Age is the Best Marker to Predict Intracytoplasmic Sperm Injection Cycles Outcome

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Authors' contributions

This work was carried out in collaboration between all authors. Author HAT is the principal author who designed and implemented the study, and conducted the bulk of the research. Author ZAK provided assistance with participant selection and conducted any vaginal ultrasound examinations. Author ZAM supervised the work and all the statistical analysis. All authors read and approved the final manuscript.

Study Protocols

ABSTRACT

Aims: To assess the ovarian reserve for subpopulations of Iraqi infertile couples seeking Intra Cytoplasmic Sperm Injection (ICSI) treatment. 2. To evaluate the effectiveness of ovarian reserve markers in predicting the response to Controlled Ovarian Stimulation and Intra cytoplasmic sperm injection cycles outcome.
Study Design: A prospective observational controlled trial.
Patients and Methods: Eighty -seven participants were enrolled during their attendance to the fertility center of Al-Sadar Medical Teaching city. Twenty subjects as a control group (N=20), and 67 as a study group (N=67). Ovarian reserve parameters were assessed and ICSI were performed. The female were divided into two groups: Those under 35 years of age and those above 35 years of age. They were compared for: Number (No.) of follicles obtained, No. of mature oocytes, Fertilization rate, Cleavage rate, Embryo scoring, No. of embryo transferred and Pregnancy rate (biochemical pregnancy).
Results: The old age group has significantly higher serum follicle stimulating hormone and body mass index while they have significantly lower anti mullerian hormone than the younger age group. The ICSI outcome parameters regarding No. of oocyte, No. of mature

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oocytes, fertilization rate, cleavage rate, No. of best quality embryos, and no. of embryo transferred and pregnancy rate were significantly lower in old age group.
Conclusion: Age is strongly associated with ovarian response and it provides the most powerful basal estimate for ICSI outcome.

1. INTRODUCTION

The primary function of the female ovary is the production of a mature and viable oocyte capable of fertilization, subsequent embryo development and implantation. Even before birth, a woman's eggs begin to diminish in number. The number of eggs decline as the woman ages [1]. In general, ovarian age parallels with chronological age, but since that is not always the case, it is vitally important for reproductive endocrinologists routinely to perform some screening tests to assess their patients' ovarian reserve (OR). This is particularly true for women over the age of 35, whose egg loss will dramatically increase [2,3].

Ovarian reserve can be defined basically as an estimate of how many oocytes are left in the ovaries, and that often translates into how many eggs we are going to be able to work with over the course of any given monthly treatment. By estimating the OR, a prediction of the remaining reproductive lifetime could be assessed as well as the likely success of assisted reproductive techniques (ART) such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) [4]. Because more women are delaying childbearing to pursue higher education and career opportunities, chances to conceive are further threatened. When they are ready to start a family, these women are often turn to ART [5]. Although ART offer a popular solution, they come with a high patient burden, expensive drug regimens, and a substantial chance of failure or developing complications. To minimize these effects, it is important to be able to accurately predict treatment outcome prior to treatment initiation and to consequently tailor protocols to the individual response [6]. The associated clinical factors for a successful treatment of IVF/ICSI have been studied extensively [7,8]. The major clinical factors related to pregnancy outcome in ART cycles include the following: age of the patient [8], embryo morphology [9], cause of infertility, and number of embryos transferred [7]. Among these factors, the age of the patients and the cause of infertility, together with the OR markers are available prior to Controlled Ovarian Stimulation (COS) in the general practice of IVF treatment. It has been reported that the prevalence of unexplained infertility increases in female patients of advanced age (>35 years) seeking infertility treatment [10,11]. A putative cause of such unexplained infertility was attributed to diminished OR. The OR markers would probably connect with the outcome of pregnancy for such patients. Herein we propose in one of Iraqi IVF centers, for the first time as for the best to our knowledge , the efficiency of OR markers to predict the outcome of ICSI treatment in couples with exclusive female cause due to tubal factor or unexplained infertility.

1.1 The Aim of this Study was

1 - To assess the OR for subpopulations of Iraqi infertile couples seeking ICSI treatment, who were referred to the fertility center of Al-Sadder Medical Teaching City in Al -Najaf province and were selected for ICSI programme.

