Original Article

Effect of Administration of Single Dose GnRH Agonist in Luteal Phase on Outcome of ICSI-ET Cycles in Women with Previous History of IVF/ICSI Failure: A Randomized Controlled Trial

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Abstract

Background: GnRH agonist administration in the luteal phase has been suggested to beneficially affect the outcome of intracytoplasmic sperm injection (ICSI) and embryo transfer (ET) cycles. This blind randomized controlled study evaluates the effect of GnRH (Gonadotropine Releasing Hormone) agonist administration on ICSI outcome in GnRH antagonist ovarian stimulation protocol in women with 2 or more previous IVF/ICSI-ET failures.

Methods: One hundred IVF failure women who underwent ICSI cycles and stimulated with GnRH antagonist ovarian stimulation protocol, were included in the study. Women were randomly assigned to intervention (received a single dose injection of GnRH agonist (0.1 *mg* of Decapeptil) subcutaneously 6 days after oocyte retrieval) and control (did not receive GnRH agonist) groups. Implantation and clinical pregnancy rates were the primary outcome measures.

Results: Although the age of women, the number of embryos transferred in the current cycle and the quality of the transferred embryos were similar in the two groups, there was a significantly higher rate of implantation (Mann Whitney test, p=0.041) and pregnancy (32.6% vs. 12.5%, p=0.030, OR=3.3, 95%CI, 1.08 to 10.4) in the intervention group.

Conclusion: Our results suggested that, in addition to routine luteal phase support using progesterone, administration of 0.1 *mg* of Decapeptil 6 days after oocyte retrieval in women with previous history of 2 or more IVF/ICSI failures led to a significant improvement in implantation and pregnancy rates after ICSI following ovarian stimulation with GnRH antagonist protocol.

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Received: Sept. 2, 2014
Accepted: Nov. 23, 2014

Keywords: Decapeptil, GnRH agonist, GnRH antagonist, ICSI, Implantation failure, Intracytoplasmic sperm injection, IVF failure, Luteal phase support.
To cite this article: Zafardoust S, Jeddi-Tehrani M, Akhondi MM, Sadeghi MR, Kamali K, Mokhtar S, et al. Effect of Administration of Single Dose GnRH Agonist in Luteal Phase on Outcome of ICSI-ET Cycles in Women with Previous History of IVF/ICSI Failure: A Randomized Controlled Trial. J Reprod Infertil. 2015;16(2):96-101.

Introduction

Production of progesterone during the luteal phase is necessary for successful implantation of the embryo. Defect in progesterone production in luteal phase leads to implantation failure. Luteal phase support in assisted reproduction technology (ART) is important, because it increases rate of conception in assisted reproduction cycles. Providing hormonal supplementation during the luteal phase with either progesterone, or human chorionic gonadotropin (hCG), which stimulates progesterone production, may improve implantation and, thus, pregnancy rates (1, 2). The fertility drugs that are administered to women who undergo assisted reproductive treatment (such

as ICSI and IVF), may disturb the natural hormones that are involved in establishment of pregnancy. On the other hand, the aspiration of the granulosa cells that surround the oocyte, the use of gonadotropin analogs (agonist or antagonist) during assisted reproduction technology (ART) treatment and supraphysiologic estradiol level after application of ovarian stimulation drugs can interfere with the production of progesterone during the luteal phase (3-6). The most important problem whit HCG administration in luteal phase support is OHSS (Ovarian Hyper Stimulation Syndrome) (7). It is suggested that co-administration of a single dose of GnRH agonist in midluteal phase significantly improves implantation and live birth rates in women undergoing intracytoplasmic sperm injection (ICSI) (8). Beneficial effect of single dose GnRH agonist administration in luteal phase in ICSI cycles has also been shown in some recent data (9-13). The action mechanism of GnRH agonist administration in luteal phase is not clear. A prospective randomized study evaluated the effect of administration of GnRH agonist in luteal phase of ICSI cycles (GnRH agonist and antagonist protocols) and helped to clarify its mechanism. The scholars indicated the direct action of GnRH agonist on the embryo (10). It was suggested that GnRH agonist can affect the maintenance of corpus luteum by LH secretion, act via local receptors on endometrium and has effect on embryos (14, 15). In this randomized controlled study, an attempt was made to assess the effect of single dose GnRH agonist (0.1 *mg* Decapeptyl), 6 days after oocyte retrieval in antagonist protocols for prevention of premature LH surge in ICSI cycles. The most important difference between our study and other studies is our distinctive population. Our women had 2 or more previous implantation failures with good embryo quality in IVF-ET or ICSI-ET cycles. The main outcome of the study was implantation and clinical pregnancy rates.

