IRAQI JOURNAL OF EMBRYOS AND INFERTILITY RESEARCHES (IJEIR)
Muhjah et al., Vol. 10, Issue 2, Pp. 51-74 (2020) Original Research
DOI: http://doi.org/10.28969/IJEIR.v10.i2.r4.20 E-ISSN: 2616-6984 P-ISSN: 2218-0265

Intra cytoplasmic sperm injection (ICSI) outcome comparison among normal, poor and hyper responder females following controlled ovarian stimulation using GnRH antagonist protocol.

Muhjah F. Hassan [1], Wasan A. Abdulhameed [1]

[1] **High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq.**

doctor89muhjah@gmail.com

ABSTRACT

The aim of any given ICSI cycle is to achieve normal response and avoid poor or hyper response. A part from ovarian hyper stimulation syndrome (OHSS), pregnancy rate in hyper responder females usually low which could be related to the adverse effect of hyper estrogenic state on oocytes, embryos quality and uterine endometrium. To evaluate the effect of hyper response on ICSI outcome represented by oocytes, embryos quality, fertilization and pregnancy rates. Study showed that total number of retrieved oocytes, mature, immature oocytes, total embryos, good quality embryos and blastocyst count were significantly higher in hyper followed by normal and poor responders respectively. Fertilization rate was significantly higher in both hyper and normal responders than poor ones, while pregnancy rate was significantly higher in hyper responders 47.6% followed by normal 40.7% and then poor ones 16.7%. Conclusions: Irrespective of abnormal hormonal milieu, excessive ovarian response and higher number of produced oocytes improve ICSI outcome in hyper responder females by increasing the chance of getting higher number of embryos, good quality embryos and even blastocyst numbers which in turn increase the chance of pregnancy.

Received: 10-July-2020
Accepted: 30-July-2020
Published: 06-Aug-2020

How to cite:

Muhjah F. Hassan and Wasan A. Abdulhameed; Intra cytoplasmic sperm injection (ICSI) outcome comparison among normal, poor, and hyper-responder females following controlled ovarian stimulation using GnRH antagonist protocol; Iraqi Journal of Embryos and Infertility Researches (IJEIR), (2020); 10 (2): 51-74.
Doi: http://doi.org/10.28969/IJEIR.v10.i2.r4.20

KEYWORDs

Hyper response, Poor response, Normal response, ICSI.

1. Introduction

Ovarian stimulation is increasingly used following the introduction of assisted reproductive technologies as treatment modalities for infertility in reproductive medicine (Souter et al., 2022 [1]). It is defined as the ovarian and endocrine reaction of the females' ovaries to stimulated drugs (Kumari, Hendarto, and Dwiningsih, 2021 [2]). During in vitro fertilization (IVF), ovarian stimulation supersedes the natural cycle aims of getting multiple oocytes as much as possible. It reduces the risk of having no or little oocytes (Fischer-Holzhausen and Röblitz, 2022 [3]). Together, they improve IVF efficiency and the chance of pregnancy by obtaining more embryos and choosing only the best ones for transfer (Shin et al., 2022 [4]) .

It is the second phase of the IVF procedure after pituitary down-regulation; during it, exogenous hormonal medications are given at regular intervals for approximately 1-2 weeks to increase the number of oocytes (Li et al., 2023 [5]). Considerable variability in ovarian response to stimulation among females necessitates close monitoring (Razafintsalama-Bourdet et al., 2022 [6]). Pregnancy achievement following ovarian stimulation is subjected to many factors, most commonly an ovarian reserve (Ho et al., 2022 [7]). The ovarian response can be classified into three categories: normal (good) response, poor (hypo or sub-optimal), and hyper (exaggerated) response, which is based on the number of growing follicles that had been seen by transvaginal ultrasound (TVUS) during stimulation course and a number of retrieved oocytes following the stimulation during pickup (Datta et al., 2021 [8]). When a female produced oocytes between 4-14 after conventional protocol, this is defined as a normal response, poor response when she produced three or fewer oocytes following maximum stimulation (Polat et al., 2021 [9]). Hyperresponse is defined as having more than 14 oocytes

together with a high serum estrogen level, which is sometimes complicated by ovarian hyperstimulation syndrome (OHSS), cycle cancellation, and freeze of all embryos, which increases the time to pregnancy (Palomba et al., 2023 [10]) .

Apart from 'more oocytes mean better outcome,' the exaggerated ovarian response is usually associated with high estrogen levels; this abnormal hormonal environment may negatively affect the quality of oocytes by increasing the number of immature oocytes and morphologically abnormal ones (Braga et al., 2020 [11]) and resulting in either decrease the number of resulting in embryos, fertilization failure or bad quality embryos (Meng et al., 2023 [12]). Meanwhile, implantation potential is also affected, resulting in implantation failure or pregnancy loss (Huang et al., 2023 [13]). So, this study aims to evaluate oocytes, embryo quality, and pregnancy rate in female responders in comparison with normal and poor respondents.

2. Materials and Methods

A cohort observational study was conducted at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies / Al-Nahrain University / Baghdad / Iraq. One hundred fourteen sub-fertile couples were included in this study. The Local Medical Ethical Committee approved the study. Informed consent was taken from all infertile females to be included in the study. The age of female partners ranged from 18-43 years old. All females were evaluated on 2nd day of the menstrual cycle depending on medical and gynecological history, physical examination, anthropometric measures (weight, height, and BMI) and fertility investigations; hormonal analysis (E2, LH, FSH, Prolactin, TSH, and AMH) and TVUS for the assessment of ovarian reserve (AFC), ovarian pathology (ovarian cyst and PCO morphology), adnexal mass and

