Precycle Estradiol in synchronizatıon and scheduling of antagonist protocol

Soumaya Mohamed Hassan, Hoda Mohamed Abdel Aal, Mohamed fekryyoussef, RehamAbdelwahed Mohamed Abdelsalam

Obstetrics and Gynaecology department, Faculty of Medicine, Cairo University

Corresponding author: RehamAbdelwahed Mohamed Abdelsalam
Email:

ABSTRACT

Introduction: Controlled ovarian hyperstimulation (COH) is the initial stage of in vitrofertilization/intracytoplasmic sperm injection (IVF/ICSI) procedures. COH aims to produce multiple follicular development in order to harvest a suitable number of oocytes, which can later be fertilized.

Methods: Women aged 20 to 40 years old who attended at the IVF unit (Kasr El- aini hospital - Faculty of Medicine - Cairo University) and private centers for IVF, the study included 72 infertile women who were candidate to receive the conventional antagonist protocol.They randomly divided into 2 groups:Group(A)(n=36):Estradiolvalerate(E2)isadministeredatdailydoseof4mgfrom day20oftheprecedingcycleonwardsformaximum10days.StimulationwithFSH should be started with the gap of 1 day after the last dose of E2. Group (B) (n=36): served as controls that remain untreated during the lutealphase.

Results: All statistical calculations were done using computer program IBM SPSS. The mean follicular size at day 8 among the pretreatment group (13.1mm ±0.1)was lessthanthatamongcontrolgroup(14.6mm±0.4).Themeannumberoffollicles ≥16mmatthedayoftriggeramongthepretreatmentgroup(9.8±0.4)wasmorethan thatamongthecontrolgroup(7.7±0.7).Themeannumberofmaturefolliclesamong the pretreatment group (7.3±0.4) was more than that among the control group (6.3±0.6).However the mean days of stimulation among the pretreatment group (11.0±0.9)waslongerthanthatamongcontrolgroup(10.1±1.3).ThetotalFSHdose

amongthepretreatmentgroup(2668.2±62.5)washigherthanthatamongtheco ntrol group (2439.8±110.5).There were no significant difference regarding number of embryo transfer and occurrence of chemicalpregnancy.

Conclusions: The present study confirmed that a luteal estradiol pre-treatment resulted in more synchronized follicles and increased number of mature follicles with prolongation of stimulation and increased FSH dose of stimulation, without deleterious impact on the chemical pregnancy rates.

Key words: Lutealestradiol, Estradiol valeratepretreatment, GnRH antagonistprotocol, Synchronization offollicles, ovarianstimulation, IVF/ICSI.

INTRODUCTION

Controlled ovarian hyperstimulation (COH) is the initial stage of in vitrofertilization/intracytoplasmic sperm injection (IVF/ICSI) procedures. COH aims to produce multiple follicular development in order to harvest a suitable number of oocytes, which can later be fertilized **(1).**

There are three basic COS protocols including the long agonist protocol, antagonist protocol, and the minimal stimulation protocol. Different nomenclature is used for these protocols, but the basic components are the same **(2).**The agonist and antagonist protocols have comparable results, while the acceptance of antagonist protocol on a much wider scale has as yet not been observed.Thisisowingtotheperceivedlackofflexibilityoftheantagonistproto col **(3).**

Follicular recruitment starts in the luteal phase of the preceding cycle under theinfluenceoftheintracycleFSHrisestarting3–5daysbeforetheonsetofmenses. Hence, the cohort which will respond to stimulation started on day 2 or 3 ofmenses as the antagonist protocol has already been decided before the start of stimulation. ThisexplainstheslightreductioninoocyteyieldandreductioninOHSSwithant agonist protocol compared with long agonist protocol. This is of advantage in hyper-responders where limitation in response of follicular growth is desired. Asynchronousmultimoleculargrowthseenmoreinantagonistcyclesascompa redto long protocol may be a direct consequence of size heterogeneities of early antral follicles during early follicular phases of controlled ovarian stimulation **(4).**

Ovarian E2 exerts negative feedback within the reproductive axis that includes inhibition of GnRH secretion and suppression of GnRH responsiveness. The concept of luteal E2 was first suggested by Fan chin and associates, (5),during lute-follicular transition; FSH preserves early antral follicles from atresia and ensures their growth.

