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ARTICLE

GnRH agonist plus vaginal progesterone for luteal phase support in ICSI cycles: a randomized study

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Abstract In this prospective randomized study, the effect of daily gonadotrophin-releasing hormone agonist (GnRHa) in the luteal phase on IVF and intracytoplasmic sperm injection (ICSI) outcomes was assessed. Women ($n = 446$) were counselled for IVF–ICSI, and randomized on the day of embryo transfer to group 1 (daily 0.1 mg subcutaneous GnRHa until day of beta-HCG) ($n = 224$) and group 2 (stopped GnRHa on day of HCG injection) ($n = 222$). Both groups received daily vaginal progesterone suppositories. Primary outcome was clinical pregnancy rate. Secondary outcome was ongoing pregnancy rate beyond 20 weeks. Mean age, oestradiol on day of HCG, number of oocytes retrieved, number of embryos transferred, and clinical and ongoing pregnancy rates were 28.9 ± 4.5 years, 2401 ± 746 pg/mL; 13.5 ± 6.0 oocytes; 2.6 ± 0.6 embryos, and 36.2% and 30.4% consecutively in group 1 compared with 29.7 ± 4.7 years, 2483 ± 867 pg/mL, 13.7 ± 5.5 oocytes, 2.7 ± 0.6 embryos, 30.6% pregnancy rate, and 25.7% ongoing pregnancy rate in group 2. No significant difference was found between the groups. Subcutaneous GnRHa during the luteal phase of long GnRHa protocol cycles does not increase clinical or ongoing pregnancy rates after IVF–ICSI.

KEYWORDS: clinical pregnancy rate, GnRHa, IVF–ICSI, luteal phase, randomized study

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Introduction

Luteal phase deficiency is a common problem in current assisted reproduction technique, and has been described in cycles using pituitary down-regulation with a gonadotrophin-releasing hormone agonist (GnRHa) (Pritts and Atwood, 2002), as well as GnRH antagonist cycles (Macklon and Fauser, 2000). It has long been recognized that supporting the luteal phase with progesterone or human chorionic gonadotrophin (HCG) is associated with higher pregnancy and delivery rates (Daya and Gunby, 2004). Oetrogen was tried for luteal phase support in combination with progesterone, but it was found to be ineffective in improving the implantation and pregnancy rates (Jee et al., 2010). Some recent data, however, have suggested a beneficial effect of GnRHa administered in the luteal phase on the outcome of assisted reproduction tehniques (Pirard et al., 2005; Tesarik et al., 2004).

The mechanism of the presumed beneficial effect of luteal-phase GnRHa administration is not clear. It was hypothesized that GnRHa may support the corpus luteum by stimulating the secretion of LH by pituitary gonadotroph cells or by acting directly on the endometrium through the locally expressed GnRH receptors (Pirard et al., 2005). It is unlikely, however, that the mechanism of adding GnRHa is through secretion of LH by the pituitary gonadotropin cells in down-regulated agonist cycles as the pituitary action is suppressed. The administration of a single dose of GnRHa in the luteal phase was also shown to increase pregnancy, implantation, delivery and birth rates in recipients of donated oocytes in whom ovulation was suppressed and the corpus luteum was thus absent, suggesting a direct effect of GnRHa on the embryo (Tesarik et al., 2004). Currently, available data on the beneficial effect of administering GnRHa on the probability of pregnancy is still controversial (Kyrou et al., 2011).

The aim of this randomized study was to assess the effect of administering GnRHa in the luteal phase in intrtacytoplasmic sperm injection (ICSI) cycles stimulated by the long GnRHa protocol on the pregnancy rate. The study was registered under ISRCTN13123887.