endometrium (endometrial thickness (ET) and endometrial pathology). Simultaneously, their male partners were assessed by seminal fluid analysis according to WHO, 2010. Seminal fluid from all of them was assessed in the ICSI lab for fitness for ICSI. All the sub-fertile couples were included in the ICSI program, and the females were subjected to pituitary down-regulation/COS by GnRH antagonist protocol (Cetrotide, Merck KGaA Darmstadt, Germany 0.25 mg 1*1 S.C (flexible protocol). Controlled ovarian stimulation was performed by the administration of different types of gonadptropins; human menopausal gonadotropin (HMG) in the form of in vitro fertilization-Menotropin (IVF-M), LG Chem Ltd, Korea 75-150 IU (75 IU FSH+75 IU LH) or recombinant FSH (r-FSH) in form of Gonal–F, Merck KGaA Dermstadt, Germany 75-300 IU. Ovulation trigger was done by the administration of 500 micrograms of recombinant human chorionic gonadotropin (r-hCG); Ovitrelle, Merck Global, Germany S.C or GnRH agonist (Decapeptyl Ferring Pharmaceuticals, Switzerland 0.2 IU) S.C in hyper responder patients whom at risk of ovarian hyperstimulation syndrome (OHSS) when the leading follicles diameter ranged between 18–20 mm with adequate E2 concentration in the serum > 1500 p/ml. Oocyte pickup under TVUS was done. Those infertile couples were divided according to their response to controlled ovarian stimulation into three groups: Group I: Poor responder/hypo responder who either produced oocytes ≤ 3 or produced oocytes ≥ 5 but needed higher doses of gonadotropin > 3000 IU and longer duration > 10 days (n=30). Group II: Normal responder who produced oocytes ≥ 5 and ≤ 14 (n=54) and Group III: Hyper responder who produced oocytes ≥ 14 (n=30). Inclusion Criteria: Females between 18 and 43 years of age, BMI 19-30 kg/m2, and COS by GnRH antagonist. Exclusion Criteria: Females with hypogonadotropic

hypogonadism, severe endometriosis, previous ovarian surgery, radiation or chemotherapy, premature ovarian failure, COS with GnRH agonist, and male partners with severe oligo-astheno-terato-zo-spermia, azoospermia or testicular sperm biopsy. Microscopic evaluation of oocytes (germinal vesicle (GV), meiosis I (MI), and meiosis II (MII) and embryo quality were done depending on special grading systems (Pierson et al., 2023 [14]). The fertilization rate was calculated based on the number of zygotes and the number of injected mature oocytes. Fresh embryo transfer using 2-3 best-quality embryos was done. Progesterone supplementation as luteal phase support was started on the evening of oocyte retrieval day for two weeks. Beta-hCG in serum was measured to assess chemical pregnancy. The pregnancy rate calculation was followed.

3. Statistical analysis

Data was analyzed using SPSS, V. 26. Categorical variables were represented by number and percentage. Numeric variables were first evaluated for normality distribution using the Kolmogorov-Smirnov test. Normally distributed numeric variables were expressed as the mean and standard deviation in addition to the range. The Chi-square test was used to evaluate the association between any two categorical variables to test the degree of significance, provided that less than 20 % of cells have an expected count of less than 5. One-way analysis of variance (ANOVA) was used to evaluate the difference in the mean of numeric variables among more than two groups, provided that these numeric variables were normally distributed. One-way ANOVA was followed by a post hoc LSD test to evaluate individual differences in mean values between any two groups.

4. Results

With respect to mean age, table 1 shows there was a significant difference among groups; the group (hyper) had a mean age that was significantly lower than that of both (poor and normal) groups who exhibited no difference. There was no significant difference regarding other parameters. Table 2. Exhibits the causes of infertility in the included females. The PCOS cause was predominant in group (hyper), accounting for 76.7 %, and it was reported to a lesser extent in groups normal and poor, 1.9 % and 3.3 %, respectively ($p < 0.001$). A comparison of serum hormonal levels among study groups is shown in Table 3. There was a significant difference in mean AMH and E2 among groups; the highest level was seen in group (hyper), which was significantly higher than that of both (normal and poor) groups ($p < 0.05$), who exhibited no difference. While mean FSH was significantly higher in the normal response group, there was no significant difference in mean LH and TSH among the study groups ($p = 0.238, 0.575$). There was a significant difference in mean prolactin among groups ($p < 0.001$); the highest level was seen in (hyper) followed by group (normal) and least in group (poor). Table 4 shows oocyte characteristics and fertilization rates among study groups. There was a significant difference in mean total oocyte count, MII, and MI among groups; the highest level was seen in hyper, followed by normal, and lastly, in the poor group ($p < 0.001$), while GV oocyte count was highest hyper, which was significantly higher than that of both (normal and poor) groups. There was a significant difference in mean fertilization rate (FR) among groups ($p < 0.001$); the highest level was seen in groups (normal and hyper), being significantly higher than that of the group (poor). A comparison of embryo characteristics among study groups is shown in Table 5. There were significant differences in mean total embryo count, good quality embryo count, and blastocyst count

among groups ($p < 0.001$) in such a way that the highest levels were seen in group (hyper) followed by group (normal) and lastly by group (poor). Figure 1. shows the pregnancy rate among three groups. In groups poor and normal, all women were subjected to embryo transfer, whereas, in group (hyper), nine women were not subjected to embryo transfer, and embryos were cryopreserved. The highest pregnancy rate was achieved by hyper (47.6%), followed by normal (40.7%), and then by poor (16.7%) ($p = 0.001$). The total positive pregnancy rate in all enrolled women was 37 out of 105 (35.3 %).

Table (1): Demographic characteristics of patients enrolled in this study.

Characteristic	**Poor** ***n*** **= 30**	**Normal** ***n*** **= 54**	**Hyper** ***n*** **= 30**	***p***
Age (years)				
Mean ±SD	34.87 ±5.44a	33.76 ±6.37a	28.50 ±6.19b	<0.001 O ***
Range	23 -42	18 -43	18 -42	
BMI (kg/m^2)				
Mean ±SD	24.28 ±3.51	22.77 ±2.30	23.38 ±3.77	0.100 O NS
Range	19 -32.5	19 -32	18 -31	
Duration of infertility (years)				
Mean ±SD	8.63 ±4.63	7.65 ±5.08	6.77 ±3.07	0.281 O NS
Range	1 -21	1 -20	1 -15	
Type of infertility				
Primary, *n* (%)	21 (70.0 %)	44 (81.5 %)	25 (83.3 %)	0.368 C NS
Secondary, *n* (%)	9 (30.0 %)	10 (18.5 %)	5 (16.7 %)	

O: one-way ANOVA and C: Chi-square test.