2 - To evaluate the effectiveness of OR markers in predicting the response to COS and ICSI outcome.
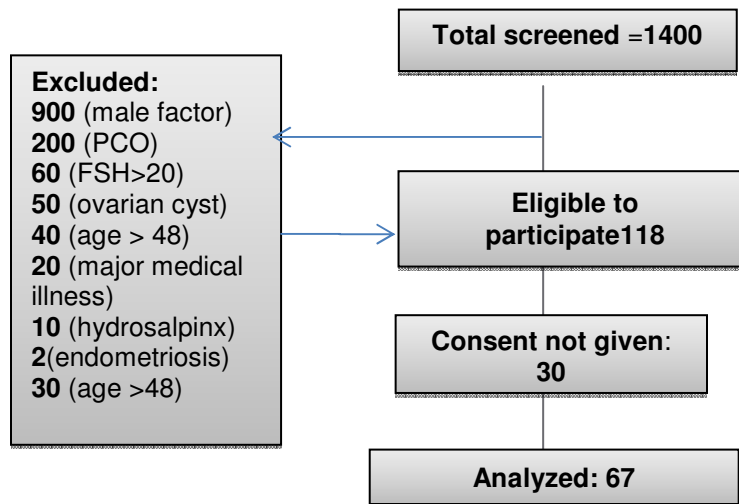
2. MATERIALS AND METHODS

2.1 Work Design

This study was designed as a prospective observational controlled trial. Eighty -seven participants were enrolled; Twenty subjects as a control group (N=20), and 67 as a study group (N=67). These subjects were referred from many Iraqi governorates to the fertility center of Al-Sadar Medical Teaching City in Al-Najaf province from December 2010 - September 2011. These participants were seeking treatment for their infertility, and we design to treat them by ICSI.

2.2 Participants Selection

Sixty-seven females constitute the study group were selected from 1400 attender to the fertility center during the period of the study. All couples were surveyed for their etiology of infertility by semen analysis for the males, and the females were asked about their gynecological and medical history.They underwent complete medical examination, height and weight measurements. Gynecological examination was performed. Cycle day two (CD2) vaginal ultrasound (U\S) and blood tests for FSH, LH, estradiol (E2), serum prolactin, testosterone and thyroid function test were performed. Hysterosalpingiography was arranged and some have been prepared for laparoscopy. Once the couple have been screened and found to be eligible according to our selection criteria, they were selected to go on with the designed programme. See flow chart.

Flowchart for subject enrolment

Total screened =1400

Excluded:
900 (male factor)
200 (PCO)
60 (FSH>20)
50 (ovarian cyst)
40 (age > 48)
20 (major medical illness)
10 (hydrosalpinx)
2(endometriosis)
30 (age >48)

Eligible to participate118

Consent not given: 30

Analyzed: 67

2.2.1 Selection criteria

1. Male partner with normal semen analysis according to World Health Organization guideline, (concentration> 15 million/ml, motility> 32% and strict morphology >4% (12).
2. Regular menstrual cycle of 21-35 days.
3. Female partnerhas normal both ovaries; Visible on U\S not having cystic ovariesdefined by The Rotterdam ESHRE/ASRM criteria (2003).
4. The cause of infertility is either unexplained (N=43) and the couple underwent at leastthree trials of Intrauterine insemination, or tubal factor infertility (N=24) but not hydrosalpinx.
5. Vaginal U\S show no uterine fibroid, anomaly or ovarian cyst measuring 20mm or morein diameter.
6. No endocrine cause for their infertility such as hyperprolactinaemia or hyperandrognesim.
7. Screening tests for hepatitis B and C as well as for human immune deficiency viruses (HIV) proveto be negative.
8. The participants have their first or previous trials of ART.
9. Informed written consent was obtained from the patients.

The females were divided in to two groups: those under 35 years and those above 35 years and they were compared for: No. of follicles obtained, No. of mature oocytes, Fertilization rate, Cleavagerate, Embryo scoring, No. of embryo transferred and Pregnancy rate (biochemical pregnancy).

2.2.2 Hormonal assay

Five mL of blood was drown on CD 2. The blood was centrifuged and sera were stored at -20ºC. AMH sera levels were measured with AMH\Gen II analysis kit (Beckman coulter, USA) using Enzyme Linked Immuno Sorbant Analysis (ELISA). Kits for measurement of FSH was (bioMérieux® France) using Mini VIDAS analysis. The kit for E2 measurement was (bioMérieux® France) using Mini VIDAS analysis.

Ultrasound was performed CD 2 by the gynecologist of the center using real time ultrasound device (Philips 11*E), using vaginal probe (7 MHZ) , Follicles measuring 2-8mm were counted from the lateral to medial margin of each ovary to determine the antral follicle cohort. The total number of the follicles per patient counted in both ovaries was used for calculation. In all patients treated for ICSI, the protocol for pituitary down-regulation was by short Protocol. The patient received 0.1mg/day of GnRH analogue as a morning dose and FSH as an evening dose. These are given subcutaneously. Decapeptyl in the form of Diphereline 0.1mg (triptoreline Beaufour phrma, France) and FSH in the form of (Gonal-f, Follitrop alfa 75IU/ampul, serono. Switzerland). The dose can be administered either via step up or step down protocol where the dosage of FSH is maintained or gradually increased or initial high *doses* are tailored down.

