Effect of Ovarian Stimulation Protocol on Embryo Quality in IVF-ICSI

Mufeda A. Jwad [1] , Ban A.Abdulmajeed [2] ,Ula M. Al-Kawaz [1]
1-High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University,Baghdad-IRAQ.
2-College of Medicine,Al- Nahrain University,Baghdad-IRAQ.

Abstract:

Background:Defects in reproduction that may involve both the male & female partners result in the inability to procreate which is known as infertility. The use of assisted reproductive technologies (ART's) has been increasing over the past three decades. Many efforts have been done to compensate for unsatisfactory results in in-vitro fertilization (IVF) procedures, so that patients undergo different ovarian stimulation protocols to control retrieval of multiple oocytes in a single cycle. Although ovarian stimulation has an important role in ART's, it may also have detrimental effects on oocyte quality, embryo quality, and IVF outcomes.

Objectives: To assess the effect of long agonist versus antagonist protocols on oocyte and embryo quality in IVF- Intracytoplasmic sperm injection (ICSI).

Patients,Materials and Methods:

This comparative study was done on the embryos of seventy one infertile females who were undergoing Intracytoplasmic sperm injection (ICSI) treatment regardless to the presence or absence of previous trials. These patients were divided into two groups, 45 of them treated with agonist protocol and other 26 patients treated with antagonist protocol. First oocyte retrieved was used for further assessment. Of the 71 oocytes, there were eight immature oocytes (3 at germinal vesicle (GV) stage, 5 at metaphase I (MI) stage) and three abnormal oocytes, and these were excluded from the following ICSI treatment. Assessment and comparison of oocyte maturity, fertilization rate, cleavage rate and resulting embryo quality were done.

Results: The results of the present study showed significant difference ($p<0.05$) in oocyte number and maturity but no significant ($p>0.05$) difference between the group treated with long agonist and those on antagonist protocol regarding fertilization rate, cleavage rate, and embryo quality.

Conclusions: The type of protocol used in *in-vitro* fertilization has no detrimental effect on oocyte or embryo quality.

Keywords: IVF protocol, agonist, antagonist, embryo quality.

Introduction:

TThe clinical definition of infertility used by the World Health Organization (WHO) is "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse"(1).

Assisted reproduction includes all the methods used for fertilization, which is not achieved through sexual intercourse (2). Intracytoplasmic sperm injection (ICSI) is, currently, the most efficient modification of micromanipulation- assisted fertilization, whereby one spermatozoon is selected, aspirated into a microinjection needle, and injected into the oocyte cytoplasm (3).

Today, assisted reproductive technologies (ART's) has become a well-established and highly efficient therapy for infertility (4). In ART's, it is well understood that the most important factors for maximizing the success rate of *in vitro* fertilization (IVF) are retrieving greater numbers of high-quality oocytes using controlled ovarian hyperstimulation (COH) and establishing a receptive endometrium. Therefore, COH plays a principal role in achieving a high ART success rate (5,6).

Gonadotropin-releasing hormone (GnRH) agonists and antagonists have been widely used to prevent premature LH surge during ovarian stimulation for IVF and embryo transfer (7). GnRH agonist suppresses gonadotropin secretion through both pituitary desensitization and GnRH receptor down-regulation whereas GnRH antagonist competes with endogenous GnRH for receptor binding and therefore rapidly inhibits secretion of gonadotropin. The introduced GnRH antagonist, Cetrorelix, has been shown to be efficient in preventing the LH rise during stimulation in IVF protocols (8)

A successful *in vitro* fertilization (IVF) program can be achieved through an accurate embryo quality evaluation (9). In most IVF clinics around the world, this quality assessment depends mainly on the morphological evaluation of cleavage stage embryos before embryo transfer which is correlated to implantation potential of each particular embryo and pregnancy rate after IVF (10). For this purpose many classification systems developed (11).