Table (2): Comparison of causes of infertility among study groups.

Causes of infertility	Poor *n* = 30	Normal *n* = 54	Hyper *n* = 30	*p*1	*p*2
Advanced age	7 (23.3 %)	7 (13.0 %)	0 (0.0 %)	<0.001 C ***	0.022 C *
Endometrial cause	1 (3.3 %)	0 (0.0 %)	0 (0.0 %)		0.244 C NS
Male factor	3 (10.0 %)	15 (27.8 %)	1 (3.3 %)		0.008 C **†
Male factor +advanced age	6 (20.0 %)	14 (25.9 %)	0 (0.0 %)		0.010 C ***†
PCOS	1 (3.3 %)	1 (1.9 %)	23 (76.7 %)		<0.001 C ***†
Tubal factor	0 (0.0 %)	4 (7.4 %)	0 (0.0 %)		0.100 C NS
Unexplained	12 (40.0 %)	13 (24.1 %)	6 (20.0 %)		0.171 C NS

Table (3): Comparison of serum hormonal levels among study groups.

Characteristic	Poor *n* = 30	Normal *n* = 54	Hyper *n* = 30	*p*
AMH (ng/ml)				
Mean ±SD	1.41 ±0.68 b	1.50 ±0.73 b	3.10 ±1.33 a	<0.001 O ***
Range	0.37 -3	0.1 -4.9	0.97 -5.9	
FSH (miu/ml)				
Mean ±SD	5.07 ±1.98 b	6.15 ±2.36 a	4.91 ±1.60 b	0.014 O *
Range	1.05 -11	1.55 -10.7	2.16 -9.3	
LH (miu/ml)				
Mean ±SD	4.29 ±1.94	4.43 ±2.15	5.13 ±2.09	0.238 O NS
Range	1.18 -10.4	1.26 -9.2	1.8 -9.8	
E2 (pg/ml)				
Mean ±SD	31.01 ±8.33 b	32.55 ±5.28 b	39.57 ±6.77 a	<0.001 O ***

Range	22.55 -50.12	23.34 -45.88	23.23 -56.9	
Prolactin (miu/ml)				
Mean ±SD	17.69 ±2.37 c	19.47 ±3.40 b	22.88 ±3.44 a	<0.001 O ***
Range	12.12 -22.08	12.45 -27.78	14.67 -28.88	
TSH(miu/l)				
Mean ±SD	0.60 ±0.26	2.79 ±13.35	1.69 ±0.40	0.575 O NS
Range	0.18 -0.99	0.25 -99	0.77 -2.3	

Table (4): Comparison of oocyte characteristics and fertilization rate among study groups

Characteristic	Poor *n* = 30	Normal *n* = 54	Hyper *n* = 30	*p*
Total oocytes				
Mean ±SD	3.27 ±1.08 c	8.56 ±2.24 b	17.23 ±2.70 a	<0.001 O ***
Range	0 -4	5 -13	14 -24	
MI				
Mean ±SD	0.53 ±0.63 c	1.24 ±0.95 b	2.03 ±0.85 a	<0.001 O ***
Range	0 -2	0 -4	0 -3	
GV				
Mean ±SD	0.10 ±0.31 b	0.31 ±0.61 b	1.00 ±1.08 a	<0.001 O ***
Range	0 -1	0 -2	0 -5	
MII				
Mean ±SD	2.53 ±1.20 c	7.00 ±2.34 b	14.23 ±2.56 a	<0.001 O ***
Range	0 -4	2 -12	10 -19	
FR				
Mean ±SD	72.22 ±31.81 b	85.81 ±15.85 a	84.21 ±6.82 a	0.010 O ***
Range	0 -100	60 -100	69.23 -93.75	

Table (5): Comparison of embryo characteristics among study groups

Characteristic	Poor *n* = 30	Normal *n* = 54	Hyper *n* = 30	*p*
Total embryo				
Mean ±SD	2.00 ±1.05 c	6.02 ±2.32 b	12.03 ±2.61 a	<0.001 O ***
Range	0 -4	2 -12	9 -17	
Good quality embryo (Grade I & II)				
Mean ±SD	1.53 ±1.01 c	4.22 ±1.87 b	9.23 ±2.05 a	<0.001 O ***
Range	0 -4	0 -9	6 -13	
Blastocyst count				
Mean ±SD	0.00 ±0.00 c	0.83 ±0.88 b	3.40 ±1.16 a	<0.001 O ***
Range	0 -0	0 -3	1 -6	