The aim of work was to assessment of the effectiveness of recycle estradiol to improve size homogeneity of antral follicles and increase the number of follicles reaching maturation at the same time during antagonist protocol.

PATIENTS AND METHODS

ThisstudywasarandomizedcasecontroltrialconductedinIVFunit(KasrEl-Aini hospital - Faculty of Medicine - Cairo University) and private centers in the period between October 2019 to August2021.

The study included 72 infertile women who were candidate to receive the conventional antagonist protocol.

They randomly divided into 2 groups:

Group(A)(n=36):Estradiolvalerate(E2)isadministeredatdailydoseof4mgfrom day 20 until next cycle day 1 (for maximum 10 days). Stimulation with FSHshould be started with the gap of 1 day after the last dose ofE2.

Group (B) (n=36): served as controls that remain untreated during the luteal phase.

Women with infertility (primary or secondary) (male or tubal or unexplained) candidate for IVF, the study was done at Kasr Al-Aini hospital, Obstetrics &Gynecology department, IVF unit and private centers.

Inclusioncriteria:

Normal day 3 FSH level and FSH to LHratio, Good ovarianreserve, regularmenses and candidate forICSI.

Exclusioncriteria:

Age above 40years, untreated thyroiddysfunction, untreatedhyperprolactinemia, functional ovariancysts, autoimmune disease likeSLE, presence ofendometriosis and patients diagnosed withPCO.

Written Consent: It was obtained from women included in thestudy.Full History TakingIncluding:Name,age, 1st day of last menstrual period(LMP), medical and surgicalhistory, types of infertility: (primary or secondary) (due to male or female factors or unexplained), duration ofinfertility and history of previous trial of controlled ovarian stimulations: by which protocol and itsresult.Thorough ClinicalExamination:Height (in cm) and weight (in kg)measurements and basal Ultrasound: to assess uterine cavity and for AFC.

Investigations:Day 2 or 3 of menstruation FSH, LH ,E2 and AMH, Prolactin,TSH.

The statistical analysis: Datawerestatisticallydescribedintermsofmean±standarddeviation(SD), median and range, or frequencies (number of cases) and percentages when appropriate. Because the groups are large enough, comparison of numerical variables between the study groups was done using Student *t* test for independent samples. For comparing categorical data, Chi-square (x^2) test was performed.Exact testwasusedinsteadwhentheexpectedfrequencyislessthan5.Two-sided*p*values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for MicrosoftWindows.

RESULTS

This study was a randomized case control one conducted in IVF unit (Kasr El-aini hospital - Faculty of Medicine - Cairo University) and private centers in the period between October 2019 to August 2021.

Seventy two (72) infertile women were included in the study. Women candidate for IVF and included in the study were divided into two groups, group A received E2 before starting ovarian stimulation and group B started stimulation without E2 before. Ovarian stimulation was achieved through antagonist protocol.

	Pretreatment (36)	**Control (36)**	**P Value***
Age(y)	29.47 ±4.766	30.72 ±5.854	0.324
BMI (kg/m²)	27.53 ±5.36	27.75 ±5.97	0.869
Medical history	1 (2.8%)	3 (8.4%)	0.499
Surgical history	9 (25%)	10 (27.8%)	0.789

Table 1: baseline characteristics of the included study

there were no statistically significant difference in the term of age, BMI, medical history, surgical history, (p-value >0.05

	Pretreatment group (N= 36)	**Control group (N= 36)**	**P-value**
Follicular size at day8	13.1 ±0.1	14.6 ±0.4	≤0.001
Days of stimulation	11 ± 0.9	10.1 ± 1.3	≤0.001
Total fish dose(IU)	2668.2 ±62.5	2439.8±110.5	≤0.001
Egg retrieved	9.2±0.7	7.2±0.4	≤0.001
MII oocytes	7.3±0.4	6.3±0.6	≤0.001

Number of Embryo transfer	2.03±0.167	2±0.414	0.688
Chemical pregnancy (positive)	24 (66.7%)	24 (66.7%)	1

Table 2: outcomes of the included study

there were statistically significant difference in the term of Follicular number at day 8, Days of stimulation, Total FSH (IU)dose, Egg retrieved and MII oocytes (p-value < 0.05).on the other hand there were statistically significant difference in Number of Embryo transfer and Chemical pregnancy (positive) (p-value > 0.05).