Methods

Design and population: This randomized controlled study IRCT.ir Trial registration number 2012111111430N1was conducted to evaluate the hypothesis that a single 0.1 *mg* dose of Decapeptil administered 6 days after oocyte retrieval in women with a history of 2 or more previous IVF-ET or ICSI-ET failures would increase the implantation and clinical pregnancy rates in women undergoing GnRH antagonist protocol. The study population consisted of 100 women who underwent ICSI after ovulation induction by gonadotropins and administration of GnRH antagonist for the prevention of a premature LH surge. This study was conducted between February 2013 and January 2014 in Avicenna infertility Clinic affiliated to Avicenna Research institute, Tehran, Iran. It involved 100 infertile couples with history of 2 or more previous IVF-ET or ICSI-ET failures treated by GnRH antagonist protocol for ICSI. This study was approved by the Ethical Committee of Avicenna Research Institute and informed consent was obtained from all participants. Women with history of 2 or more previous IVF-ET or ICSI-ET failures who were stimulated with GnRH antagonist protocol and were planned to undergo ICSI were included in the study. The women were under 42 years old and had FSH levels <12 *mIU/ml* on 2nd or 3rd day of menstrual bleeding with normal thyroid and prolactin levels and the couples had at least one embryo available for transfer. Women with hydrosalpinx or anatomical uterine disorders or those with thrombophilia disorders were excluded from the study. In addition, couples suffering from azoospermia who required testicular sperm retrieval and those who had undergone Preimplantation Genetic Diagnosis (PGD) were also excluded from the study.

Randomization protocol: Computer-generated randomization list was used for randomization. Selection was performed on the day of OCP administration for GnRH antagonist cycle. There were two groups. Intervention group received Decapeptil (Ferring, Germany) 0.1 *mg* S.C., 6 days after oocyte retrieval and control group did not receive Decapeptil. All women received routine luteal phase support with 800 *mg* vaginal progesterone daily.

GnRH antagonist protocol: Contraceptive pill (Aboreihan, Iran) was administered during the cycle preceding ovarian stimulation. On day 2nd or 3rd of menstrual bleeding following withdrawal of contraceptive pill, recombinant human FSH (Gonal F: Merck Serono, Germany; Fostimon: IBSA, Switzerland) or HMG (Merional: IBSA, Switzerland) administration was started. FSH and HMG doses were adopted according to size of ovarian follicles and their growth rates in vaginal ultrasonography. GnRH antagonist (Cetrotide: Merck Serono, Germany) was started when the leading follicle reached 14 *mm* in diameter monitored by vaginal ultrasonography (Honda 2000-5 MHz, Japan). It was administered at a daily dose

of 0.25 *mg* S.C. until at least 2 follicles reached a diameter of ≥18 *mm*. HCG (Choriomon-IBSA, Switzerland), 10 000 *IU* intramuscularly was then administered and after 36 *hr*, oocyte retrieval was undertaken under vaginal ultrasonography guide. Fertilization was achieved with ICSI in all couples. ET was performed on day 3. All women after oocyte retrieval were given 400 *mg* Cyclogest (vaginal progesterone suppository, PhEur-Actavis) daily until ET and after that they were given 800 *mg* daily until 12 weeks of pregnancy.