Participants were monitored for follicular recruitment and growth by serial transvaginal ultrasound and serum E2 from the 6th day of stimulation with gonadotropins. Titration of FSH upward or downward are based on response of follicular genesis. Eighteen consecutive cycles which were cancelled because of a poor follicular response were initially selected. Those participant that did develop less than 3 follicles measuring 18mm in diameter after 14 days of FSH treatment, or E2 level <100Pg/ml, have their treatment cycle cancelled or was

converted to have intra uterine insemination depending on their clinical factor of infertility (poor responder, n=18. This was according to the ESHRE consensus on the definition of' poor response' to ovarian stimulation for in vitro fertilization: The Bologna criteria ,at least two of the following three features must be present: (i) advanced maternal age or any other risk factor for POR; (ii) A previous POR; and (iii) An abnormal ovarian reserve test (ORT) [13,14]. These were considered to have an oocyte retrieval = zero.

The remaining 49 women having a completed ICSI cycle. When at least 2 dominant follicles of 17mm in diameter in each ovary are ready, then ovulation is triggered by human chorionic gonadotropin (HCG), pregnyl, 5000-10000 IU (HCG, Preygnyl, Organon, Holland) intramuscular. Thirty six hours after HCG trigger, follicular aspiration is done by transvaginal ultrasound guidance. The oocytes were incubated and evaluated for maturity after their denudation. ICSI was done under inverted microscope with manipulators (Bickland industrial, UK). On day 1, pronuclear stage was assessed and fertilization rate calculated. On day 2, subsequent divisions were assessed and cleavage rate was calculated. Embryos were graded and scored under the microscope according to Steer et al., (15); and Bączkowski et al., [16], Embryo transfers were done 48-72hours after ICSI .The transfer to the uterus was done with Labotec catheter. Luteal phase was supported by Progesterone vaginal cream (Crinon 8%,Serono,U.K), Duphastone tablets (10mg,Solvay pharmaceutical, Holland),Aspirin tablet(Aspin-100mg/ enteric coated tab. SDI. Iraq), and Folic acid tablet (5 mg/tab. SDI- Iraq). On the fourteenth day of embryo transfer serum B- HCG was performed to confirm pregnancy, by that time pregnancy rate is stated.

2.3 Statistical Analysis

Descriptive statistics were expressed as mean and standard deviation, categorical variable as percentages. Student's t-test or chi-square test was used accordingly to compare groups. *P*-values <0.05 were considered to be significant. Statistical analysis was performed using SPSS version 17.

Demographic characters of the study group are illustrated in Table 1.

Table 1. Demographic data regarding the study group (n= 67) participating in ICSI treatment of different age groups. Values are mean ± standard deviation or %

Base line characteristic	
Age(years) female	31.76±6.85
male	38.65±54
female BMI(Kg/m2)	28.23±5.08
Duration of infertility(years)	8.09±4.79
Type of infertility	
Primary (no)%	(54)80.59
Secondary(no) %	(13)19.41
Etiology	
Unexplained (no)%	(43)64.2
Blocked tube (no)%	(24)35.8
Cycle day 2	
Basal FSH(mIU/ml)	5.75±2.61
Basal E2(pg/ml)	39.64±19.67
Basal AMH(ng/ml)	2.90±3.44
Basal inhibin B(pg/ml)	74.2±1.55
Total AFC	7.42±2.87

The control group was volunteers either relatives or staff of the fertility center. Their ages ranged from 22- 45 years who had delivery at least within the last 4 years and now are using either rhythm method or intra uterine contraceptive device, as methods for birth control. They underwent height and weight measurements. Cycle day 2 blood samplings for FSH, LH, E2, AMH and Inhibin B measurement. Vaginal U/S was done for AFC (Table 2).

Table 2. Demographic character of the control group. Values are mean ± SD (n=20)

Age (years)	**32.5±7.41**
Duration of child free(years)	2.25±1.06
Basal FSH(mIu/ml)	4.71±1.35
Basal E2(Pg/ml)	41.83±15.79
Basal AMH(ng/ml)	2.54±1.48
Basal Inhibin B(Pg/ml)	118.91±1.00
AFC	7.60±1.87
BMI(Kg/m2)	28.02±3.46

2.4 Semen Analysis and Sperm Preparation

Semen sample was collected at the time of oocyte pickup (opu) by masturbation (After 2-5 days of sexual abstinence) and prepared by centrifugation method.