Materials and methods

This randomized study was conducted in accordance with CONSORT guidelines (http://www.consort-statement.org). A total of 610 patients were assessed for eligibility. Those who did not meet the inclusion criteria were patients over the age of 39 years, patients who had two or more failed IVF trials, patients with intramural, submucous fibroids or uterine anomalies, as well as patients who had a poor response and did not reach the stage of HCG administration in a previous IVF cycle or in the current trial. Patients who had an HCG injection to trigger ovulation but had failed fertilization, or those who had less than two embryos available for transfer (**Figure 1**) were also excluded. Approval of our ethical committee was obtained on 23 December, 2010 (reference: Aboulghar172010). Signed consent was obtained from 466 patients who were enrolled for randomization. The study was conducted at the

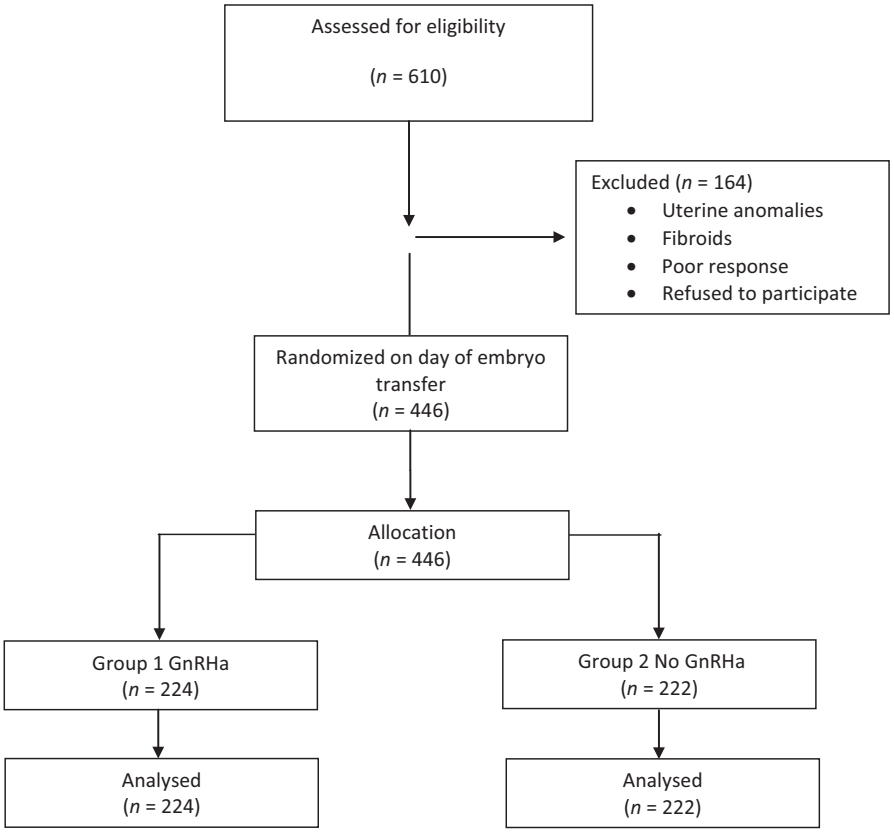

Figure 1 Randomization.

Egyptian IVF centre, Cairo, Egypt between 1 January, 2011 until 31 December, 2012.

Randomization

A computer-generated randomization table was created for the study population on the basis of 1:1. A nurse not involved in the study picked one envelope for each patient from sequentially numbered envelopes on the day of embryo transfer and informed the patient about their allocated arm. Allocation concealment was ensured by the use of dark, sealed envelopes containing the assigned intervention.

Power of the study

Sample size calculation was based on comparing two proportions from independent samples using chi-squared test; the alpha error level was fixed at 0.5 and the power was set at 80%. It is assumed that using GnRHa in the luteal phase will improve clinical pregnancy rate from 24% to 36%. Accordingly, the optimum sample size should be 228 cases in each arm. PS Power and Sample size Calculations software, Version 2.1.30 for MS Windows, was used to calculate sample size (Dupont and Vanderbilt, USA).