-Parameters of Embryo Classification:

On day 3, embryo quality assessment is based on morphological criteria (blastomeres number, blastomere regularity and fragmentation rate) (12).

One of suggested grading system which depends on morphological criteria of cleaving embryo is classified as follows (with grade 1 representing the best morphological condition):

-Grade I: Symmetrical blastomeres and no fragmentation

-Grade II: Even or uneven blastomeres and 10-20% fragmentation.

-Grade III: Uneven blastomeres and> 20% fragmentation (10).

Patients,Materials and Methods:

This prospective comparative study included seventy one women who underwent ICSI at the infertility clinic of High Institute of Infertility Treatment and Assisted reproductive technologies/ Al- Nahrain University/ Baghdad/ Iraq, during the period from December 2015 until December 2016. Their age range was 20 to 43 years. The infertility duration range 1-20 years with both primary and secondary infertility. These patients were divided into two groups, 45 of them treated with agonist protocol and other 26 patients treated with antagonist protocol. The long agonist protocol and antagonist protocols are summarized in figure (1)

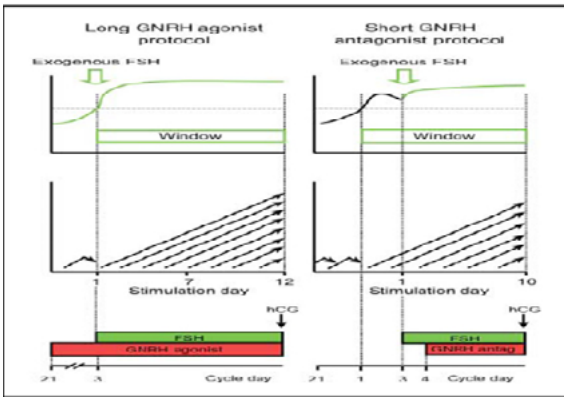
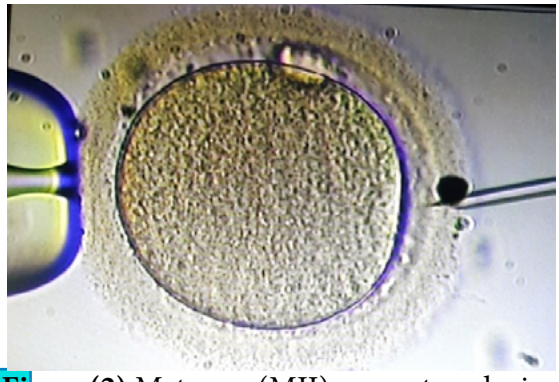


Figure (1): long GnRH agonist regimen; GnRH antagonist regimen. The window represents the duration of time at which FSH concentrations are above the threshold. Each arrow represents a developing follicle (13).

All the 71 women, had a normal ovarian reserve, based on the serum AMH level and antral follicle count (AFC), evaluated at day 3 of the menstrual cycle. Of the 71 oocytes, there were eight immature oocytes (3 at germinal vesicle (GV) stage, 5 at metaphase I (MI) stage) and three abnormal oocytes ((figure 2, 3,4 and 5), and these were excluded from the following ICSI treatment. Of the remaining sixty mature oocytes undergoing ICSI treatment (38 from agonist group and 22 from antagonist group) all formed two pronuclei 14–16 hours after the treatment. From these fertilized oocytes of both groups 12 arrested at 2PN and 48 undergo cleavage giving embryos with different grades of maturity. These embryos were classified on day three according to the number of blastomeres and percentage of fragmentation (12).



Figure(2):Mature (MII) oocyte during ICSI.