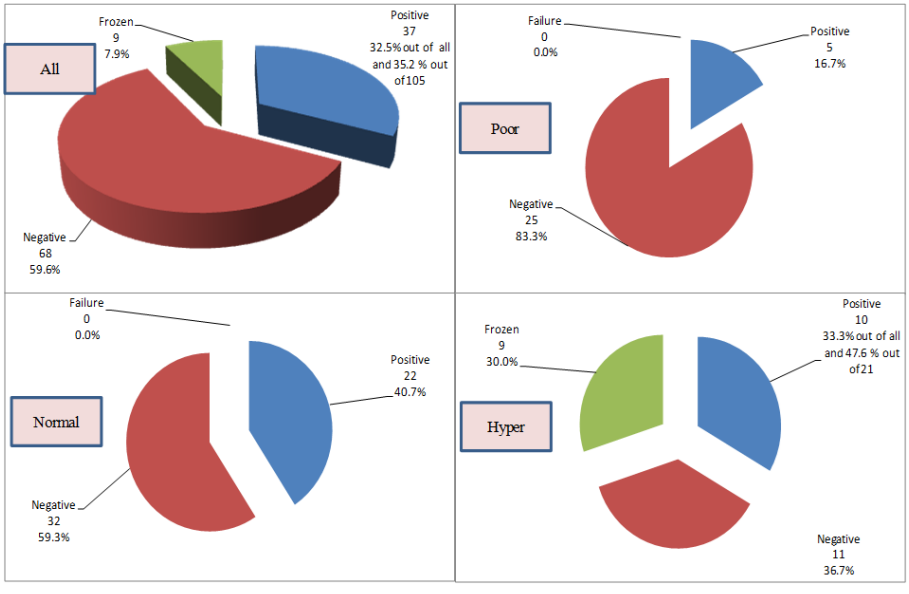

Figure (1): Pie chart showing a comparison of pregnancy rates among study groups

4-Discussion

The study exhibited a significant difference regarding the age of females among the three groups; the females in the hyper response were significantly younger than those in the normal and poor response groups, who showed no significant difference. Several studies go with these results and showed that females who exhibit a hyper response or are predicted to be hyper responders are significantly younger than those who exhibit normal or poor response (Knez et al., 2015 [15]; Yadav et al., 2019 [16]), while Kamel, 2017 did studies [17] and Hassan, Al-Yasiry, and Ghaleb 2020 [18]) showed that hyper response females are non-significantly younger. A study by Tasks et al. showed no significant difference in age among the three groups (Tsakos et al., 2014 [19]). This could be explained by the fact that young females usually have a good ovarian reserve and tend to exhibit normal responses. Still, when certain characteristics exist, these females exhibit hyperresponse (presence of PCOS or multi-cystic ovaries, excessive gonadotropins stimulation, high starting dose of gonadotropins, and a high AMH) (Vembu and Reddy, 2017 [20]; Di Paola et al., 2018 [21]) .

Regarding BMI, the study exhibited no significant difference among the three groups. This was consistent with the results of the studies done by (Tsakos et al., 2014 [19]; Hassan, Al-Yasiry, and Ghaleb, 2020 [18]). While others showed that females who exhibited hyperresponse significantly had a lower BMI (Kamel, 2017 [17]; Yadav et al., 2018 [16]). The detrimental effect of high BMI on the response to ovarian stimulation can be explained via the effects of certain mediators that are associated with obesity e.g., leptin and ghrelin (Esinler, Bozdag, and Yarali, 2008 [22]), high leptin within ovarian follicles inhibits steroidogenesis by its antagonistic effect to stimulatory factors (IGF-1, TGF-b, insulin, and LH) making the obese female's response to ovarian

stimulation lower than normal. The absence of these mediators in lean or normal-weight females puts them at risk of excessive or hyperresponse (Hassan, Al-Yasiry, and Ghaleb, 2020 [18]). Also, higher doses of gonadotropin and longer durations of stimulation cycles were needed to stimulate overweight/obese PCOS women than normal-weight women, which led to higher E2 levels and more stimulated follicles and increased the risk of hyperresponse and even development of OHSS (Cakiroglu et al., 2017 [23]).

The current study showed no significant differences regarding the type and duration of infertility, which was reported in a study by (Amin et al., 2021 [24]). However, a study by Satwik et al. showed that females in the poor response group had a significantly longer duration of infertility than those in the normal and hyper response groups, which could be related to poor reserve, female age, and treatment failure (Satwik et al., 2012 [25]).

Regarding the cause of infertility of the couples involved in the study, there was a significant difference among the three groups. About 23.3 % of the poor response group age were≥ 38 years old vs. 13.0% and 0.0 % in the normal and hyper response groups, respectively. This was approved by several studies that had the same results, which could be related to decrease ovarian reserve with increasing age, especially above 35 years (Chang et al., 2023 [26]). About 76.7 % of females in the hyperresponse group had PCOS vs 3.3% and 1.9% in poor and normal response groups, respectively .

These inconsistent with the results of several studies that were done on females with PCOS following COS/ ICSI, and most of them concluded that PCOS females are usually at risk of excessive response to ovarian stimulation and OHSS, which is due to the high number of small follicles that.

Are arrested in the antral stage, which is highly sensitive to gonadotropins

even at low doses (Fouks et al., 2023 [27]; Sudhakaran et al., 2023 [28]).

Regarding the females in the normal response group, they significantly had male factor cause 27.8% or male factor and advanced female age 25.9 % compared with 10.0% and 3.3% in poor response, 20.0% and 0.05 in hyper response group, respectively .

Regarding the serum levels of AMH, FSH, and LH of the females in the three groups, the study showed a significant difference in the serum level of AMH being highest in the group of hyper response with no significant difference between normal and poor response groups. Serum LH levels did not show any significant difference among the females of the three groups .

Similar results had been reported by several studies that showed hyperresponse females had significantly higher AMH than those of other groups, and LH exhibited no.

Significant differences among hyper, poor, and normal response females (Tsakos et al., 2014 [19] Amin et al., 2021[24]).

Regarding serum FSH level, it was significantly higher in the normal response group with no significant difference between both hyper and poor response groups, which is not in agreement with the results of the above studies that showed a significant high FSH level in poor response group and with the study by Sandeep and Sangisapu, that when compared serum FSH levels between normal and poor responders, it was significantly higher in poor response than normal response group (Sandeep and Sangisapu, 2019 [29]) .

Regarding serum E2 level, it was significantly higher in the response group than in the normal and poor response one who exhibited no significant difference between them. Serum prolactin level was significantly higher in the response group, followed by normal and poor response groups, respectively, while serum TSH level exhibited no significant difference

among the three groups. In agreement with the current study, results is a study by Hu et al. that showed serum E2 level was significantly higher in hyper responder females than normal responder ones, and serum prolactin level was insignificantly higher in hyperresponder than normal responder females (Hu et al., 2020 [30]). A study by Sandeep and Sangisapu showed no significant difference in serum E2 levels between normal and poor responder females (Sandeep and Sangisapu, 2019 [29]). No significant difference in basal serum levels of E2, prolactin, and TSH between normal and poor responder females had been reported in a study by (Amin et al., 2021 [24]). While a study by Laqqan and Yassin showed that serum E2 level was significantly higher in poor response followed by hyper response and normal response groups, respectively, serum LH level was significantly higher in hyper response than both normal and poor response, while serum FSH, prolactin and TSH levels exhibited no significant differences among the three groups (Laqqan and Yassin, 2021 [31]).