Fretilization rate (FR) was calculated as follows:

$$FR = (No\ of\ 2PN\ on\ day\ 1/WholeNo.of oocytesinjected) \times 100$$
PN=pronuclear

Cleavage Rate (CR):

The cleavage rate was calculated as follows:

$$CR = (No.\ of\ cleaved\ embryos\ on\ day/Total\ no.\ of\ (2PN)) \times 100$$

The same assessment was repeated on day 3 after injection for cases where embryos were transferred on day 3 rather than day 2.

Detection of pregnancy and pregnancy rate: Unfortunately this was not possible to be made for all pregnant women in our study because of loss of follow-up after positive B-HCG; may be due to social embarrassment to perform such technology in our culture as they believe that it will scratch their paternity. So, only biochemical pregnancy is stated. In general, pregnancy rate (PR) was defined as the number of pregnant ladies after ICSI divided by the whole number of patients who underwent ICSI cycles and ET all multiplied by 100.

Female patients were categorized into two groups according to age. Those of 35 years; and those of ≥35 years in order to study the effect of age on the outcome of ICSI.

3. RESULTS AND DISCUSSION

Table (3) compares the base line characteristic of the females of the study group and the control group. No statistically significant differences existed in age of the study and control groups (p>0.05) (31.76±6.83; 3 2.5±7.41 years) respectively. The duration of voluntary infertility which corresponds to the period of last child birth in which the non-hormonal contraception which was used in the control group was (2.25±1.06 years), and it differs significantly from the involuntary period of infertility of the study group (8.09±4.79 years), (p<0.05). Only Significant differences (P<0.05) were identified in CD2 serum levels of FSH. The control group demonstrate 5.75±2.61mIu/ml value while 4.71±1.35mIu/ml in the study group, while other markers demonstrate no significant difference (P>0.05).

Table 3. Comparison between study and control groups regarding base line evaluation. Values are mean±SD

Parameter	Study group (n=67)	Control group (n=20)	*P*-value
Age(years)	31.76±6.83	32.5±7.41	*P*>0.05
Duration (years)	8.09±4.79	2.25±1.06	*P*<0.05[**]
FSH (mIu/ml)	5.75±2.61	4.71±1.35	*P*< 0.05[**]
E2 (Pg/ml)	39.64±19.67	41.83±15.79	*P*>0.05
AMH (ng/ml)	2.90±3.44	2.54±1.48	*P*>0.05
Inhibin B (Pg/ml)	74.25±1.55	118.91±1.00	*P*>0.05
AFC	7.41±2.87	7.60±1.87	*P*>0.05
BMI (Kg/m2)	28.24±5.07	28.02±3.46	*P*>0.05

[**] *Significantly different from the corresponding group*

Basal serum level of E2 was: 39.64±19.67Pg/ml in the study group while it was 41.83±15.79Pg/ml in the control. Basal serum level of AMH was: 2.90±3.44 in the study group vs. 2.54±1.48 (ng/ml), in the control. Inhibin B at CD2 (74.25±1.55 for study group vs.118.91 ± 1.00 (Pg/ml) in the control. Basal count for antral follicles was (7.41±2.87in the study group vs. 7.60±1.87 in the control). BMI measurements for both groups (28.24±5.07 vs. 28.02±3.46) (Kg/m2). All the mentioned difference was insignificant.

At this point, we divided the females according to age to: those < 35 and ≥ 35; as shown in Table 4.

Cycle day 2 measurements of FSH and AMH showed a significant difference between young and advanced age patients (P<0.05). (5.16±2.06 vs. 6.68±3.11mIu/l) for FSH; (3.80 ±3.69 vs.1.48±2.46ng/ml) for AMH. While other markers demonstrate no significant difference between the 2 studied groups (serum levels of E2 40.60±19.16 for those <35 and 38.13±20.76Pg/ml for ≥35 years), inhibin B (91.29 ±189.03 vs. 47.38±72.33Pg/ml) and AFC (7.66±2.82 vs. 7.04±2.96). BMI shows higher significant (p<0.05) level in the older age group (27.29±4.91) compared to young ones (29.73±5.06) Kg/m2.

