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WILL THE TYPE OF GnRH ANALOGUE AFFECT THE EMBRYO CLEAVAGE

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ABSTRACT

Aim and Objective: The present study is aimed to carry out the effect of the type of GnRH analogue on embryo cleavage. **Materials and Methods:** A total of 403 patients who underwent Intra Cytoplasmic sperm injection were included in the study. They were divided in to three groups. Group I- Embryos which cleaved before 27 hours after injection. Group II- Embryos which cleaved after 27 hours, Group III-Embryos which cleaved before and after 27 hours. The effects of GnRh agonist and antagonist on embryo cleavage were compared between the three groups. **Results:** All the 403 patients were analysed. There was no difference in the mean age, duration of ovarian stimulation, number of oocytes retrieved, fertilization, cleavage rates and embryo quality between the three groups. Out of 403 patients, early cleavage was observed in 165 patients (40.94 %). Late cleavage was observed in 129 patients (32.01%), both early and late cleavage was observed in 109 patients (27.05%). Out of 227 patients in the agonist protocol the early cleavage was observed in 98 patients (43.17%), late cleavage was observed in 71 patients (31.28%), and both early and late cleavage was observed in58 patients (25.55%). Out of 176 patients in the antagonist protocol the early cleavage was observed in 67 patients (38.07%), late cleavage was observed in 58 patients (32.95%), and both early and late cleavage was observed in51 patients (28.98%). P 0.563. We observed there was no statistical significant difference between agonist and antagonist stimulation protocol on embryo cleavage **Conclusion:** The embryo cleavage was not affected by the type of GnRH analogue used.

KEYWORDS: Early cleavage, Embryo quality, Intracytoplasmic sperm injection, Ovarian stimulation.

INTRODUCTION

In order to decrease multiple pregnancies and attain a maximal rate of implantation, selection of the most viable embryo for transfer has become a high concern in assisted conception treatment. Conventionally, embryo selection is performed by using embryo morphology. Other selection methods include oocyte and zygote morphology, blastomere symmetry and blastocyst culture. In recent times, observation of embryonic early cleavage has been highlighted. Numerous studies have shown that embryonic early cleavage, which occurs at 25–27 hours post insemination for *in vitro* fertilization (IVF) /intracytoplasmic sperm injection (ICSI), can be an additional marker of viable embryos. Most of these earlier studies were only using the gonadotrophin-releasing hormone (GnRH) agonist long protocol for pituitary suppression.[1]

Recently, a GnRH antagonist protocol has become available in assisted reproductive treatment. The advantages of GnRH antagonist are associated with a lower utilization of gonadotropins, a shorter period of stimulation, a lower risk of ovarian hyperstimulation syndrome (OHSS), and a lower cancellation rate, especially in poor responders. Previous studies have shown that using the GnRH antagonist protocol had the comparable pregnancy rate when compared with the GnRH long agonist protocol. However, some studies have shown that GnRH receptors are expressed in human and mouse preimplantation

embryos, and addition of GnRH antagonist to mouse embryo culture media inhibits preimplantation embryo growth. We investigated whether the effects of these two different protocols upon embryonic development were the same. In the previous study, they found that early-cleavage is a reliable predictor for embryo implantation.[1]

In 1980s the GnRH agonist protocol is introduced to suppress the release of pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by desensitizing the pituitary receptors. In late 1990s, the GnRH antagonists have also been found effective for ovarian stimulation by directly binding to the GnRH receptors, and through which they block GnRH receptor activity in a competitive manner and induce an immediate, reversible, and quick suppression of gonadotropin release. As a result, the GnRH antagonist protocol has also been commonly employed recently in the clinical settings with Invitro fertilization and embryo transfer treatment. There is evidence that application of GnRH antagonist protocol decreases the duration of ovulatory stimulus and reduces the occurrences of ovarian hyperstimulation syndrome. While these observations are exciting and encouraging, controversial results have also been reported. [2]

In recent years, it has become evident that ovarian stimulation, although a central factor of IVF, may itself have detrimental effects on oogenesis, embryo quality, endometrial receptivity and perhaps also perinatal outcomes. [3]

Therefore, the aim of this study was to investigate the effect of the GnRH agonist and GnRH antagonist protocol on the embryonic early-cleavage rates.