However, in the current study, FSH was within the accepted normal range at cycle day 2 (2.9-12.0 miu/ml), so it could be an accidental finding or could be explained that the mean age of poor response group did not show a significant difference with that of normal response, so both groups were in the same age range that made serum FSH levels of poor response did not exhibit such a wide range of difference .

Although serum levels of both E2 and prolactin were also within the normal range (18-147 pg/ml and 5-35 miu/l respectively) in the response group, A study by Zhang et al. showed that when basal serum prolactin level was higher than 16 ng/ml, it usually increases the rate of producing higher number of retrieved mature oocytes, embryos and high cumulative pregnancy rate (Zhang et al., 2020 [32]). In addition, as the current study previously showed, most of the females in the hyperresponse

group had PCOS, and as suggested by researchers, about 10% of PCOS females have an associated high serum prolactin level and even hyper prolactinemia which could explain why females in hyper response group exhibited higher serum prolactin level in this study (Hassan, 2020 [33]). Similarly, a study by Barrenetxea et al. showed that there was a significant positive correlation between the total number of retrieved oocytes during IVF and basal serum E2 level in such a way that higher E2 level (within normal range) usually increases the rate of producing high number of oocytes (Barrenetxea et al., 2019 [34]), in contrast to high basal level of E2 > 70 pg/ml which usually predisposes to and predicts poor ovarian response (Udumudi, Lava and Hegde, 2023 [35])

Regarding the total number of retrieved oocytes during pick up and their maturity status, the current study showed that the total number of retrieved oocytes and total number of mature MII oocytes was significantly more in the hyper response followed by normal then poor response respectively. Similar results have been suggested by some studies (Amin et al., 2021 [24]; Abbasi et al., 2023 [36]). These studies explained that "the more oocytes retrieved, the better" is the general opinion. Together, hyper-responder females are usually young; already, their oocyte quality is good compared to the age-related decline in oocyte quality that presents in poor-responder females (Sasaki et al., 2019 [37]).

The total number of immature oocytes (MI) was significantly more in hyper response followed by normal and poor, while the total number of immature oocytes (GV) was significantly more in hyper response than both normal and poor response groups who exhibited no significant difference. Similar results were shown in studies by Astbury et al. and Kok et al. The latter showed that hyper-responder females had an increased tendency to produce immature oocytes

with no negative effects on fertilization rate and pregnancy potential (fertilization rate in hyper-responders was similar to that in normal ones, while PR was significantly higher in hyper than normal responders) (Kok et al., 2006 [38]; Astbury et al., 2020 [39]). This is due to the detrimental effects of ovarian stimulation and high estrogen (E2) level that occurs in excessive ovarian response (Zhang et al., 2023 [40]), which was proved while studying the oocyte quality in hyperstimulated donor females (Ertzeid and Storeng, 2001[41]).

Together, in the current study, the majority of hyperresponse females were triggered by GnRH agonists instead of hCG for either decreasing the risk or preventing OHSS .

Studies exhibited that GnRH agonist triggers an increase in the rate of getting immature oocytes (Alyasin Mehdinejadiani and Ghasemi, 2016 [42]. FR was significantly higher in hyper and normal response groups than in the poor response group despite a higher rate of male factor in the normal response group. This could be explained by the higher number of total oocytes and mature oocytes in both hyper and normal response groups, and the male partners were selected in such a way that only those with moderate impairment were included, and those with severe impairment were excluded. Together, during ICSI, the embryologists usually select the best sperm (progressively motile and morphologically normal). This result is similar to what was shown in studies (Kok et al., 2006 [38]; Astbury et al., 2020 [39]; Abbasi et al., 2023 [36]).

Insignificant differences regarding FR among normal, poor, and hyperresponders also had been suggested (Liu et al., 2023 [43]).

The study showed that there were significant differences in mean total embryo count, good quality embryo count, and blastocyst count among groups in such a way that the highest number was seen in the hyper response group, followed by the normal response

group, and lastly, the poor response group. This is in agreement with what was suggested by several studies that concluded the total number of embryos and good-quality embryos were significantly higher in hyperresponder females (Mee et al., 2014 [44]; Tsakos et al., 2014 [19]). Similarly, a study by Amin et al. that compared the total number and score of embryos between normal and poor response females showed that normal response ones significantly produced a higher number of embryos and good quality embryos (Amin et al., 2021 [24]) .

This could be explained by the fact that hyper-responder females produce a higher number of oocytes, so their chance of getting embryos of good quality is high. Evenly following embryo transfer, the surplus good quality embryos can be cultured for more than three days up to 5 days, which increases the chance of successfully reaching the blastocyst stage .

In addition, as shown in the current and previously mentioned studies, the hyper-responder females are younger than normal and poor, so their chance of producing good-quality embryos and blastocysts tends to be higher (Wu et al., 2023 [45]). Together, only 1% of females in the response group had male factor cause, which increased the chance of producing good quality embryos and blastocyst count in such a group of females.

Regarding the overall PR of all included females in the study, it was 35.3%, which is accepted to global PR following ICSI (Adebayo et al., 2023 [46]) .

Regarding the PR in each group, it was significantly higher in the females of hyperresponse at 47.6%, followed by the normal response group at 40.7% and then the poor response group at 16.7% .