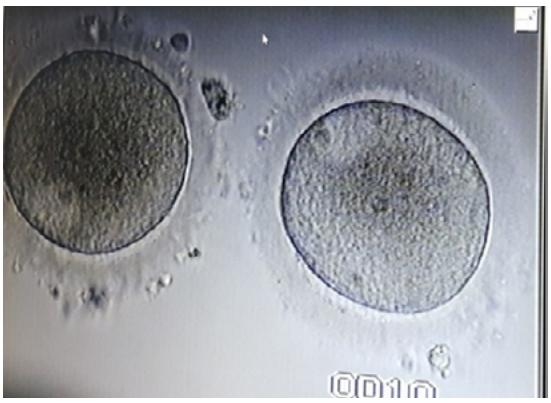


Figure (3): 2 oocytes in MI grade of maturity were excluded from the study

Figure (4): 4 oocytes of GV grade of maturity with abnormally wide PVS in upper left oocyte these were excluded from the study

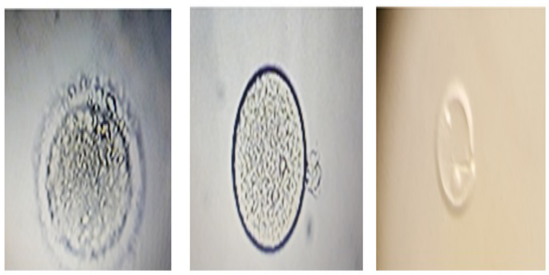


Figure (5): Abnormal oocytes. A-Atretic, B- Absent zona pellucida, C- Empty zona. Examples of abnormal oocytes which were excluded from the study

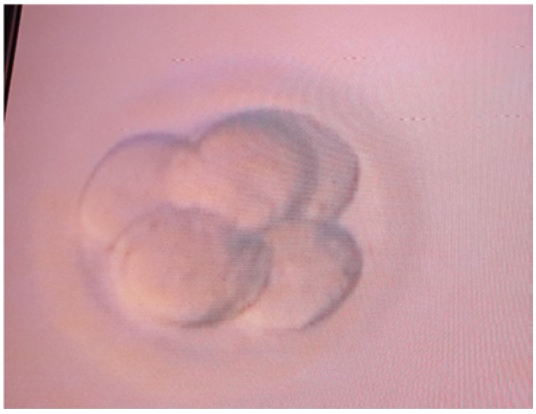


Figure (6): Day three (6-cells grade I embryo)

Results:

-Cycle outcome and oocyte quality:

After completing the ovarian stimulation protocols the ovarian response was assessed by using an ultrasound for calculating the number of dominant follicles which have at least a diameter of 17 mm so that when at least 3 dominant follicles were present the triggering of ovulation by HCG injection was done. The number of dominant follicles was recorded for all patients which were 864 follicles (12.17±0.19 (mean±SEM).

Thirty six hours after HCG triggering oocyte retrieval was done. The number of oocytes retrieved was calculated which were 543 oocytes (7.65±0.18). After denudation the oocyte maturity assessment was done. The oocytes were classified according to degree of maturity into normal mature oocyte (metaphase II- MII) with one polar body and normal morphological characteristics. The number of normal MII oocytes in all patients was 351 (4.94±0.18) while abnormal oocytes were 64 (0.9±0.13). The other type of oocyte according to the maturity classification was the immature type which is either metaphase I (MI) oocyte n=77 (1.08±0.14) or germinal vesicle (GV) n= 51 (0.72±0.23) (mean±SEM).

These results of the cycle outcome and oocyte quality were compared between the group of females who were treated with agonist protocol (G1) and the group who were treated with antagonist protocol (G2). The number of dominant follicles in G1 was n= 612 (13.6±0.24) while in the other group (G2) it was n= 252 (9.69±0.25) (mean± SEM). The statistical analysis showed highly significant difference ($p<0.01$) in number of dominant follicles between the two groups.