Table 4. Comparison between basal ovarian reserve tests of females <35 years and ≥35 years. Values are mean±SD

Parameter	Age<35 n=41	Age≥ 35 n=26	*P*-value
FSH(MIu/l)	5.16±2.06	6.68±3.11	*P*<0.05[**]
E2 (Pg/ml)	40.60±19.16	38.13±20.76	*P*>0.05
AMH (ng/ml)	3.80±3.69	1.48±2.46	*P*<0.05[**]
InhibinB(Pg/ml)	91.29±189.03	47.38±72.33	*P*>0.05
AFC	7.66 ± 2.82	7.04±2.96	*P*>0.05
BMI(Kg/m2)	27.29±4.91	29.73±5.06	*P*<0.05[**]

[**] *Significant difference from the corresponding value*

Table 5. Comparison between outcome data of different age groups participating in ICSI treatment. Values are mean ± SD or %. Bold no. for calculation

Parameter	Age <35 n=41	Age≥35 n=26	*P*-value
Total gonadotropin dose (IU)	1538.02±27.78	1646.15±932.38	*P*>0.05
Serum E2 level on day of HCG (Pg/ml)	2437.78±1326.39	754.65 ±136.50	*P*<0.05[**]
Total no. of follicles	14.02±6.95	5.34±6.53	*P*<0.05[**]
Total no. of oocytes retrieved	9.75±5.56	3.00 ± 4.95	*P*<0.05[**]
Total no. of (MII) injected	256 / 6.40±4.19	51 / 2.55±3.27	*P*<0.05[**]
Total no. of2-PN oocyte	165 / 4.34±2.83	29 / 1.55±2.17	
% Fertilization Rate	53.74	9.44	*P*<0.05[**]
Total no. of cleaved embryo D2	113	24	
Cleavage Rate%	58.54	12.43	*P*<0.05[**]
No. of best quality embryos	3.48±1.95	2.36±1.91	*P*< 0.05[**]
No. of bad quality embryos	1.63±0.92	1.00±0.00	*P*<0.05[**]
Embryos transferred	2.98±1.47	1.54±1.29	*P*<0.05[**]
Pregnancy Rate%	18.6	4.65	*P*<0.05[**]

NS: non-significant difference. [**] *significant difference from corresponding value*

The patients ≥35 years of age consumed higher doses of exogenous gonadotrophins, (1646.15±932.38 IU) compared to those used by <35 years (1538.02±527.78 IU), but this difference is insignificant. The old age group has significantly lower estradiol levels on the day of hCG administration (754.65±136.50Pg/ml) compared to that reached by the younger age group (2437.78±1326.39 Pg/ml) (p<0.05) as shown in (Table 5).

Table (5) demonstrate that the No. of follicles obtained after COS in the young age group was (14.02±6.95) which was significantly higher than that obtained from the old age group (5.34±6.53) (p<0.05). Total number of oocyte retrieved after follicular aspiration was (9.75±5.56) mean ± SD, for <35 years, and 3.00±4.95 for those ≥35 years, t –test showed that this No. is significantly relevant to the chronologic age of the patients, (p<0.05).

Of (9.75±5.56) oocyte retrieved only (6.40±4.19) was MII oocyte and was injected by ICSI, for those <35 years and out of (3.00±4.95) only (2.55±3.27) was MII and was injected for those ≥ 35 years. The difference was significant (p<0.05). (Refer to Table 5).

Of (6.40±4.19) MII injected oocytes only (4.34 ± 2.83) were fertilized for those <35 years. Those having 2PN twenty four hours after ICSI were (1.55±2.17) out of (2.55±3.27) MII injected oocytes for those ≥35 years (Table 4). FR% (53.74 vs. 9.44) .The difference in FR was significant. The cleavage rate for both groups was calculated and it showed significant difference. (58.54% for those <35 years vs. 12.43 for those ≥35 years) P<0.05. Assessment of embryos 48hours after ICSI revealed that the mean No. of best quality embryos was (3.48±1.95) for <35 years and (2.36±1.91) for ≥35 years, the difference was significant (p<0.05). No. of bad quality embryos was 1.63±0.92 for young age group vs. 1.00 ± 0.00 for old group (p>0.05). Total No. of ET to the uteri of the females was (2.98±1.47) for less than 35 years vs. 1.54±1.29 for those above 35 years) mean ± SD (P<0.05).Pregnancy rate was 18.6% in the young age group and 4.65% in the old age group which was significantly different from corresponding value (p< 0.05). (Table 5).