These results were similar to studies by Huber et al. and Bansiwal et al., that showed a higher pregnancy rate and clinical pregnancy rate in hyper

responder females when compared with normal and poor responders (Huber et al., 2013 [47] and Bansiwal et al., 2023 [48]) which could be due to younger age and more good quality oocytes and embryos. No significant differences in pregnancy rate between normal and poor response females had been reported in a study by (Amin et al., 2021 [24]). A study by Mee et al. showed that normal-response females significantly had higher pregnancy and clinical pregnancy rates than both hyper and poor-response females, which is related to the negative effects of high E2 on endometrium and embryo implantation potential in hyper-responder females (Mee et al., 2014 [44]; Xu et al., 2023 [49]).

6- Conclusion

Irrespective of abnormal hormonal milieu, excessive ovarian response and a higher number of produced oocytes improve ICSI outcome in hyper-responder females by increasing the chance of getting a higher number of embryos, good quality embryos, and even blastocyst numbers, which in turn increase the chance of pregnancy.

Acknowledgment

We would like to acknowledge the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/ Al Nahrain University, Baghdad, Iraq.

Funding

This work received no funding .

Author Contribution

Muhjah F. Hassan performed the study, and Wasan A. Abdulhameed supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The Ethical Approval Committee approved the study.

Financial Disclosure

There is no financial disclosure.

References

[1] Souter I, Sun F, Zhang H, Diamond MP, Legro RS, Wild RA, Hansen KR, Santoro N. A personalized medicine approach to ovulation induction/ovarian stimulation: development of a predictive model and online calculator from level-I evidence. Fertility and

Sterility. 2022 Feb 1;117 (2):408-18. https://doi.org/10.1016/j.fertnstert.2021.10.024.

[2] Kumari RK, Agung A, Hendarto H, Dwiningsih SR. The Relationship of Antral Follicle Count (AFC) with Levels of Estradiol Serum and Mature Ovarian Follicles in Women Undergoing the IVF Program at the Graha Amerta Fertility Clinic of General Hospital of Dr. Soetomo in the Period 2018. Indian Journal of Forensic Medicine & Toxicology. 2021 Jul 1;15.(3)

[3] Fischer-Holzhausen S, Röblitz S. Mathematical modelling of follicular growth and ovarian stimulation. Current Opinion in Endocrine and Metabolic Research. 2022 Aug 6:100385. https://doi.org/10.1016/j.coemr.2022.100385.

[4] Shin J, Kwon H, Choi DH, Park C, Kim JH, Kim J, Kang YJ, Koo HS. Accumulated Vitrified Embryos Could Be a Method for Increasing Pregnancy Rates in Patients with Poor Ovarian Response. Journal of Clinical Medicine. 2022 Aug 23;11 (17):4940. https://doi.org/10.3390/jcm11174940 .

[5] Li NJ, Yao QY, Yuan XQ, Huang Y, Li YF. Anti–müllerian hormone as a predictor for live birth among women undergoing IVF/ICSI in different age groups: an update of systematic review and meta-analysis. Archives of Gynecology and Obstetrics. 2023 Jul;308 (1):43-61. https://doi.org/10.1007/s00404-022-06683-1.

[6] Razafintsalama-Bourdet M, Bah M, Amand G, Vienet-Lègue L, Pietin-Vialle C, Bry-Gauillard H, Pinto M, Pasquier M, Vernet T, Jung C, Levaillant JM. Random antral follicle count performed on any day of the menstrual cycle has the same predictive value as AMH for good ovarian response in IVF cycles. Journal of Gynecology Obstetrics and Human Reproduction. 2022 Jan 1;51 (1):102233. https://doi.org/10.1016/j.jogoh.2021.102233.

[7] Ho CY, Chang YY, Lin YH, Chen MJ. Prior salpingectomy impairs the retrieved oocyte number in in vitro fertilization cycles of women under 35 years old without optimal ovarian reserve. Plos one. 2022 May 4;17 (5):e0268021. https://doi.org/10.1371/journal.pone.0268021

[8] Datta AK, Maheshwari A, Felix N, Campbell S, Nargund G. Mild versus conventional ovarian stimulation for IVF in poor, normal and hyper-responders: a systematic review and meta-analysis. Human reproduction update. 2021 Mar 1;27 (2):229-53. https://doi.org/10.1093/humupd/dmaa035.

[9] Polat M, Mumusoglu S, Yarali Ozbek I, Bozdag G, Yarali H. Double or dual stimulation in poor ovarian responders: where

do we stand? Therapeutic Advances in Reproductive Health. 2021 Jun;15:26334941211024172. https://doi.org/10.1177/26334941211024172.

[10] Palomba S, Costanzi F, Nelson SM, Caserta D, Humaidan P. Interventions to prevent or reduce the incidence and severity of ovarian hyperstimulation syndrome: a systematic umbrella review of the best clinical evidence. Reproductive Biology and Endocrinology. 2023 Jul 21;21 (1):67. https://doi.org/10.1186/s12958-023-01113-6.

[11] Braga DP, Zanetti BF, Setti AS, Iaconelli Jr A, Borges Jr E. Immature oocyte incidence: Contributing factors and effects on mature sibling oocytes in intracytoplasmic sperm injection cycles. JBRA Assisted Reproduction. 2020 Jan;24 (1):70. https://doi.org/10.5935%2F15180557.20190056.

[12] Meng Y, Tao L, Xia T, Zhu J, Lin X, Zhou W, Liu Y, Ou J, Xing W. Elevated estradiol levels on hCG trigger day adversely effects on the clinical pregnancy rates of blastocyst embryo transfer but not cleavage-stage embryo transfer in fresh cycles: a retrospective cohort study. PeerJ. 2023 Jul 18;11:e15709. https://doi.org/10.7717/peerj.15709.

[13] Huang J, Lu Y, He Y, Wang Y, Zhu Q, Qi J, Ding Y, Zhao H, Ding Z, Sun Y. The effect of peak serum estradiol level during ovarian stimulation on cumulative live birth and obstetric outcomes in freeze-all cycles. Frontiers in Endocrinology. 2023;14. https://doi.org/10.3389%2Ffendo.2023.1130211.