All patients were stimulated by our standard routine GnRHa (Decapeptyl; Ferring Pharmaceuticals, Kiel, Germany) long protocol (Aboulghar et al., 1994). A total of 446 patients undewent ICSI cycles. Randomization resulted in group 1 (n = 224) patients who continued to receive daily 0.1 mg subcutaneous GnRHa injection (Decapeptyl; Ferring, Kiel, Germany) until day of serum beta-HCG detection. In group 2 (n = 222), patients stopped GnRHa on the day of HCG trigger. Both groups received daily vaginal progesterone suppositories (total dose 600 mg) (Prontogest, IBSA, Egypt). Clinical pregnancy was diagnosed by ultrasound detection of fetal echoes and pulsations. A questionnaire was sent to 10 reputable IVF centres to enquire if they were using GnRHa for luteal phase support in IVF.

The primary outcome measure was clinical pregnancy rate and the secondary outcome measures were ongoing pregnancy and ectopic pregnancy rates.

Statistical analysis

The data were statistically described in terms of mean ± SD or frequencies (number of cases) and percentages when appropriate. Student's t-test was used to compare numerical variables between the study groups for independent samples. Chi-squared test was used for comparing categorical data. Exact test was used instead when the expected frequency was less than 5. $P < 0.05$ was considered statistically significant. Statistical Package for the Social Sciences (SPSS) version 15 for Microsoft Windows (SPSS Inc., Chicago, IL, USA) was used for all statistical calculations.

Results

This study was completed and the results are reported in accordance with the CONSORT Statement.

No significant difference was found in age, the dose of FSH used for stimulation or the oestradiol level on the day of HCG between the two groups, whereas only a small significant difference in body mass index was found between the two groups ($P = 0.012$) (Table 1).

The mean number of transferred embryos in both groups were 2.6 ± 0.6 versus 2.69 ± 0.7, and the good quality embryos were 2.5 ± 0.6 versus 2.5 ± 0.7; both showed no significant difference between both groups. No significant difference was found in clinical and ongoing pregnancy rates or ectopic pregnancy rate between the two groups (Table 1). The questionnaire showed that none of the 10 IVF centers were using GnRHa for luteal phase support.

Discussion

This is a powered randomized study, which investigated the effect of GnRHa administration in the luteal phase on the outcome of IVF in long protocol GnRH cycles. The study assessed the effect of continuing a daily 0.1 mg dose of GnRHa until the day of serum beta HCG on clinical and ongoing pregnancy rates. No statistically significant differences in the clinical or ongoing pregnancy rates were found between the groups

Table 1 Patients' characteristics and clinical outcome.

	Group 1 (agonist)	*Group 2 (no agonist)*
Number of patients	224	222
Mean age (years)	28.9 ± 4.5	29.7 ± 4.7
Mean body mass index (kg/m^2)[a]	28.4 ± 7.9	30.0 ± 5.8
FSH ampoules (75 IU)	32.2 ± 10.3	33.7 ± 10.3
Mean oestradiol on day of HCG (pg/ml)	2401 ± 746	2483 ± 867
Mean number of oocytes retrieved	13.5 ± 6.0	13.7 ± 5.5
Mean number of embryos transferred	2.6 ± 0.6	2.7 ± 0.6
Good quality embryos	2.5 ± 0.6	2.5 ± 0.7
Clinical pregnancies n (%)	81 (36.2)	68 (30.6)
Ongoing pregnancies n (%)	68 (30.4)	57 (25.7)
Ectopic pregnancies n (%)	1 (0.4)	4 (1.8)

[a] $P = 0.012$.

with or without continuation of the GnRHa for 2 weeks in the luteal phase.

One merit of the study is that the study population consisted of consecutive infertile patients treated by the long GnRHa protocol. Although clinical pregnancy and ongoing pregnancy rates were slightly higher in the GnRHa group compared with the controls, the observed differences were short of reaching statistical significance. The study was powered to detect a difference of 50% between the two arms in clinical pregnancy rate, hence, there is a probability of an improved clinical pregnancy rate not being detected by this power.