Assisted reproduction cycles have revolutionized the treatment of infertility and they are being increasingly used. Because assisted reproduction treatments are costly, time-consuming and stressful for patients and not universally successful, attempts have been made to determine the factors which predict a successful outcome in a given patient. A major challenge to the IVF teams is to predict prospective patients who will be low responders and to appropriately counsel women who are potential candidates for assisted reproduction. A woman's age is considered as a prognostic factor when assisted reproduction treatment is proposed to infertile couples as a marked decline in success rates is observed at 35–37 years [17,18]. Oocyte donation has demonstrated that the age-related decline in female fecundity is due predominantly to ovarian rather than uterine factors, presumably due to a decrease in OR [17,16]. Traditional methodology used to assess OR has consisted of baseline serum levels of hormones such as FSH, estradiol and inhibins, and chronological age [15,18]. Also, a number of provocative tests have been devised to indirectly assess OR and identify patients who might not be detected by basal hormone screening alone [17,19,20]. However, neither basal hormone measurements nor such dynamic tests provide direct information concerning the responsiveness of the ovaries to exogenous gonadotropins used in ovarian stimulation for assisted reproductive treatment.

AMH is formed in females' ovaries from the 36th week of gestation, during the female life until menopause it isexprimed in granulosa cells of small growing follicles (primary and preantral). The biological activity of AMH in women is not completely understood, but data along the last years suggest that AMH modulates follicular growth in a way that it inhibits the recruitment of follicles from the primordial pool by modifying the FSH sensitivity of those follicles and regulating ovarian steroidogenesis and intrafollicular androgen to estrogen ratio [21,22,23]. There is a linear decline of AMH levels over time [22,20]. The fact that AMH acts first of all paracrine and is not involved in feed-back mechanisms of hypothalamo-pituitary-gonadal axis, and that AMH is expressed at a constant level and demonstrates less individual intra- and inter-cycle variation, makes AMH very attractive, promising and reliable economic marker as a direct measurement of OR [22,24,25,26].

The basic demographic characters of the control were not different significantly (p>0.05) from the study group regarding age, basal E2, AMH inhibin B, AFC nor BMI as demonstrated in (Table 3). These findings are expected since our study group has extra ovarian cause for their infertility. The only difference was in duration of the involuntary infertility of our study group which is significantly higher than the voluntary infertility of the control group. This difference can't be explained on scientific bases since the involuntary one is out of the wish of the couple and the other is according to the need of the couples. Basal serum FSH level is

significantly higher in the study group .This may be a contributing cause to their infertility since high FSH serum levels are associated with decrease fecundity [27] (Table 3).

We initially divided the females' patients into two groups by age (i.e., <35 years and ≥35 years) and then calculated the difference for the biomarkers of OR, as shown in Table 4. A slow but steady decrease in fertility is observed in women aged between 30 and 35 years, which are followed by an accelerated decline among women aged over 35 years (28).

In the present study, some of the surveyed OR markers, including baseline FSH levels and baseline AMH levels, were significantly relevant to the chronologic age of the female partner of infertile couples as demonstrated in Table 4. Serum concentrations of AMH decreased over time in young normo-ovulatory women .These finding are in consistent with that of other workers [29,30].

This study highlights the significant increase in the BMI with age, a finding that agrees with other team works [31,32]. It seems that there is a reduction in basal metabolic rate (BMR) adjusted for differences in body composition in older female subjects compared with younger ones. However, at present, we cannot offer any plausible metabolic mechanism explaining this observation, and further research is needed.

Other markers in the present study that is associated with ovarian aging such as basal E2 and Inhibin B haven't changed (Table 4). Van Rooij and his team measured serum Inhibin B and E2 at 4 years interval within the same female group and they concluded that serum inhibin B and seum E2 did not change, Inhibin B only change after the age of 40, since the mean age of our old group was 38.65.Their finding may be applied to our study population as well [33].

Studies in fertile and IVF-treated populations show a close negative correlation between AFC and age, and a positive relation between AFC and time to menopause, [34,35,36,37]. The fact that our results showed no effect of age on AFC levels, contrary to other studies, may be related to familial factors in our study population and limitations to the decrement in AFC with age since the AMH and FSH serum levels in the older age group of our study population still maintain a good reasonable reproductive limits (Table 4).

There is a significant difference between the two age groups in serum E2 levels at day of HCG injection, No. of follicles obtained and No. of oocytes retrieved (Table 4). This is mostly due to the significantly higher level of FSH and lower level of AMH in the old age group, since these two parameters effect the No. of follicles and oocytes negatively [30,31]. Also Table 4 demonstrates that mean No. of MII, FR, CR, and No. of good and bad quality embryos is significantly higher in the young age group. The results are in concordant with previous reports as that of de Vet et al. [38]; Eldar-Geva et al. [39] and Lee et al. [30]. These differences also reflect the fewer ET and add a reason for lower pregnancy rate in older age group (Table 4).The significant difference in pregnancy rate between the two age groups is widely accepted since the probability of embryo implantation and successful live birth after IVF also declines progressively in women over the age of 35 years [40]. The higher pregnancy rate attained in the age group <35 , may be attributed to the better OR as reflected by significant difference in basal serum level of FSH and AMH No. of MII and good quality embryos obtained (Table 4). It also may be due to the detrimental effect of the higher gonadotropins used in those ≥35, which seems to have a drawback effects on oogenesis, embryo quality, endometrial receptivity and perinatal outcomes. This at least is in animal model [41,42,43].