[14] Pierson HE, Invik J, Meriano J, Pierson RA. A novel system for rapid conversion of Gardner embryo grades to linear scale numeric variables. Reproductive BioMedicine Online. 2023 May 1;46 (5):808-18. https://doi.org/10.1016/j.rbmo.2023.01.008.

[15] Knez J, Kovačič B, Medved M, Vlaisavljević V. What is the value of anti-Müllerian hormone in predicting the response to ovarian stimulation with GnRH agonist and antagonist protocols? Reproductive Biology and Endocrinology. 2015 Dec;13:1-7. doi.org/10.1186/s12958-015-0049-5.

[16] Yadav V, Malhotra N, Mahey R, Singh N, Kriplani A. Ovarian sensitivity index (OSI): validating the use of a marker for ovarian responsiveness in IVF. Journal of reproduction & infertility. 2019 Apr;20 (2):83 PMID: 31058052; PMCID: PMC6486569.

[17] Kamel A. Using Anti-Müllerian Hormone and Serum Estradiol to Predict Ovarian Hyperstimulation Syndrome in Patients Using Antagonist Protocol During

IVF/ICSI Cycles. Evidence-Based Women's Health Journal. 2017;7 (2):63-68.

[18] Hassan MF, Al-Yasiry RZ, Ghaleb RA. Possible predisposing and predictable factors for the development of ovarian hyperstimulation syndrome (OHSS) in women with polycystic ovary syndrome (PCOS) whom have undergone intra cytoplasmic sperm injection (ICSI). Malaysian Journal of Biochemistry and Molecular Biology. 2020:1:37-40.

[19] Tsakos E, Tolikas A, Angelos Daniilidis, Asimakopoulos B. Predictive value of anti-mu¨llerian hormone, follicle-stimulating hormone, and antral follicle count on the outcome of ovarian stimulation in women following GnRH-antagonist protocol for IVF/ET. Arch Gynecol Obstet. 2014. Springer.

[20] Vembu R, Reddy NS. Serum AMH level to predict the hyper response in women with PCOS and non-PCOS undergoing controlled ovarian stimulation in ART. Journal of Human Reproductive Sciences. 2017 Apr;10 (2):91. PMID: 28904496; PMCID: PMC5586096.

[21] Di Paola R, Garzon S, Giuliani S, Laganà AS, Noventa M, Parissone F, Zorzi C, Raffaelli R, Ghezzi F, Franchi M, Zaffagnini S. Are we choosing the correct FSH starting dose during controlled ovarian stimulation for intrauterine insemination cycles? Potential application of a nomogram based on a woman's age and markers of ovarian reserve. Archives of gynecology and obstetrics. 2018 Nov;298:1029-35. doi.org/10.1007/s00404-018-4906-2 .

[22] Esinler I, Bozdag G, Yarali H. Impact of isolated obesity on ICSI outcome. Reproductive biomedicine online. 2008 Jan 1;17 (4):583-7. https://doi.org/10.1016/S14726483(10)60249-0.

[23] Cakiroglu Y, Doger E, Vural F, Kopuk SY, Vural B. Impact of insulin resistance and obesity on intracytoplasmic sperm injection outcomes in young women with polycystıc ovary syndrome. North Clin Istanb. 2017 Oct 20;4 (3):218-24. doi: 10.14744/nci.2017.79663.

[24] Amin AH, Sherif N, Askalani A, SaadAlla S. Comparative Study Between Ovarian Response Prediction Index Versus Anti-Müllerian Hormone, Antral Follicular Count as Predictors of Ovarian Response in Women Undergoing IVF/ICSI Cycles. Evidence-Based Women's Health Journal. 2021 May 1;11 (2):146-56. https://dx.doi.org/10.21608/ebwhj.2019.17205.1020.

[25] Satwik R, Kochhar M, Gupta SM, Majumdar A. Anti-mullerian hormone cut-off

values for predicting poor ovarian response to exogenous ovarian stimulation in in-vitro fertilization. Journal of Human Reproductive Sciences. 2012 May;5 (2):206 https://doi.org/10.4103%2F09741208.101023.

[26] Chang J, Xu L, Qin Y, Liu R, Li C, Gao S, Dang Y. Low anti-mullerian hormone decreased clinical pregnancy and increased risk of poor ovarian response in women over 35 years of age. Chinese Medical Journal. 2023 Feb 20;136 (04):499-501.

[27] Fouks Y, Neuhausser W, Ryley D, Penzias A, Sakkas D, Vaughan D. ART outcomes in lean compared to obese phenotypes of polycystic ovarian syndrome. Journal of Assisted Reproduction and Genetics. 2023 May 22:1-9. https://doi.org/10.1007/s10815-023-02804-0.

[28] Sudhakaran G, Babu SR, Mahendra H, Arockiaraj J. Updated experimental cellular models to study polycystic ovarian syndrome. Life Sciences. 2023 Apr 5:121672. https://doi.org/10.1016/j.lfs.2023.121672.

[29] Sandeep K, Sangisapu S. Comparative study of ovarian reserve tests and ovarian response to controlled ovarian stimulation in IVF. Journal of Evidence-Based Medicine Healthc. 2019;6 (1):1-8.

[30] Hu L, Sun B, Ma Y, Li L, Wang F, Shi H, Sun Y. The relationship between serum delta FSH level and ovarian response in IVF/ICSI cycles. Frontiers in Endocrinology. 2020 Nov 5;11:536100. https://doi.org/10.3389/fendo.2020.536100.

[31] Laqqan MM, Yassin MM. Investigation of the Predictive Factors of Diminished Ovarian Reserve in Women Aged Less Than 40 Years and Undergoing ICSI Cycle. Reproductive Sciences. 2023 Mar;30 (3):873-82. https://doi.org/10.1007/s43032-022-01055-1.

[32] Zhang D, Yuan X, Zhen J, Sun Z, Deng C, Yu Q. Mildly higher serum prolactin levels are directly proportional to cumulative pregnancy outcomes in in-vitro fertilization/intracytoplasmic sperm injection cycles. Frontiers in Endocrinology. 2020 Aug 28;11:584. https://doi.org/10.3389/fendo.2020.00584.