MATERIALS AND METHODS

It was a prospective observational study conducted in the Department of Reproductive Medicine, at a tertiary care centre from Oct 2010-Jan 2014. A total of 403 patients who underwent Intra Cytoplasmic Sperm Injection (ICSI) were included in the study in the age group of 21-45 years. Inclusion criteria: All patients enrolled for ICSI during this study period were included in the study. The patient having only early cleavage embryos, the patient having only late cleavage embryos and the patient having both early and late cleavge embryos for transfer were included in the study.

Exclusion criteria: Patient age <21 and >45 yrs were excluded from the study.

Short and ultra short protocols for stimulations were excluded from the study.

Embryos beyond Grade III for transfer were excluded from the study.

Informed consent was taken before the enrollment of each participant and the Institutional ethical committee approval was obtained (IEC/10/JULY/83/29).

Two stimulation protocols were used in this study. Patients with young age with good ovarian reserve, we used agonist protocol. Patients with advanced age, poor ovarian reserve, low AntiMulerian Hormone (AMH) level, and PolyCystic Ovarian Syndrome(PCOS) were the indication for the use of antagonist protocol. The gonadotropin-releasing hormone (GnRH) agonist protocol- A gonodotropin releasing hormone agonist is an analogue that activates the receptors resulting in increased secretion of Follicle stimulating hormone (FSH), Luteinizing hormone (LH). The GnRH antagonist protocol -A gonadotropin-releasing hormone antagonist is an analogue that blocks the GnRH receptor resulting in an immediate drop in gonadotropin (FSH, LH). In the GnRH agonist protocol, pituitary down regulation was done with GnRH agonists. Once the patient was down regulated completely (had menses, E2 <30 pg/ml) gonadotropin injections (recombinant follicle stimulating hormone/human menopausal gonadotropin) were given until the day of hCG administration. The doses were adjusted according to the patient's ovarian response. In the GnRH antagonist protocol, without down regulation gonadotropin injections were administrated daily from the second day of the menstrual cycle. The doses were adjusted according to the patient's individual ovarian response. Once the dominant follicle reached 14 mm in mean diameter, GnRH antagonist was administered subcutaneously at a dose of 0.25 mg daily until the day of hCG administration. In both groups, ovulation was induced by the administration of either

recombinant h CG or urinary h CG when at least two follicles reached 18 mm in diameter, and oocyte retrieval was performed 34–36 hours later. Oocytes were retrieved transvaginally under ultrasound- guidance. Motile sperms were isolated by a swim-up or gradient centrifugation. Ejaculated, testicular biopsy; cryopreserved ejaculated and cryopreserved testicular biopsy semen specimens were all included in the study. Intra Cytoplasmic Sperm Injection (ICSI) was performed 3–5 h after oocyte aspiration with the prepared sperm. Normal fertilization was confirmed by the presence of two pronuclei and two polar bodies 16–20 h (day1) after Intra Cytoplasmic Sperm Injection (ICSI). Normally fertilized oocytes (Zygotes) were spherical and had two polar bodies and two PNs. PNs had approximately the same size, centrally positioned in the cytoplasm with two distinctly clear, visible membranes. The presence of nucleolar precursor bodies, their number and size aligned at the PN junction were assessed. On the same day, early cleavage examination was performed on the zygotes within 27 hours after Intra Cytoplasmic Sperm Injection (ICSI). Embryos displaying two cells at inspection were designated as 'early cleavage'. The embryos that had not yet cleaved to the 2-cell stage after 27 hours were designated as 'late cleavage'. Two or three embryos were transferred on Day2 depending on the patient's age and embryo quality. The embryos that were not transferred were cryopreserved. The luteal phase was supported by vaginal supplementation of progesterone or intramuscular injection of progesterone.

Pregnancy was determined by a serum β human Chorionic Gonodotropin (β h CG) test 14 days post transfer. The clinical pregnancy was confirmed by the presence of an intrauterine gestational sac with fetal cardiac activity by ultrasound examination at 4 weeks after embryo transfer. Patients were divided into three groups. Group I- Embryos which cleaved to two cells before 27 hours after injection. Group II- Embryos which cleaved to two cells after 27 hours. Group III- Embryos which cleaved to two cells before and after 27 hours after injection. The effects of GnRh agonist and antagonist on embryo cleavage were compared between the three groups.