4. CONCLUSION

There is a significant increase in FSH, and decrease in AMH with age, especially those ≥ 35 years. All studied ICSI outcome measures including follicles and the oocytes retrieved decrease with age. Age must always be the first marker to be considered in ovarian reserve assessment.

CONSENT

All authors declare that 'written informed consent was obtained from the patient for publication.

ETHICAL APPROVAL

All authors hereby declare that this work had been approved by the ethical and scientific committee of Medical College / Baghdad University.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1.   Wallace WHB, Kelsey TW. Human ovarian reserve from conception to menopause. Polson. 2010;5(1): 87772.
2.   Tietze C. Reproductive spans and rate of reproduction among Htterite women. Fertil Steril. 1957;8:89-97.
3.   American Society of Reproductive Medicine (ASRM). Age related fertility decline: A committee opinion. 2008; 90-s:154- 5.
4.   Baird DT, Collins J, Egozcue J et al. Fertility and aging. Hum Reprod update. 2005;11:261-76.
5.   Weinstein M, Wood AJ, Chang MC. Age pattern in fecundability. Gray R, Leridon H, Spira A, (eds). In: Biomedical and Demographic determination s of Reproduction. Clarendon Press. Oxford. 1993;209-220.
6.   Jackson MM. Using Anti-mullerian hormone to measure ovarian reserve. Clinical update. 2010;1. Available: http://www.Walgreen shealty.com.

7. Roseboom TJ, Vermeiden JP, Schoute E et al. The probability of pregnancy after embryo transfer is affected by the age of the patient, cause of infertility, numberof embryo transferred and the average morphology score ,as revealed by multilogistic regression analysis . Hum Reprod. 1995;10:3055-3041.

8. Chang CC, Chen CD, Chao KH et al. Age is a better predictor of pregnancy potential than basal level in women undergoing IVF. IVF. 2003;79:63-68.

9. Terriou P, Sapin C, Giorgetti A et al. Embryo score is a better predictor of pregnancy than the number of transferred embryos or female age. Fertil Steril. 2001;75:525-531.

10. Miller JH, Weinborg RK, Canino NL et al. The pattern of infertility diagnosis in women of advanced reproductive age. Am J Obestet Gynecol. 1999;181:952-957.

11. Maheshwari A, Hamilton M, Bhattacharya S. Effect of female age on the diagnostic categories of infertility. Hum Reprod. 2008;23:538-542.

12. Cooper TG, Noonan E, von Eckardstein S, Auger J, G Baker HW, Behre H M, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update. 2010;16(3):231–245.

13. Tarlatzis BC, Zepiridis L, Grimbizis G, Bontis J. Clinical management of low ovarian response to stimulation for IVF: A systematic review. Hum Reprod. update 2003;9(1):61-76.

14. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L; ESHRE workinggroup on Poor Ovarian Response Definition ,Bologna criteria. Hum Reprod. 2011;26(7):1616-24.

15. Steer CV, Mills CL, Tans L, Capbell S, Edward RG. The cumulative embryo scoring: A predictive embryo scoring technique to select the optimal numbers of embryo to transfer in an IVF and ET programs. Hum Reprod. 1992;7:117-119.

16. Bączkowski T, Kurzawa R, Glabowski W. Method of embryo scoring in *In vitro* Fertilisation. Reprod Biology. 2004;4(1):5-22.

17. Scott RT Jr, Hofmann GE. Prognostic assessment of ovarian reserve. Fertil Steril. 1995;63:1–11.

18. Barnhart K, Osheroff J. Follicle stimulating hormone as a predictor of fertility. Curr Opin Obstet Gynecol. 1998;10:227–232.

19. Sharara FI, Scott RT, Seifer BB. The detection of diminished ovarian reserve in infertile women. Am J Obstet Gynecol. 1998;179:804–12.

20. Bukman A, Heinemann MJ. Ovarian reserve testing and the use of prognostic models in patients with subfertility. Hum Reprod Update. 2001;7:581–590.

21. Durlinger AL, Kramer P, Karels B, de Jong FH, Uilenbroek JT, Grootegoed JA, et al. Control of primordial follicle recruitment by anti-Mullerian hormone in the mouse ovary. Endorinology. 1999;12:5789-5796.