[33] Hassan MF. THE FREQUENCY OF ELEVATED PROLACTIN LEVEL IN POLYCYSTIC OVARY SYNDROME WOMEN (PCOS) AND ITS EFFECT ON PREGNANCY RATE. Global Journal of Public Health Medicine. 2020 Mar 1;2 (1):109-17. https://doi.org/10.37557/gjphm.v2i1.28.

[34] Barrenetxea G, Martínez E, De las Heras M, Arambarri JI, Axpe M, Barrenetxea J. Lack of predictive value of ovarian reserve tests for pregnancy likelihood. The a huge

difference between quantity and quality. Am J Biomed Sci & Res. 2019:133-41. https://doi:10.34297/AJBSR.2019.03.000649

[35] Udumudi A, Lava KC, Hegde, V. High-risk genotypes associated with poor response to controlled ovarian stimulation in Indian women. Journal of Human Reproductive Sciences. 2023;16 (1):70.

[36] Abbasi F, Davar R, Shamsi F, Dashti S. Insulin-like growth factor-1 on cycle day two and assisted reproductive techniques outcome: A cross-sectional study. International Journal of Reproductive Biomedicine. 2023 Feb;21 (2):139. https://doi.org/10.18502%2Fijrm.v21i2.12804.

[37] Sasaki H, Hamatani T, Kamijo S, Iwai M, Kobanawa M, Ogawa S, Miyado K, Tanaka M. Impact of oxidative stress on age-associated decline in oocyte developmental competence. Frontiers in Endocrinology. 2019 Nov 22;10:811. https://doi.org/10.3389/fendo.2019.00811.

[38] Kok JD, Looman CW, Weima SM, te Velde ER. A high number of oocytes obtained after ovarian hyperstimulation for in vitro fertilization or intracytoplasmic sperm injection is not associated with decreased pregnancy outcomes. Fertility and sterility. 2006 Apr 1;85 (4):918-24. https://doi.org/10.1016/j.fertnstert.2005.09.035.

[39] Astbury P, Subramanian GN, Greaney J, Roling C, Irving J, Homer HA. The Presence of Immature GV− Stage Oocytes during IVF/ICSI Is a Marker of Poor Oocyte Quality: A Pilot Study. Medical Sciences. 2020 Jan 16;8 (1):4. https://doi.org/10.3390/medsci8010004.

[40] Zhang F, Zhang H, Du H, Li X, Jin H, Li G. Application value of Early-Follicular Phase Long-Acting Gonadotropin-Releasing Hormone Agonist Long Protocol in patients with resistant ovary syndrome. BMC Pregnancy and Childbirth. 2023 Dec;23 (1):1-9. https://doi.org/10.1186/s12884-023-05477-w .

[41] Ertzeid G, Storeng R. The impact of ovarian stimulation on implantation and fetal development in mice. Human reproduction. 2001 Feb 1;16 (2):221-5. https://doi.org/10.1093/humrep/16.2.221.

[42] Alyasin A, Mehdinejadiani S, Ghasemi M. GnRH agonist trigger versus hCG trigger in GnRH antagonist in IVF/ICSI cycles: A review article. International Journal of Reproductive BioMedicine. 2016 Sep;14 (9):557. PMID: 27738657; PMCID: PMC5054292.

[43] Liu S, Guo Y, Li F, Jin L. Influence of ovarian reserves on assisted reproductive and perinatal outcomes in patients with endometriosis: a retrospective study. Frontiers in Endocrinology. 2023 May 12;14:1084927. https://doi.org/10.3389/fendo.2023.1084927.

[44] Mee L, Aw TC, Hum SC, Lee SN, Chung SF, Yu SL. Basal serum anti-müllerian hormone and antral follicle count are predictors of ovarian response for Asian women in Singapore. Asian Pacific Journal of Reproduction. 2014 Sep 1;3 (3):169-75. https://doi.org/10.1016/S23050500(14)60021-X.

[45] Wu R, Chen Z, Xu W, Yang C, Zhou H, Xu W, Huang G, Zhao S. Impact of having surplus blastocysts cryopreserved on the ongoing pregnancy rate following a fresh transfer. Gynecological Endocrinology. 2023 May 26;39 (1):2217281. https://doi.org/10.1080/09513590.2023.2217281.

[46] Adebayo FO, Ameh N, Adesiyun AG, Ekele BA, Wada I. Correlation of female age with the outcome of IVF in a low-resource setting. International Journal of Gynecology & Obstetrics. 2023 Apr;161 (1):283-8. https://doi.org/10.1002/ijgo.14545.

[47] Huber M, Hadziosmanovic N, Berglund L, Holte J. Using the ovarian sensitivity index to define poor, normal, and high response after controlled ovarian hyperstimulation in the long gonadotropin-releasing hormone-agonist protocol: suggestions for a new principle to solve an old problem. Fertility and sterility. 2013 Nov 1;100 (5):1270-6. https://doi.org/10.1016/j.fertnstert.2013.06.049.

[48] Bansiwal R, Mahey R, Malhotra N, Singh N, Saini M, Bhatt A, Nilima N, Mani K, Cheluvaraju R, Rajput M, Bhatla N. Comparison of Cumulative Live Birth Rate (CLBR) According to Patient-Oriented Strategies Encompassing Individualized Oocyte Number (POSEIDON) Stratification Among Low Prognosis Women Undergoing IVF-ICSI Cycles. Journal of Reproduction & Infertility. 2023 Apr 18;24 (2):117-31. https://dx.doi.org/10.18502/jri.v24i2.12497.

[49]- Xu X, Yang A, Han Y, Wang W, Hao G, Cui N. Determining the Association between Serum Estradiol Levels on hCG Trigger Day and Live Birth Rates in Non-PCOS Patients. Research Developments in Medicine and Medical Science Vol. 8. 2023 Apr 10:13-44. 10.9734/bpi/rdmms/v8/18901D.