STATISTICAL ANALYSIS

The collected data were analysed with SPSS 16.0 version. To describe about the data descriptive statistics frequency analysis, percentage analysis, means and standard deviation were used. For the numerical data nonparametric Mann–Whitney *U* test was used to find the significance. To find the significance in categorical data Chi - Square test was used. In all the statistical tools, the probability value of $p<0.05$ was considered as significant level.

RESULTS

A total of 403 patients were analyzed. The baseline characteristics were shown in (Table 1).

There was no difference in the mean age, duration of ovarian stimulation, number of oocytes retrieved, fertilization, cleavage rates and embryo quality between the three groups. In our study about 67.25 % of the patients were in the age group of 26-35 years. The type of gonadotrophin used for ovarian stimulation was similar in the two groups. Out of 403 patients, early cleavage was observed in 165 patients (40.94 %). Late cleavage was observed in 129 patients (32.01%), both early and late cleavage was observed in 109 patients (27.05%).

Out of 403 patients 227 patients (56.33%) were given GnRH agonist protocol and 176 patients (43.67%) were given antagonist protocol. (Table 2) (Figure 1). Out of 227 patients in the agonist protocol the early cleavage was observed in 98 patients (43.17%), late cleavage was observed in 71patients (31.28%), and both early and late cleavage was observed in58 patients (25.55%). Out of 176 patients in the antagonist protocol the early cleavage was observed in 67 patients (38.07%), late cleavage was observed in 58 patients (32.95%),and both early and late cleavage was observed in 51 patients (28.98%).P 0.563.(Table 3) (Figure 2).

In this study significantly more MII oocytes in group I than in Group II and Group III. 47.02 % MII oocytes in the Group I, 22.09% MII oocytes in the Group II, 30.89% MII oocytes in the Group III. P 0.051 (Table 4). The results showed that the good quality oocytes were 69.27% in the group I, 19.72% in group II and 11.01% in group III.P 0.001 which was statistically significant (Table 5). But when we compared the good quality oocytes in agonist and antagonist there was no significant difference. 59.47% vs 47.15% (Table 6).

Table 1. Baseline Characteristics

Parameters	**Earlycleavage Group i (165)**	**Latecleavage Group ii (129)**	**Earlycleavage &latecleavage Group iii (109)**	**P value**
No of patients	165	129	109	-
Mean age (yrs)	31 ± 5	31 ± 5	32 ± 5	0.265
Mean duration of infertility (yrs)	7 ± 4	7 ± 4	8 ± 5	0.698
No of oocytes retrieved (mean ± SD)	15 ± 8	14 ± 8	15 ± 9	0.308
No of mii oocytes (mean ± SD)	12 **± 7**	10 ± 7	12 ± 7	0.051 *
No of mi oocytes (mean ± SD)	1 ± 2	1 ± 1	1 ± 1	0.072
No of gv oocytes (mean ± SD)	1 ± 2	2 ± 3	1 ± 2	0.505
No of o ocytes injected (mean ± SD)	12 **± 7**	10 ± 6	12 ± 7	0.028 *
No of o ocytes fertilized (mean ± SD)	10 ± 6	8 ± 5	9 ± 6	0.006 **
No. of grade i embryos (mean ± SD)	7 ± 5	5 ± 4	7 ± 6	0.005**

Kruskal-Wallis Test was applied to compare these three groups and get the significance.