22. La Marca A, Volpe A. Anti-Müllerian hormone (AMH) in female reproduction: is measurement of circulating AMH a useful tool? Clin Endocrinol (Oxf). 2006;64:603-610.

23. Fic¸icio˘glu C, Kutlu K, Baglam E, Bakacak Z. Early follicular antim¨ullerian hormone as an indicator of ovarian reserve. Fertil Steril. 2006;85(3):592–596.

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

25. Hehenkamp WJ, Looman CW, Themmen AP, de Jong FM, Te Velde ER, Broekmans FJ . Anti-Müllerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. J Clin Endocrinol Metab. 2006;91:4057–4063.

26. van Disseldorp J, Lambalk CB, Kwee J, Looman CW, Eijkemans MJ, Fauser BC, Broekmans FJ. Comparison of inter- and intra-cycle variability of anti-Mullerian hormone and antral follicle counts. Hum Reprod. 2010;25(1):221-7. 19.

27. Sharif K, Elgendy M, Lashen H, Afnan M. Age and basal follicle stimulating hormone as predictors of in vitro fertilisation outcome. Br J Obstet Gynaecol. 1998;105:107-112.
28. Gougeon A, Ecochard R, Thalabard JC. Age-related changes of the population of human ovarian follicles: increase in the disappearance rate of non-growing and early-growing follicles in aging women. Biol Reprod. 1994;50:653–663.
29. de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC. Antimullerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril. 2002;77:357-362.
30. Lee TH, Liu CH, Huang CC, Hsieh KC, Lin PM, Lee MS . Impact of female age and male infertility on ovarian reserve markers to predict outcome of assisted reproduction technology. Reproductive Biology and Endocrinology. 2009;7:100.
31. El-Hazmi MA, Warsy AS. Relationship between Age and the Prevalence of Obesity. Bahrain Medical Bulletin. 2002;24:44-51.
32. Wang Z, Heshka S, Heymsfield SB, Shen W, Gallagher DA. Cellular-level approach to predicting resting energy expenditure across the adult years. Am J Clin Nautr. 2005;81:799–806.
33. Van Rooij IA, Broekmans FJ, Scheff er GJ, Looman CW, Habbema JD, De Jong FH et al. Serum anti-Müllerian hormone levels best refl ect the reproductive decline with age in normal women with proven fertility: a longitudinal study. Fertil Steril. 2005;83:979-87.
34. Ruess ML, Kline J, Santos R, Levin B, Timor-Tritsch I. Age and the ovarian follicle pool assessed with transvaginal ultrasonography. Am J Obstet Gynecol. 1996;174:624-7.
35. Haadsma ML, Bukman A, Groen H et al. The number of small antral follicles (2–6 mm) determines the outcome of endocrine ovarian reserve tests in a subfertile population. Hum Reprod. 2007;22:1925–1931.
36. Fanchin R, Schonäuer LM, Righini C, Guibourdenche J, Frydman R, Taieb J . Serum anti-Müllerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. Hum Reprodl. 2003;18:323–327.
37. Visser JA & Themmen APN. Anti-Müllerian hormone and folliculogenesis. Molecular and Cellular Endocrinology. 2005;234:81–86.
38. de Vet A, Laven JS, De Jong FH, Themmen AP, Fauser BC. Antimullerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril. 2002;77:357-362.
39. Eldar-Geva T, Ben Chetrit A, Spitz IM, Rabinowitz R, Markowitz E, Mimoni T et al. Dynamic assays of inhibin B, anti-Mullerian hormone and estradiol following FSH stimulation and ovarian ultrasonography as predictors of IVF outcome. Hum Reprod. 2005;20:3178-3183.
40. Alebić MŠ, Stojanović N, Žuvić-Butorac M: The IVF Outcome Counseling Based on the Model Combining DHEAS and Age in Patients with Low AMH Prior to the First Cycle of GnRH Antagonist Protocol of Ovarian Stimulation. International Journal of Endocrinology. 2013, Article ID 637919, 7 pages.
Available: http://dx.doi.org/10.1155/2013/637919.
41. Bopp B, Seifer D. Age and Reproduction Fertility Glob. libr. Women's med; 2011. Available: http://www.glowm.com/index.html.
42. Brodin T. Ovarian Reserve and Assisted Reproduction. ACTA UNIVERSITATIS UPSALIENSIS UPPSALA; 2013. Ph.D thesis. ISSN 1651-6206; ISBN 978-91-554-8592-4; urn: nbn: see: uu: diva-192998.

43. Himabindu Y, Gopinathan KK, Pandey AK, Sriharibabu M. Correlation of age and antimullerian hormone in assisted reproductive technology program outcome. Indian J Physiol Pharmacol. 2013;57(1):9–15.