Assisted Reproduction Techniques: The oocytes surrounding by granolosa cells were primarily kept in culture medium. They were then denuded from the cumulus oophorus and corona radiate cells by mechanic and Enzymatic dissection using hyaluronidase and followed by incubation in G1-medium (Vitrolife) at 37℃ with 6% CO_2 for 1-3 *hr*. Mature (metaphase II) oocytes were selected for ICSI (16). Then, they were incubated in G1-medium. Fertilization was assessed 16-18 *hr* post ICSI. They were kept in the medium for 3 days after ICSI. At the time of medium change on day 2 and day 3 (10-30 *min* before embryo transfer), cleaving embryos were evaluated by morphologic criteria (16). Embryos that had more than 6 cells at 3 days after ICSI, or more than 3 cells at 2 days after ICSI and showed less than 10% of their space was occupied by cell fragments were in good morphologic conditions and were selected for embryo transfer (ET). Embryo transfer was performed with the use of embryo transfer set (Cook, Queensland, Australia) three days after ICSI. Number of transferred embryos was 1-3 in each cycle according to condition of women and quality of embryos. In our study, only fresh embryos were transferred.

Embryo selection: Selection of embryos was based on number of blastomers, evaluation of symmetric blastomers and fragmentation. Embryos that had more than 6 cells at 3 days after ICSI, or more than 3 cells at 2 days after ICSI and showed less than 10% of their space was occupied by cell fragments were in good morphologic conditions and were selected for embryo transfer (ET).

Outcome measures: Pregnancy was tested by measuring serum beta-hCG levels 14 days after ET. The implantation rate was calculated as the ratio of the number of embryonic sacs detected by ultrasonography to the total number of embryos transferred. Clinical pregnancy was defined as the presence of a fetus with a heart beat by vaginal ultrasonography at 6 weeks of pregnancy. Multiple pregnancies were defined by presence of more than one fetus in vaginal ultrasonography.

Statistical analysis: Data were analyzed by SPSS 11.5. The results are shown as mean and standard deviation. Chi-squared test, Student's t-test, Fisher's exact test and Mann-whitney U test were used for comparing the study groups. The p-<0.05 was considered statistically significant.

Results

Out of 100 women, there were 17 dropouts. Finally, 83 women were included in the final analysis. Among the women assigned to GnRH agonist (intervention group n=43) group, 2 discontinued the ovarian stimulation, 1 had no embryos for transfer, 3 were OHSS and their embryos were

Table 1. Demographic characteristics of patients in GnRH agonist and control groups

Parameter	GnRH agonist	Control	p-value
Number of patients	43	40	
Age (years)	34.4±4.6	35.4±5.2	0.361
BMI	24.9±2.8	24.1±2.4	0.117
Number of previous IVF or ICSI-ET	2.7±1	2.3±0.7	0.064
Number of previous transferred embryos	3.1±0.8	3.3±1	0.365
FSH on 2nd or 3rd day of menstrual bleeding (*mIU/ml*)	7.6±2.5	7.4±3.2	0.770
Number of gonadotropins in current cycle	39.2±14	45.6±19	0.089
Days with gonadotropin administration in current cycle	9.3±1.5	9.4±1.6	0.993
Sperm *(count/ml)*	(40±45)×10^6	(46 ±41)×10^6	0.596
Mean number of oocytes retrieved in current cycle	9.8±5.6	9.5±6.8	0.794
Mean number of transferred embryos per transfer in current cycle	3.2±0.9	2.9±1.02	0.121
Number of total embryos in current cycle	5.7±4.1	6.6±6.2	0.448
Embryo quality (good quality. A)	58.1%	49.9%	0.149