**** Highly significant, * Significant.**

Table 2. Protocol and No. of patients

DESCRIPTION	**AGONIST PROTOCOL**	**ANTAGONIST PROTOCOL**
NO OF PATIENTS (403)	227 (56.33%)	176 (43.67%)

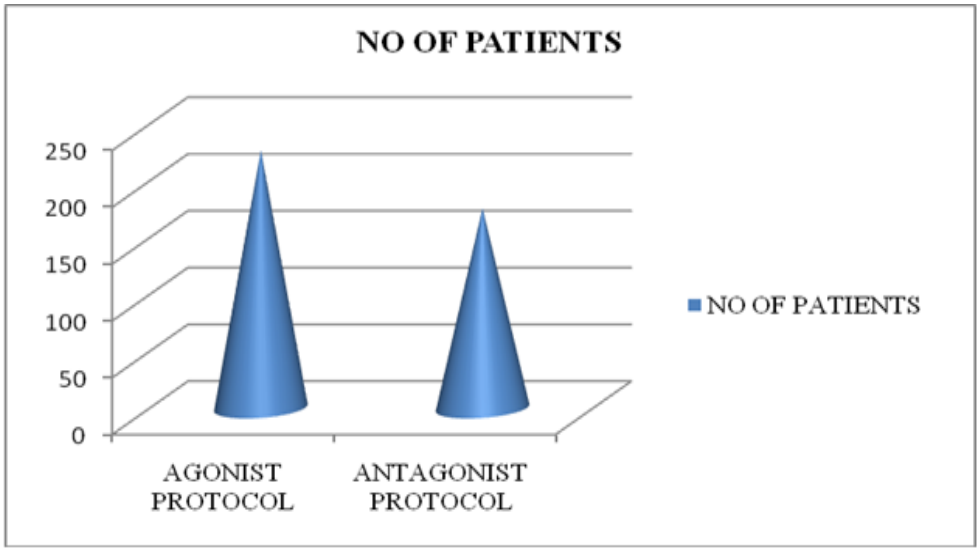

Figure 1: Protocol and No. of patients

Table 3. Effect of protocol and cleavage

PROTOCOL	EARLYCLEAVAGE GROUP I (165)	LATECLEAVAGE GROUP II (129)	EARLYCLEAVAGE &LATECLEAVAGE GROUP III (109)	P VALUE
AGONIST (227)	98 (43.17%)	71 (31.28%)	58 (25.55%)	0.563
ANTAGONIST (176)	67 (38.07%)	58 (32.95%)	51 (28.98%)	

When we compared the agonist and antagonist protocol with cleavage of Group I , Group II, and Group III, there was no statistical difference between these groups. p 0.0563.

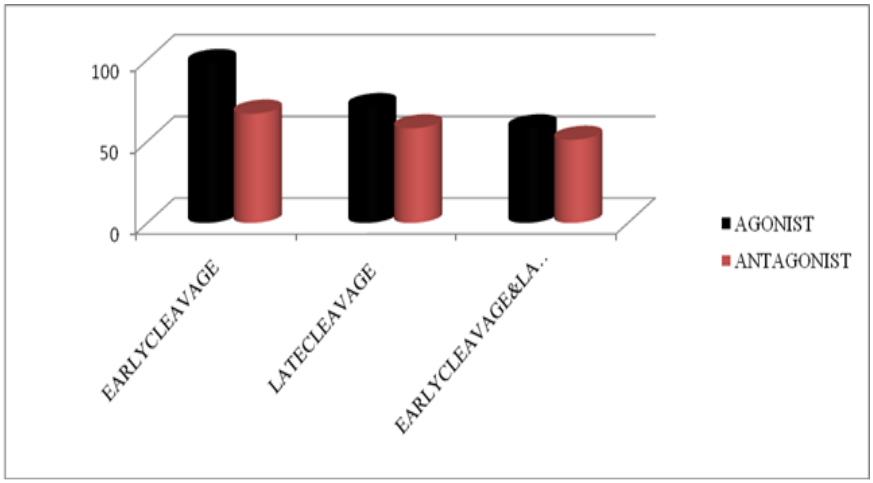

Figure 2: Effect of protocol and cleavage

Table 4. Comparison of No. of MII oocytes and cleavage

NO OF MII OOCYTES	EARLYCLEAVAGE GROUP I (165)	LATECLEAVAGE GROUP II (129)	EARLYCLEAVAGE & LATECLEAVAGE GROUP III (109)	P VALUE
TOTAL NO OF MII OOCYTES (4662)	2192 (47.02%)	1030 (22.09%)	1440 (30.89%)	0.051*