Regarding the total number of oocytes retrieved was n= 382 (8.49±0.22) in G1 and n= 161 (6.19±0.29) in G2 (mean± SEM). Statistical analysis of these results documented significant difference ($p<0.05$) between the two groups. When the number of mature metaphase II (MII) oocytes was compared between two studied groups it was found to be n= 238 (5.29±0.23) and n= 113 (4.35±0.27) (mean± SEM) in G1 and G2 respectively and the statistical analysis recorded no significant difference ($p>0.05$) between them. While comparing the number of metaphase I oocytes between the two different groups of females (G1, G2) included in the present study recorded n=62 (1.38±0.16) and n= 15 (0.58±0.25) respectively and statistically highly significant difference ($p<0.01$) was documented between them.

The abnormal oocytes were estimated in the two different groups and results were n=41 (0.91±0.17) and n=23 (0.88±0.2) in G1 and G2 respectively and statistically there was no significant difference ($p>0.05$) between them.

After estimation the number of oocytes according to degree of maturity the maturity index was calculated by using the following equation:

Maturity index = Number of normal matured MII oocytes / Total number of oocytes collected

According to that equation maturity index was calculated for all females present in this study and it was 64.64%. A comparison of the maturity index between the two tested groups was done using Fisher exact test it was 62.3%% and 70.19% in G1 and G2 respectively.

The statistical analysis showed no significant difference (p>0.05) between the two groups. These results were summarized in table (1).

Table (1): Comparison of cycle outcome and oocyte quality (mean± SEM) between long agonist (G1) and antagonist treated groups (G2)

Variable	(G1) Long agonist protocol (mean± SEM)	(G2) Antagonist protocol (mean± SEM)	Total (mean± SEM)	P-Value
Number of mature follicles	n= 612 13.6±0.24	n= 252 9.69±0.25	n= 864 12.17±0.19	0.002**
Number of oocytes retrieved	n= 382 8.49±0.22	n= 161 6.19±0.29	n= 543 7.65±0.18	0.019 NS
Number of Metaphase II oocytes (MII) retrieved	n= 238 5.29±0.23	n= 113 4.35±0.27	n= 351 4.94±0.18	0.225 NS
Number of Metaphase I oocytes (MI) retrieved	n=62 1.38±0.16	n= 15 0.58±0.25	n=77 1.08±0.14	0.005 **
Number of abnormal oocytes retrieved	n=41 0.91±0.17	n=23 0.88±0.2	n=64 0.9±0.13	0.916 NS
Number of germinal vesicle (GV) retrieved	n=41 0.91±0.3	n=10 0.38±0.24	n=51 0.72±0.23	0.110 NS
Maturity index %	No. (%)	No. (%)	No. (%)	
	238/382 (62.3)	113/161 (70.19)	351/543 (64.64)	0.095 NS

*P<0.05: significant. **P<0.01: Highly significant.

-Fertilization, cleavage rates:

16-17 hours after making ICSI the oocytes were assessed for being fertilized by appearance of two pronuclei (2PN). Fertilization rate was calculated according to the following equation:

Fertilization rate = (Number of fertilized oocytes (2PN) / total number of mature (MII) oocytes retrieved) ×100

According to this equation the total fertilization rate was 73.5 % (n= 258).A comparison was done for fertilization rate between the two tested groups of females who underwent stimulation with long agonist and antagonist protocols and results was 76.05% (n= 181) and 68.14% (n= 77) respectively. The statistical analysis showed no significant differences (p>0.05) between the two groups.

In day two after fertilization the cleavage of embryos was monitored and recorded to be used for measuring the cleavage rate of embryos according to the following equation:

Cleavage rate= (number of cleaved embryos / total number of fertilized oocytes 2PN) ×100

According to this equation cleavage rate was calculated for all females included in the present study which and it was 75.19% (n= 194). A comparison was done for cleavage rate between two groups of females which was 72.37% (n= 131) and 81.81% (n= 63) in G1 and G2 respectively. The statistical analysis showed no significant difference (p>0.05) between the cleavage rate of two groups(table 2).