Table 2. Outcome measures in GnRH and control groups

Parameter	GnRH group	Control	p-value
Pregnancy rate (%)	32.6	12.5	0.030
Gestational sac (%)	27.9	10	0.039
Clinical pregnancy rate (%)*	14	7.5	0.485

* Fisher's Exact Test

frozen and one was lost to follow-up. Among control group (without GnRH agonist n=40), 5 women discontinued the ovarian stimulation or had no embryos for transfer and 5 women did not transfer because of OHSS. There were no significant differences in the demographic characters of the two groups and their ovulation stimulation cycle characters were also comparable (Table 1). They received equal numbers of embryos with similar qualities. The level of beta-HCG, 14 days after ET in both groups, did not differ significantly (p= 0.588). Pregnancy rate increased significantly in GnRH group compared to controls (p=0.030, OR= 3.3, 95% CI, 1.08 to 10.4). In this regard, 14 women in GnRH agonist group (32.6%) and 5 women in control group (12.5%) had beta-HCG levels (positive chemical pregnancy). Gestational sacs were detected in 12 women (27.9%) in GnRH agonist group and in 4 women (10%) in the control group (Table 2) and this difference was significant (p=0.039, OR=3.4, 95% CI, 1.01 to 11.9). Implantation rate significantly improved in the GnRH agonist group compared to the control group (Mann-whitney test, p=0.041). All pregnancies were with one gestational sac. Fetal heart rates were detected in 6 women in GnRH agonist group and 3 women in the control group (14% *vs*. 7.5%, p=0.485).

Discussion

Aspiration of granulosa cells and suppression of LH following GnRH agonist and GnRH antagonist administration in ovarian stimulation cycles leads to luteal phase deficiency and this is suggested in several lines of evidence (3-6). The most common form of luteal phase support in these women is progesterone or HCG administration in luteal phase (1, 2). Recently, the effect of GnRH agonist administration in luteal phase of ICSI cycles in different studies has been the center of attention. Most of these studies agree that administration of GnRH agonist in luteal phase of ICSI cycles does not guarantee the continuation of pregnancy resulting from assisted reproduction attempts but rather seems to support implantation (17-19). This randomized controlled study was undertaken to evaluate the effect of a single 0.1 *mg* dose of Decapeptil administration 6 days after oocyte retrieval in women with 2 or more previous IVF-ET or ICSI-ET failures who were stimulated with antagonist protocol. In addition to vaginal progesterone 800 *mg* daily, these women received 0.1 *mg* Decapeptil S.C. 6 days after oocyte retrieval. Tesarik et al. (2006) reported that receiving a single dose GnRH agonist 6 days after oocyte retrieval in GnRH antagonist ovarian stimulation protocol significantly increased serum concentration of E2 on days 7 and 15 after oocyte retrieval, progesterone on both 7 and 15 after oocyte retrieval and beta-HCG 15 days after oocyte retrieval, compared with placebo. They suggested that GnRH agonist has a direct action on embryo and/or endometrium (10). In our study, no significant differences in beta-HCG concentrations were detected in the two groups (p=0.588). They had 15 multiple births (all twins) in GnRH agonist group as compared with only two multiple births (all twins) in placebo group but there were no multiple pregnancies in our study. This may be due to fewer samples in our study. On the other hand, our population were women with 2 or more IVF/ICSI-ET failures with good quality embryos and there were lower pregnancy rates among these women (20). Tesarik et al. (2006) reported significant increase in clinical implantation rates (29.8% *vs*. 18.2%, p<0.05), live birth rates (27.4% *vs*. 18.2%, p<0.05) and nonsignificant increase in clinical pregnancy and ongoing pregnancy rates in GnRH agonist group as compared with placebo group in GnRH agonist ovarian stimulation cycle. They also reported significant improvement in clinical implantation (27.1% *vs*. 17.4%, p<0.05), ongoing pregnancy (43.3% *vs*. 30.7%, p<0.05) and live birth rates (25.2% *vs*. 14.6%, p<0.05) in GnRH antagonist ovarian stimulation cycles and also a trend toward a higher clinical pregnancy rate (p=0.083) in GnRh agonist group. Razieh et al. (2009) observed that administration of 0.1 *mg* of GnRH agonist triptorelin on day 3 after embryo transfer led to a significant improvement in implantation rate (12.3% *vs*. 7.3%) and clinical pregnancy rate (25.5% *vs*. 10.0%) as compared with placebo (13). In a study by Isik et al. (2009), the same results were demonstrated. All cases in that study received intravaginal micronized progesterone (600 *mg* daily) for luteal phase support in GnRH antagonist during ovarian stimulation cycles. Although the number and the quality of