*** Significant**

Table 5. Effect of oocyte quality and cleavage

OOCYTE QUALITY	EARLYCLEAVAGE GROUP I (165)	LATECLEAVAGE GROUP II (129)	EARLYCLEAVAGE &LATECLEAVAGE GROUP III (109)	P VALUE
GOOD QUALITY OOCYTES (218)	151 (69.27%)	43 (19.72%)	24 (11.01)	0.001**

**** Highly significant**

Table 6. Effect of protocol and oocyte quality

OOCYTE QUALITY	AGONIST PROTOCOL	ANTAGONIST PROTOCOL
GOOD QUALITY OOCYTES	59.47% 135/227	47.15% 83/176

Table 7. Effect of embryo grade and cleavage

EMBRYO GRADE	EARLYCLEAVAGE GROUP I	LATECLEAVAGE GROUP II	EARLYCLEAVAGE &LATECLEAVAGE GROUP III	P VALUE
GRADE I (313)	158 (50.48%)	75 (23.96%)	80 (25.56%)	0.005**

** Highly significant

Table 8. Effect of protocol embryo grade

EMBRYO GRADE	AGONIST PROTOCOL	ANTAGONIST PROTOCOL
TOTAL NO OF GRADE I EMBRYOS	66.09% (1493/2259)	68.35% (920/1346)

When we compared the grade I embryos in the three groups 50.48% in group I , 23.96% in group II, and 25.56 % in group III P 0.005, which was also statistically significant (Table 7). We analysed for good quality embryos in agonist and antagonist protocol there was no difference, 66.09% (1493/2259) vs 68.35% (920/1346) (Table 8).

But we observed the effect of GnRH analogues and early cleavage there was no significant difference between agonist and antagonist stimulation protocol on early cleavage status .43.17%Vs 38.07%. P 0.053 (Table 3).

DISCUSSION

The first assisted conception therapies were performed in natural unstimulated IVF cycles. Nowadays, gonadotrophins are given to induce multiple follicular growth and GnRH analogues for the prevention of premature LH surges in IVF.[4]

The quality of oocytes and developing preembryos is one of the most important factors determining the success of an assisted reproductive treatment. In order to improve the efficiency of the treatment, either more embryos at a time will be transferred or a well-recognised stimulation protocol and embryo-selection procedure with lower number of transferred embryos is practised. There is the need to transfer less but more viable embryos to minimise the occurrence of multiple pregnancies. As a result of better fertilization and embryo culture techniques, patients may produce more good-quality embryos and have higher pregnancy and implantation rates.[2] As ovarian stimulation protocol is one of the appropriate factors during an assisted conception treatment, its embryo quality influencing effects are necessary to know. Since 2000 the assessment of GnRH agonist vs. GnRH antagonist protocols has been well analyzed in clinical studies, most of them focused on the clinical

outcome of the two protocols only. But it does not concentrate on the on embryo early cleavage.The effects of the GnRH analogues on oocyte and embryo-quality and on early cleavage development are still not recognised in detail.[5]

The long agonist protocol for controlled ovarian stimulation is generally the most effective and is used most regularly, therefore becoming the gold standard. Meta analysis comparison of GnRH agonist and antagonist protocol have shown comparatively lower pregnancy rate for GnRH antagonist, which may have discouraged its acceptance by clinicians.[10]Since the GnRH antagonist protocol is simple, easy, convenient and flexible along with the lack of functional ovarian cyst formation and "menopausal" symptoms frequently seen in the agonist protocol, it has become a better choice by clinical doctors and patients. Conversely, data from some randomized clinical trials shown that the antagonist protocol retrieves fewere number of oocytes along with lower pregnancy rates than the agonist long protocol.[2]

GnRH agonists have been commonly used since the mid-1980 s in order to prevent the surge of LH in IVF/ICSI cycles. Since their introduction in 1986, the prevalence of severe OHSS has been reported to have increased six-fold compared with the incidence in IVF cycles stimulated by clomiphene/HMG only. In the late 1990s, the GnRH antagonists became available: these compounds suppress gonadotrophin release by competitive receptor binding resulting in an instant suppression and blockage of gonadotrophin secretion rather than pituitary desensitization. The safety and efficacy of GnRH antagonists and agonists in IVF and ICSI cycles have been reported to be same. GnRH antagonists are now part of the therapeutic beneficial options of infertility units worldwide.[6] In our study, ovarian stimulation with a GnRH antagonist proved to be more commonly successful and with a reduced risk compared with cycles using GnRH agonists. The incidence of ovarian hyper stimulation syndrome (OHSS) was significantly reduced in the GnRH antagonist cycles.