DISCUSSION

Controlled ovarian hyperstimulation (COH) is the initial stage of *in vitro*fertilization/intracytoplasmic sperm injection (IVF/ICSI) procedures. COH aims to produce multiple follicular development in order to harvest a suitable number of oocytes, which can later be fertilized **(1)**.

There are three basic COS protocols including the long agonist protocol, antagonist protocol, and the flare protocol. Different nomenclature is used for these protocols, but the basic components are the same **(2)**

The acceptance of antagonist protocol on a much wider scale has as yet not been observed. This is owing to the perceived lack of flexibility of the antagonist protocol **(6)**.

The current study was an observational one conducted in IVF unit (Kasr El- aini hospital - Faculty of Medicine - Cairo University) and private centers .It aimed to evaluate the effectiveness of recycle estradiol to improve size homogeneity of antral follicles and increases the number of follicles reaching maturation at once during antagonist protocol.

ThestudyincludedSeventytwo(72)infertilewomencandidatesforIVF.They were divided into control group (N=36) and E2 pretreatment group(N=36).

Results of our study concluded in that there was significant difference in mean size of follicles, number of follicles ≥ 16mm at the day of trigger, duration of stimulation between both groups, number of follicles at day 8, dose of fish, number of oocytes retrieved and number of mature follicles. While there was no significant difference regarding the number of embryo transfer and occurrence of chemical pregnancy between both groups.

In agreement of our findings**, fan chin et al., (5)** did prospective study on 100 female candidates for antagonist protocol. They divided into pretreatmentgroup

(47) received 4mg estradiol valerate from day 20 till day 2 and control group (43) remainuntreatedduringlutealphase.Allreceivedconventionalantagonistprotocol.

Results: the mean follicular size at day 8 among pretreatment group =9.9±0.2 while among control 11.1±0.3 (significant p value).Days of stimulation among pretreatment 11.9±0.2 while in control 10.8±0.2 (significant p value).Dose of stimulation among pretreatment 2674±91 while in control 2463±100(non-significant p value).

Aprospectivecontrolledstudydoneby**Bloc keeletal., (7)**on86normo-gonadotrophic women enrolled in an assisted reproduction program. In the control group (n=39), a standard GnRH-antagonist protocol was applied; theE2pretreatment group (n=37) underwent a modified treatment protocol withestradiolvalerate administration during 6–10 consecutive days (from cycle day 25 onwards) prior to the start of recombinant FSH (fresh)stimulation.

With regard to the outcome of ovarian stimulation in both groups, a longer duration of stimulation was observed in the pretreatment group (9.6 ± 1.4 days versus 8.6 ± 1.5daysinthecontrolgroup;P=0.004)andHighertotaldoseofgonadotropinsw as observed in the pretreatment group (1485.1 ± 248.7 versus 1295.0 ± 254.2 in the control group; P = 0.002).

The patients were randomized to receive 17β-estradiol (4 mg/d) or no pretreatment before daily recombinant FSH administration started on the first day of estrogen discontinuation or on cycle day 2 in no pretreatment women.

The mean numbers of retrieved oocytes (10.9 ± 5.7 vs. 10.2 ± 5.6) and obtained embryos (5.5 ± 3.7 vs. 4.8 ± 3.7) were not significantly different between women allocated to estrogen pretreatment (n = 238) and no pretreatment (n = 234). Total FSH amount (1,557 ± 408 vs. 1,389 ± 347 IU) and stimulation duration (10.8 ± 1.4 vs. 10.0 ± 1.5 days) were slightly but significantly increased in pretreated patients. Positivepregnancytests,ultrasoundpregnancyrate,anddeliveryratepercycle were similar (36%, 33%, and 26.6%, respectively, vs. 38.2%, 35.4%, and30%).