embryos transferred were similar in the two groups, the women in the luteal phase agonist group (0.5 *mg* leuprolide acetate, subcutaneously on day 6 after ICSI) had significantly higher rates of implantation and clinical pregnancy rates ($p< 0.05$) compared with the group that received only routine luteal phase support (9). In our study, pregnancy rate increased significantly in GnRH group compared to the control. Implantation rate significantly improved in the GnRH agonist group compared to the control group and clinical pregnancy rate improved nonsignificantly in GnRH group. Pregnancy developed to stage of gestational sac formation in the GnRH agonist group compared to the control group. One of the most frustrating problems in infertility today is IVF failure-also called implantation failure.

Various attempts to improve success in repeated IVF failure include blastocyst transfer, assisted hatching, prophylactic salpingectomy, preimplantation genetic diagnosis, and coculture methods. Petersen et al. (2005) reported that in women with repeated implantation failures, the implantation rate in those who received laser-thinned embryos was significantly higher ($p=0.02$) than in those whose embryos were not laser-thinned (10.9 and 2.6%, respectively) (21). Our population in this study had history of 2 or more IVF/ICSI failures and when compared with other treatments in IVF failure women, it seems administration of GnRH agonist in luteal phase in these women has acceptable results. The mechanism of GnRH agonist in luteal phase support is not clear. Based on the increase in level of estradiol and progestrone in luteal phase of these women scrutinized in few studies, the mechanism of GnRH agonist may be due to its effect on pituitary or ovaries (10). It was suggested that GnRH agonist can affect maintenance of corpus luteum by LH secretion, act via local receptors on endometrium and has effect on embryos (14, 15). The main difference between the present study and the previous reports was our distinctive population. Our women had a history of 2 or more previous IVF-ET or ICSI-ET failures. GnRH antagonist was used in ovarian stimulation cycles for prevention of premature LH surge. It may be speculated that stimulation of corpus luteum activity by GnRH agonist may result from stimulation of LH secretion, given that despite the blockage, the pituitary remains responsive to the administration of GnRH or GnRH agonist. Oliveira et al. (2010), based on a meta-analysis study, concluded demonstrated that the luteal phase single dose GnRH agonist administration can improve clinical outcomes after ICSI. However, because of heterogeneity between the trials, many more trials might be needed to be undertaken before one could recommend GnRH agonist administration in the luteal phase (11).

Conclusion

Our results suggested that, in addition to routine luteal phase support with progesterone, administration of one dose of Decapeptil 6 days after oocyte retrieval in women with previous history of 2 or more IVF/ICSI failures with good embryo quality, led to a significant improvement in implantation and pregnancy rates in ICSI cycles following ovarian stimulation with GnRH antagonist protocol.

Acknowledgement

We are grateful to all of the colleagues at the Avicenna fertility clinic for their inspiration and contribution. We would like to thank the patients for their kind help in this research. In addition, we are grateful to Hamid Hamzezad for helpful advice, and Zahra Akbarzadeh Pasha and Reyhaneh Tokhmechi for their kindly cooperation.

Conflict of Interest

Authors declare that there is no conflict of interest.

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