An initial meta-analysis in the Cochrane database reported that GnRH antagonists are associated with a shorter period of stimulation, a reduced gonadotropin consumption and a reduced ovarian hyperstimulation occurence than long GnRH agonist protocols.[7]

Antagonist appears to be safer due to the lower incidence of ovarian hyperstimulation syndrome (OHSS), still, the literature has shown that high dose of gonadotropins use with the agonist protocol, resulting in higher cost for patients in addition to the risk of OHSS.[8] GnRH antagonist protocol produced a similar ovarian response, embryo development and pregnancy rates to GnRH agonist regime requiring lesser amounts of gonadotrophins.[9]

The resultsfrom our study were similar to these reported studies. The results of the current study shown that the GnRH antagonist and agonist long protocols provided comparable outcomes. In a recent prospective study, there was no significant difference between antagonist and agonist groups in terms of pregnancy and delivery complications, neonatal outcome and risk of major malformations.[10]

To achieve a singleton pregnancy without minimising the implantation rate should be the primary goal in assisted reproduction treatment. Till now, embryonic morphology has been one of the most useful tools to achieve this goal. In recent years, embryonic early-cleavage observed 25–27 hours after insemination has been recommeded as another available parameter for embryo selection All these previous studies used a GnRH agonist long protocol for pituitary suppression.[1]

The results of our study showed that, the mean numbers of normal fertilized oocytes, good quality oocytes, good embryos were all comparable with those in the GnRH agonist group and antagonist protocol. We also observed that embryonic early-cleavage was a good predictor for early embryonic development. In the present study, we found that ther was no difference in embryonic early-cleavage rate in using the GnRH antagonist protocol and agonist protocol.

Dynamics of early embryonic development could reflect the developmental potential of the embryo. It is identified that early cleavage is a strong indicator of the quality and the viability of the embryos, although a recent study showed higher implantational potential for early-cleavage embryos only with

the use of GnRH agonists. We observed significantly higher rate of early cleavage in this group did not reach statistical significance with the antagonist.[2] In the the previous studies the GnRH antagonist may still have some effects on delaying the first mitosis of zygotes. [1] But in the present study, we observed that embryonic early cleavage rate was comparable in both the GnRH agonist protocol and in the GnRH antagonist 43.17% vs 38.07% p 0.563 .In the present study, the results showed that the Grade I embryos were significantly higher in the early cleavage group. The early cleavage group was comparable in both Agonist and antagonist protocol.

Bidirectional signalling between oocytes and granulose cells is necessary for follicular development and the achievement of oocyte competence .The nuclear and cytoplasmic maturity of the oocyte that accompanies follicular development plays a vital role in facilitating fertilisation and the early stages of embryonic development. When the developing oocytes are exposed to supraphysiological concentrations of gonadotrophins may disturb oocyte maturation and the completion of meiosis leading to chromosomal aneuploid oocytes and/or embryos. Therefore, the gonadotrophin stimulation compromised not only uterine receptivity but also oocyte/embryo developmental competence. So, milder ovarian stimulation protocol seem to be less detrimental to the vulnerable process of nuclear maturation and chromosomal segregation. [3]

CONCLUSION

In conclusion, the results of this study showed that the early-cleavage rate was comparable in both the GnRH antagonist protocol and in the GnRH long agonist protocol. The results also showed that early cleavage of zygote seems to be a powerful predictor for embryo implantation potential when both the GnRH antagonist protocol and GnRH agonist protocol was applied. There was no significant effect of GnRH antagonist protocol and GnRH agonist protocol on embryo cleavage. Moreover, GnRH antagonist protocol required a shorter stimulation period plus fewer side effects. Hence GnRH antagonist protocol provided means for a friendlier, convenient and cost effective protocol for patients and it can be used in routine assisted reproductive technology treatment.

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