Table (2): Comparison of fertilization outcome percentage between long agonist (G1) and antagonist treated groups (G2)

Variable (%)	G1 (Long agonist protocol) No. (%)	G2 (Antagonist protocol) No. (%)	Total No. (%)	P-Value
Fertilization rate	181/238 (76.05)	77/113 (68.14)	258/351 (73.5)	0.122 NS
Cleavage rate	131/181 (72.37)	63/77 (81.81)	194/258 (75.19)	0.118 NS

NS: Non significant.

-Embryo grading:

On day 3, embryo quality was assessed based on morphological criteria (blastomeres number, blastomere regularity and fragmentation rate) (12,13). The number and percentage of grade I, II and III embryos for all females was calculated. It was found that 52.58% (n= 102) were grade I embryos, while 19.07% (n= 37) were of grade II and 28.35% (n= 55) were of grade III embryos.

The comparison was done for the percentage of different embryo grading (GI, GII and GIII) between the two different groups (G1 and G2) which was found to be 46.56% (n=61) and 65.08% (n= 41) for grade I, 21.37 %(n= 28) and 14.29% (n= 9) for grade II, lastly 32.06% (n= 42) and 20.36% (n= 13) for grade III embryos. Statistical analysis showed non significantly higher percentage of grade I embryos in both groups compared to grade II or III embryos using Chi square test.

When the percentage of different embryo grades were compared between the two groups by Fisher exact test it was found that there was significant difference ($p<0.05$) in GI, no significant difference ($p>0.05$) in GII and highly significant difference ($p<0.01$)in GIII embryos as shown in figure 7.

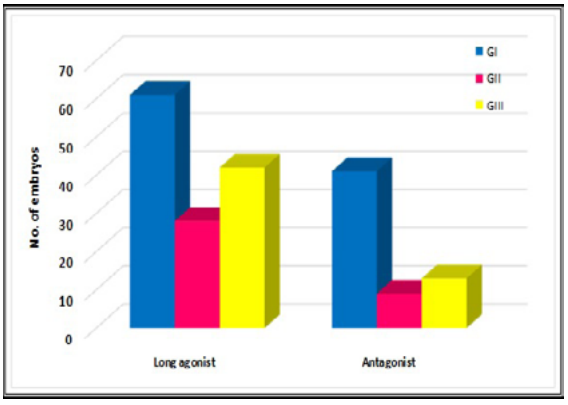


Figure (7): Embryos grading in two protocols

Discussion:

Current methods that are routinely used to determine the potential for pregnancy success with individual embryos are based primarily on the morphological characteristics of embryos or oocytes (14). The present study was designed to compare the effect of protocol type (long agonist versus antagonist) on oocyte quality and embryo during IVF-ICSI.

Regarding the number of dominant follicles the statistical analysis of the present study recorded significantly higher number of follicles and non significantly higher number of oocytes retrieved in female group treated with agonist compared to antagonist treated group. These results match that of Shrestha *et al*., 2015 who stated that the advantage of using agonist protocol is high follicular number than using antagonist protocol (15) and another study recorded that the disadvantage of the antagonist protocol is low follicles number are produced (16). On the other hand other studies disagree with present study in that the type of protocol has no effect on mean number of mature follicles (17).

The addition of GnRH agonists and antagonists significantly reduced the incidence of premature LH surges and cycle cancellations and allowed practitioners to delay HCG trigger to achieve larger multi-follicular growth, which ultimately translated to a higher number of oocytes retrieved (18). But the statistical analysis in the present study documented that the number of retrieved oocytes were higher in long agonist treated group than antagonist treated group and this result matches that of Kara *et al*., 2013 (19).

The maturity index was compared between the two tested groups in this study and statistical analysis showed no significant difference between the two tested groups and this may due to the similarity of the two groups in their ovarian reserve which is highly correlated with female age. As the recent studies have shown, that poor ovarian response is a first sign of ovarian ageing (early ovarian failure or early menopause) (20). The other factor that can affect ovarian response is basal serum follicle-stimulating hormone (FSH) levels (21,22) and these two factors were excluded in the present study.