So, the results of the previously mentioned two studies agreed with ours regarding the duration of stimulation and dose of gonadotropins as in both studies a longer duration of stimulation and a higher total dose of gonadotropins were observed in the pretreatment group.

Retrospectivecohortstudywasdoneby**Fraticellietal., (8)**toevaluate whetherthelutealestradiolprotocolforexpectedpoor-respondersimprovesembryo number and quality. The luteal phase estradiol protocol showed a statistically significantly greater number of embryos with > or = 7 cells, oocytes retrieved, matureoocytes,andembryosthandidthestandardprotocol.Therewasnodiffer ence between the two protocols with respect to basal antral follicle count, days of

stimulation,numberoffollicles.Atrendtowardimprovedpregnancyoutcomes was found with the luteal estradiolprotocol.

Anotherretrospectivecohortstudydoneby**changeetal., (9)**on155poor responder patients subjected to IVF/ICSI were analyzed. In luteal E2 treatment protocol($n = 86$),oralestradiolvalerate4mg/daywasinitiatedonlutealday21and eitherstoppedatmenstrualcycleday3(ProtocolA,$n = 28$)orcontinuedduringthe periodofovarianstimulationuntilthedayofchg.injection(ProtocolB,$n = 58$). IVF parameters and pregnancy outcome of luteal E2 treatments group were compared with a standard GnRH antagonist protocol ($n = 69$) which the patients received no hormonalpretreatment.

With regard to the outcome of the study, cancellation rate was lower with luteal E2 group(15.1%vs37.7%,$p < 0.01$).Moreover,patients treated with luteal estrogen resulted in anincreasednumberofoocytesretrieved(4.5 ± 2.9vs 3.2 ± 1.9; $p < 0.01$). A trend toward increases in number of normally fertilized embryos (2.9 ± 2.1vs 2.3 ± 1.9; $p = 0.043$), and increased prevalence of good quality embryos (51.2% vs 25%; $p = 0.047$) were noted.

So,theresultsofthepreviouslymentionedtwostudiesagreedwithoursregarding that the luteal estradiol prior to ovarian stimulation increased number ofeggsretrieved .while these results come in contrast to ours regarding the quality of embryoandthepregnancyratewhenusedinpatientsdeemedtohaveapoorovarian response toIVF.

Omar et al., (10)conducted a retrospective study to evaluate luteal estradiol pretreatment of poor and normal responders during GnRH antagonist protocol.

They included women undergoing IVF program who were given a course of 4 mg oralestradiol-17bdailyfromday20ofthesamecycleuntilday1oftheirnextcycle beforestartinganantagonistprotocol,formingpretreatment-groupbutcontrol-group started on day 3 stimulation without pretreatment. The total was divided into 2 groups (poor (group 1, n = 148) and normal responders (group 2, n = 244)). Their findings showed for group 1 a significant decrease in cancelation rate (3% vs 14%) and a significant improvement in clinical outcomes (clinical pregnancy per transfer and live birth rate respectively: 47% and 44% vs 12% and 11%). For group 2, this pretreatmentcouldincreasesignificantlythematurationrate(77%vs68%).The rate offrozenembryoswasimprovedinbothgroups:(group1:11%vs2%andgroup2 : 53% vs 41%). Luteal estradiol pretreatment increases the frozen embryos rate whatever the nature of the ovarian response, but especially

for normal responders it coordinates follicular recruitment increasing the maturation rate. In the case ofpoor responders, it affects positively clinical outcomes decreasing the canceledcycles

Concerning the second lot (Group 2, n = 244) treating a normal responder, no significant difference was observed in the number of oocytes retrieved (11.46 ±vs 12.02 ± 3.31) which comes in contrast to our result.

In the agreement with our study, the aforementioned study showed an important increase in follicular maturation.

The aforementioned study showed an important increase in embryological outcomes (maturation, fertilization and frozen embryos rate) which was not a point of study in ours.

Conclusion

The present study confirmed that a luteal estradiol pre-treatment resulted in more synchronized follicles and increased number of mature follicles with prolongation of stimulation and increased FSH dose of stimulation, without deleterious impact on the chemical pregnancy rates.

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