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Original Article

Ovarian Cysts Formation During Depot Formulation of GnRH-a Therapy and the Effect of Pretreatment with Oral Contraceptive Pills on Subsequent Implantation and Pregnancy Rate in ART Cycles

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Abstract

Long protocol of Gonadotropin-Releasing Hormone-analougue (GnRH-a) can result in the formation of ovarian cyst by the transient initial stimulatory effect which increases the levels of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH). These cysts require surgical drainage or result in poor ovarian response. Ovarian cyst formation can be prevented by taking oral contraceptives (OCs) which suppress LH and FSH after initiation of GnRH-a therapy. This study was designed to investigate ovarian cyst formation during therapy with depot formulation of GnRH-a and also the effect of taking (OCs) before starting the treatment with depot formulation of GnRH-a, on the formation of ovarian cyst, implantation and pregnancy rate in assisted reproductive tecnique (ART) cycles. Fifty four infertile women who were candidate for ART, underwent two treatment protocols in a prospective randomized trial: (a) OC+HMG+diphereline and (b) HMG+diphereline. In group (a) patients were pretreated with OC for 14 days starting from the first day of mensturation and on the day 14 received 3.75 mg IM depot diphereline. Patients in group (b) received 3.75 mg diphereline by intramuscular injection on the second day of menstruation. Sonography was performed on the first day of menstruation and also 7 and 14 days after diphereline injection. Ovarian cyst incidence, gonadotropin consumption, follicular growth, implantation rate and pregnancy in the two groups were studied. No ovarian cyst with diameter over 26 mm was developed with depot formulation of GnRH-a in any of the two groups (a and b). There was no significant difference between the two groups in the follicular growth (9.2±2.1 and 9.4±2.9), number of oocyte (5.0±2.8 and 5.4±5.7), implantation rate (0.02±0.08 and 0.03±0.10) and pregnancy rate (0.09 and 0.11). We divided the patients into two groups based on their ages: (20-34) and (≥35). It showed no significant difference in the gonadotropin consumption , mean number of follicles and mean number of embryos in groups (a and b) based on their ages. No ovarian cyst developed with depot formulation of GnRH-a. So, in women with a history of ovarian cyst formation in previous cycles depot form of GnRH-a may be considered. Pretreatment with OCs during therapy with depot formulation of GnRH-a and gonadotropin didn't increase the number of oocyte, implantation rate and pregnancy.

Keywords: Oral contraceptive; Depot formulation; GnRH-a; Ovarian cyst.

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Introduction

Using of GnRH-analogue before ovarian stimulation with gonadotropins in ART cycles has resulted in an increasing number of oocytes obtained, higher fertilization rates, better embryo quality, higher pregnancy and live birth rates. (1-3). It has also shown that long protocol GnRH-a can improve the ART results in comparison with ultrashort or short protocols (4) and when the long protocol is used, initiation of GnRH-a administration in the early follicular phase results in less profound pituitary suppression, greater number of oocytes collected and possibly higher pregnancy rates compared with initiation of GnRH-a administration in the midluteal phase (5-6). In some patients, long protocol of GnRH-a will result in the formation of ovarian cysts that require surgical drainage or prolonged exposure to GnRH-a (7). Baseline ovarian cysts have a negative impact on the quality of ovarian hyperstimulation procedure (8).

These functional ovarian cysts are formed by the transient initial stimulatory effect of the GnRH-a.

This drug increases the levels of both FSH and LH and stimulates primordial follicles to grow but due to rapid pituitary suppression, continued follicular development and ovulation does not occur and consequently follicular cysts are formed. Pretreatment with OCs will suppress of LH and FSH after initiation of GnRH-a therapy and can be effective in the prevention of ovarian cyst formation (7).

In this study, we investigated the incidence of ovarian cyst formation during depot formulation of GnRH-a administration and the effect of pretreatment with an oral contraceptive on ovarian cyst formation during GnRH-a therapy as well as its effect on subsequent implantation and pregnancy rates in ART cycles.

Experimental

We designed a prospective randomized clinical trial. Our study was carried out on fifty four patients who were undergoing in vitro fertilization (IVF) and itracytoplasmic sperm injection (ICSI) treatment from Jannary 2002 to Jannary 2003 in the IVF center of Yazd and Day hospital.