In the present study the total fertilization rate was 73.5%.

When comparison was done between the fertilization rates in the group of female underwent long agonist and those who underwent antagonist protocol the statistical analysis recorded no significant difference between them. This may be due to the similarity in the characteristic of females in the two groups regarding age, ovarian reserve, and the exclusion of couples with azoospermic male partner and those with testicular biopsy, and the unique ICSI technician. These results agree with that of Cheung *et al* (17).

The embryo cleavage of the oocytes with two pronuclei was evaluated after 24 and 48 hours of *in vitro* culture (23).The total cleavage rate in the present study was 75.19%. The statistical analysis of the present study recorded non significant higher cleavage rate in group of females treated with antagonist compared to those treated with long agonist protocols. This finding may be in accordance with that of previous studies which pointed out that high E2 could impair embryonic cleavage (24).

The morphology and cleaving status are used to evaluate embryo developmental potential before uterine transfer (10). Embryos were graded into three grades depending on morphological criteria (blastomeres number, blastomeres regularity and fragmentation rate). The statistical analysis showed that the two types of protocols (long agonist and antagonist) had no effect on embryo grading regarding the three embryo grades and these results are in accordance with that of Halvaei and colleges (25).

References:

1.Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary on ART terminology. Hum Reprod. 2009; 24: 2683–2687.

2. Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, Regional, and Global Trends in Infertility Prevalence Since 1990: A Systematic Analysis of 277 Health Surveys. PLoS Med. 2012; 9: 12.

3. Bartoov B, Berkovitz A., Eltes F, Kogosovsky A, Yagoda A, Lederman H, Artzi S, Gross M, and Barak Y. Pregnancy rates are higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic injection Fertility and sterility, 2003; 80: 6. 1413-1419

4.Parsanezhad M. E, Jahromi B. N, Rezaee S , Kooshesh L, Alaee S. The Effect of Four Different Gonadotropin Protocols on Oocyte and Embryo Quality and Pregnancy Outcomes in IVF/ICSI Cycles; A Randomized Controlled Trial. IJMS. 2017;42:1.

5. Turhan N, Pekel A, Ayrim A, Kasap B, BAYRAK Ö. Effectiveness of HP-hMG versus r-FSH in patients undergoing IVF/ ICSI cycles with moderate male-factor infertility. Turk J Med Sci. 2013; 43: 144-9.

6. Mohamed MA, Sbracia M, Pacchiartti A, Micara G, Linari A, Tranquilli D, *et al*. Urinary follicle-stimulating hormone (FSH) is more effective than recombinant FSH in older women in a controlled randomized study. Fertil Steril. 2006; 85: 1398-403.

7.Khalaf M, ´ Mittre H, Levallet J, Hanoux V, Denoual C, Herlicoviez M, Bonnamy P, Benhaim A. GnRH agonist and GnRH antagonist protocols in ovarian stimulation: differential regulation pathway of aromatase expression in human granulosa cells. Reproductive BioMedicine Online. 2010; 21: 56– 65

