markers of ovarian reserve measures the features of the ovaries and can not be considered as useful predictors of oocyte yield or oocyte pick up following stimulated IVF and when used, they are poor predictors of fertility potential of reproductive women.

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