In the primary visit, physical examination and sonography showed no ovarian cyst. Patients were divided into two groups by randomized allocation method and underwent two treatment protocols: (a) OCP+HMG+diphereline and (b) HMG+diphereline. In group (a) patients were pretreated with OCP (ethinyl estradiol 0.03 mg and levonorgestrol 0.15 mg, Iran Hormon pharmacenticals) for 14 days starting on the first day of mensturation and then 3.75 mg diphereline depot (IPSEN, Germany) was injected intramuscularly on the last day of OCP administration. Patients in group (b) received 3.75 mg dipherelin (IPSEN, Germany) intramuscularly on the second day of menstruation. In both groups, vaginal sonography was performed on the first day of menstruation and 7 and 14 days after diphereline injection to detect any cyst. For controlled ovarian hyperstimulation in both groups, HMG (75 IU of FSH and 75 IU of LH, organon, Italy) was injected intramuscularly daily that started on 2nd day of the next cycle. The initial and adjusted dosage of gonadotropins used were determined according to the patients' age and follicular response by serial sonography. Follicular growth was monitored by vaginal sonography that started on the day 9 of cycle, and then, depending on the size of follicles. When there were at least three follicles larger than 18 mm in diameter shown in sonography, Human Chorionic Gonadotropin (HCG) (10000 IU, Serono, Italy) was injected intramuscularly. Transvaginal oocyte recovery was performed 36 hours after HCG administration. IVF or ICSI was done on oocytes and the embryos were evaluated on the day of transfer (second day of IVF or ICSI). The best embryos were selected for transfer and the remaining embryos were cryopreserved.The luteal phase was supplemented by 400 mg of micronized progestrone (cyclogest, Schering, Canada) which was self-administered by the patients, vaginally. Pregnancy was defined as the presence of one or more fetal hearts detected on sonography performed at least 4 weeks after embryo transfer (ET). Cycle outcome was quantified by the number of ovarian cyst (>26 mm in diameter), the number of HMG ampules required, number of follicles at the

Table 1. Outcome of cycles in two study protocols.

variable	Protocols		
	Oc+diphereline+HMG	**diphereline +HMG**	**P value**
Cyst formation (size>28mm)	No cyst	No cyst	NS
Number of follicles >16mm	9.2±2.1	9.4±2.9	NS
Number of oocytes retrived	5.0±2/8	5.4±5.7	NS
Implantation rate(%)	0.02±0/08	0.03±0.10	NS
Pregnancy rate(%)	0.09±0/29	0.11±0.42	NS

Note: Data are presented as mean ± standard deviation

time of HCG administration, number of oocytes retrieved, number of embryos, implantation rate and pregnancy rate. The implantation rate was calculated by dividing the number of fetal hearts visualized at the time of the first sonography by the number of embryos transferred and clinical pregnancy rate was calculated by dividing the number of clinical pregnancies by the number of patients who underwent embryo transfer.

The study was approved by Shahid Sadoughi University of Medical Sciences, yazd, Iran.

The data were analysed with the use of the Chi square test and *t*-test. A 95% confidence interval was calculated for all performed tests. A *p* value of <0.05 was considered statistically significant.

Results

A total of 54 IVF and intracytoplasmic sperm injection (ICSI) cycles were performed, Sixteen patients underwent the second course of IVF and ICSI, with intervals more than 6 months. The causes of infertility among our patients were as follows: male factor in 21 cases, tubal damage in 20 cases, unexplained infertility in 6 cases and anovulation in 7 cases. The etiology and duration of infertility were equally distributed among the groups. Three patients were excluded from study because of incomplete data.

There were no statistical difference in the ovarian response variables among the two group. (Table 1). No ovarian cyst formation occurred in either of the two groups. Differences between the mean number of follicles (9.2±2.1 and 9.4±2.9), mean number of oocytes (5.0±2.8 and 5.4±5.7), mean implantation rate (0.02±0.08 and 0.03±0.10) and mean pregnancy rate (0.09 and 0.11) were not statistically significant in the two groups.

41% of patients (22/54) were >35 years old at the time of the ovulation induction. The patients in group (a) were between 22-43 years old and in group (b) were between 27-42 years old. The mean age between two groups were significantly different ((a): 31.48±5.82 versus (b): 35.27± 4.13) (p value=0.007). So we divided the patients into two groups based on their ages: (20-34) and (≥35) and then outcome of cycles were studied in the two age groups. The results showed no significant difference between two protocols in the gonadotropin consumption (p value=0.27), mean number of follicles (p value=0.76) and mean number of embryos (p value=0.81) based on their ages (Table 2).

Discussion

This study demonstrated that with the use of depot formulation of GnRH-a no cyst formation observed in ART cycles, no significant differences in the number of follicles the number of oocytes, the implantation rate and the pregnancy rate between patients who had taken OC before dipherelin and HMG and those who had not taken OC.