8. Albano, C., Felberbaum, R.E., Smitz, J. 'Ovarian stimulation with HMG: results of a prospective randomized phase III European study comparing the luteinizing hormonereleasing hormone (LHRH)-antagonist cetrorelix and the LHRHagonist buserelin. European Cetrorelix Study Group. Hum. Reprod. 2000; 15: 526–531.
9.Prados F. J., Debrock S, Lemmen J. G.,and Agerholm I. The cleavage stage embryo. Human Reproduction, 2012; 27: S1. 150–171.
10. Parsanezhad M. E, Jahromi B. N, Rezaee S , Kooshesh L, Alaee S. The Effect of Four Different Gonadotropin Protocols on Oocyte and Embryo Quality and Pregnancy Outcomes in IVF/ICSI Cycles; A Randomized Controlled Trial. IJMS. 2017; 42: 1.
11. Holte J, Berglund L, Milton K, Garello C, Gennarelli G, Revelli A, Bergh T. Construction of an evidence-based integrated morphology cleavage embryo score for implantation potential of embryos scored and transferred on day 2 after oocyte retrieval. Hum Reprod. 2007; 22:548–557.
12. Stensen MH, Tanbo T, Storeng R, Byholm T, Fedorcsak P. Routine morphological scoring systems in assisted reproduction treatment fail to reflect age-related impairment of oocyte and embryo quality. Reprod Biomed Online. 2010; 21(1): 118-125.
13. Macklon NS, Stouffer RL, Giudice LC & Fauser BC. The science behind 25 years of ovarian stimulation for *in vitro* fertilization. Endocrine Reviews. 2006; 27: 170–207
14. Adamson GD, Abusief ME, Palao L, Witmer J, Palao LM, Gvakharia M. Improved implantation rates of day 3 embryo transfers with the use of an automated time-lapse-enabled test to aid in embryo selection. Fertil Steril. 2016; 105:369-375.
15. Shrestha D , La X , Feng H. L. Comparison of different stimulation protocols used in *in vitro* fertilization: a review Ann Transl Med 2015;3(10):137
16. Kim CH, You RM, Kang HJ, *et al.* GnRH antagonist multiple dose protocol with oral contraceptive pill pretreatment in poor responders undergoing IVF/ICSI. Clin Exp Reprod Med. 2011; 38: 228-33.
17. Cheung L.P, Lam P.M, Lok I. H, Yu Chiu T.T, Yeung S.Y, Tjer C. C and Haines C.J. GnRH antagonist versus long GnRH agonist protocol in poor responders undergoing IVF: a randomized controlled trial. Human Reproduction Vol. 2005; 20: 3. 616–621.
18. Jaime M. K, James A. G, Akiva P. N, Meghan B. S, Alan S. B.Is bigger better: The association between follicle size and livebirth rate following IVF? Open Journal of Obstetrics and Gynaecology. 2012; 2: 361-366
19. Kara K, Aydin T, Aran T, Turktekin N, Ozdemir B. Comparison of GnRH agonist and antagonist protocols in normoresponder patients who had IVF-ICSI. Archives of Gynecol Obstet. 2013; 288:1413–1416
20. Beckers NGM, Macklon NS, Eijkemans MJC, Fauser BCJM. Women with regular menstrual cycles and a poor response to ovarian hyperstimulation for *in vitro* fertilization exhibit follicular phase characteristics suggestive of ovarian aging. Fertil Steril. 2002; 78: 291–7.
21. Kligman I, Rosenwaks Z. Differentiating clinical profiles: predicting good responders, poor responders, and hyperresponders. Fertil Steril 2001;76: 6. 1185–90.
22.Loutradis D. , Drakakis P. & E. Vomvolaki & A. Antsaklis. Different ovarian stimulation protocols for women with diminished ovarian reserve. J Assist Reprod Genet. 2007. 24: 597–611

23. Nagy Z. P, Dozortsev D, M.D., Diamond M, Rienzi L, Ubaldi, F, Abdelmassih R, and Greco E. Pronuclear morphology evaluation with subsequent evaluation of embryo morphology significantly increases implantation rates. Fertility and sterility; 2003. Vol. 80, No.1. 67-74.24. Valbuena D, Martin J, de Pablo JL, Remohi J, Pellicer A, Simon C.Increasing levels of estradiol are deleterious to embryonic implantation because they directly affect the embryo. Fertil Steril. 2001; 76(5):962–968.

25. Halvaei I, Khalili M. A, Razi M. H, Agha-Rahimi A, and Nottola S. A. Impact of different embryo loading techniques on pregnancy rates in *in vitro* fertilization/embryo transfer cycles. J Hum Reprod Sci. 2013; 6(1): 65–69.