It was shown that the use of a GnRH-a together with gonadotropins for ovarian stimulation in assisted conception results in significantly lower cancellation rates, increase in the number of oocyts obtained and increase in the pregnancy rate. In addition, the long protocols have better outcomes in comparison with short and ultrashort protocols. (4) Since menstrual cycles in women often are not exactly 28 days, and the stress during treatment may delay the

Table 2. Comparing mean number of HMG, follicles and embryo between the two study protocols based on age.

Age	diphereline +HMG				Oc+ diphereline +HMG			
	patient (n)	HMG[a] (n)	follicle[b] (n)	embryo[c] (n)	patient (n)	HMG[a] (n)	follicle[b] (n)	embryo[c] (n)
20-34	13	28.92±4.13	11±5.98	2.76±1.78	16	33.11±8.23	10.40±4.30	3.75±1.94
≥35	16	30.81±6.17	8.18±7.41	2.12±1.92	6	39.57±11.92	6.50±3.44	2.83±2.04
total	29	33.56±9.10	9.44±6.84	2.41±1.86	22	31.30±6.99	9.28±4.38	3.50±1.97

Note: Data are presented as mean±standard deviation
[a] (p value=0.27)
[b] (p value=0.76)
[c] (p value=0.81)

time of ovulation, the patients who undergoing midluteal initation of GnRH-a therapy actually may receive the drug in the early luteal phase, which may be associated with poorer outcomes (5-6). In addition, it may interfere with early pregnancy (9). So, follicular initition of GnRH-a is recommended (10). The only disadvantage of follicular over midluteal initiation of GnRH-a administration is the possibility of an increased incidence of ovarian cyst formation (7). In these patients the period leading to ovarian suppression frequently is prolonged and unpredictable, requiring repeated blood tests and additional US scans. Moreover, a prolonged period of ovarian suppression causes additional stress and anxiety for the patients who are undergoing IVF treatment. Therefore, it would be of considerable interest if the occurrence of ovarian cysts could be eliminated without a negative effect on subsequent follicular development, embryo quality and pregnancy rate.

Functional ovarian cysts probably are formed by the transient initial stimulatory effect of GnRH-a, which stimulates primordial follicles to grow. Owing to rapid pituitary suppression, however, continued follicular development and ovulation dose not occur and follicular cysts are formed (11). The initial stimulatory effect of the GnRH-a increases the levels of both FSH and LH. However, early follicular development is caused primarily by FSH and therefore the surge of FSH rather than LH probably is responsible for functional cyst formation (12). Therefore, to abolish the formation of functional ovarian cysts in the follicular phase of the cycle, the initial FSH surge should be attenuated.

Vizziello et al (13) studied flare-up of gonadotropins during treatment with triptoreline and confrimed that LH levels show an increase six-fold the basic values while FSH levels increases two-fold after two days of treatment.

Sonntag et al (10) showed that serum LH and FSH levels were significantly lower in patients who received depot formulation of GnRH-a (triptorelin acetate) and discussed that higher FSH stimulation dose requirment and lower oocyt number and fertilization rate indicating a need for minimal LH activity in folliculogenesis and oocyts development.

Another study (14) showed that low dose of triptorelin produces more suppression on endogenous LH in pituitary than buserelin long protocols. Dalprato, et al reported that low doses of short GnRH-a requiring lower amount of gonadotropins in comparison with depot formulations (15). Given the results of these studies it seems that depot formulation of GnRH-a cause deeper pituitary suppression.

Our data confirm that with administration of depot formulation of GnRH-a ovarian cyst was not increased. It can be due to the more decrease in FSH level in comparison with LH levels in flare up. So, in patients with the history of ovarian cyst with GnRH-a adminstration in the previous cycles depot formulation of GnRH-a can be considered.

In an attempt to program IVF cycles and abolish the occurrence of a premature LH surge, Templeton et al (16) studied the effects of norethindrone and a combined OC on pituitary suppression. They reported a significantly slower response to combined treatment with

clomiphene citrate and HMG in patients who were pretreated with OC. They suggested that OCs cause a deeper pituitary suppression.

In another study, Forman et al (17) reported a similar level of suppression of LH, but a significantly deeper suppression of FSH with the OC compared with norethisterone.

Combination of depot GnRH-a and OC in our study did not show any difference in comparison with depot formulation alone. This can be because OC has negligible effect as compared with depot formulation of GnRH-a on pituitary supression. Due to this fact no significant differences in growth of follicles, number of oocyt and embyo, implantation rates and pregnancy in patients who had received OC and depot form of GnRH-a, in comparison with the patients who received depot formulation of GnRH-a alone were found.

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