On the basis of our results, it is possible that administration of GnRHa in the luteal phase had no benefit because, in these cycles, the GnRH receptor in the endometrium is already saturated, whereas adding GnRHa to a GnRH antagonist cycle may show different results.

Our power calculation used an overly optimistic improvement in clinical pregnancy outcome (50%). This likely resulted in the study being underpowered. The clinical pregnancy rates in both groupts were 36.2% versus 30.6%. This difference many be clinically relevant but it would take a much larger study to determine statistical significance.

Several studies have reached similar conclusions to our study – that administration of GnRHa in luteal phase does not improve pregnancy rate (Ata et al., 2008). In a large randomized, double-blind study by Ata et al. (2008) one dose of GnRHa was injected 6 days after embryo transfer. No difference was found in pregnancy rate after GnRHa administration. It may be argued that delaying the GnRHa injection to day 6 in the luteal phase may be the reason for not improving the pregnancy rate. In another randomized study in IVF cycles with the long GnRHa protocol, three additional injections of 0.1 mg GnRHa on day 6 after embryo transfer did not affect the pregnancy rate (Inamdar and Majumdar, 2012). In a retrospective study Geber and Sampaio (2013) studied the effect of duration of GnRHa in the luteal phase on the outcome of assisted reproduction technique cycles, and found that duration did not affect the pregnancy rate.

The continuous administration of the GnRHa in the luteal phase will continue the down-regulated state of the GnRH receptors in the reproductive organs, which may cause ineffectiveness of GnRHa in improving the pregnancy rate (Inamdar and Majumdar, 2012). Although molecular studies have suggested a direct effect of GnRHa on endometrial function, research failed to show any clinical relevant effect (Fauser and Devroey, 2003; Takeuchi et al., 1998).

On the other hand, our study did not confirm the value of administration of GnRHa plus progesterone in the luteal phase suggested by earlier studies (Cedrin-Durnerin et al., 2000; Tesarik et al., 2004, 2006). Our study differs from previous studies because GnRHa administration continued for the whole luteal phase by daily injection of 0.1 GnRHa compared with the much shorter duration of GnRHa injection in previous studies. The continuation of daily injection of GnRHa from day 1 of the luteal phase avoided interruption of its effect if it is given from day 6 of the luteal phase (Ata et al., 2008).

Several studies have reported a positive effect of administration of GnRHa in luteal phase. Tesarik et al. (2006), in a prospective randomized study, found a higher implantation rate and live birth rate after a single dose of 0.1 mg of GnRHa was administered 3 days after embryo transfer. The pregnancy rate, however, was not significantly improved. In a previous study, the same investigators found that GnRHa administration in the luteal phase improved implantation rate in egg donation model (Tesarik et al., 2004). Fujii et al. (2001) in a quasi-randomized study compared continuous administration of GnRH a after HCG injection until day of embryo transfer with cessation of GnRHa 1 day before HCG injection, and found that administration of GnRHa in the luteal phase improved implantation, pregnancy rate and live birth rate. The investigators found it difficult to assume that the altered luteal function or endometrial receptivity might be the reason why GnRHa continuation resulted in a higher implantation rate.

Various studies have attempted to explain the positive effect of administering GnRHa in the luteal phase. It was suggested that interruption of GnRHa administration is followed by an abrupt fall in the serum concentration of the alpha-subunit of gonadotrophin (Oppenheimer et al., 1992). Recovery of hypophyseal synthesis is usually observed within about a week (Winslow et al., 1992). If GnRHa administration was interrupted early in the follicular phase, a profound LH suppression after stopping GnRHa lessens the ovarian response to FSH and increases the cancellation rate (Cedrin-Durnerin et al., 2000; Fujii et al., 1997).

The biological mechanism of action for an improved pregnancy rate could be a direct effect on the embryo or on the endometrium, as the GnRHa was added to a long-luteal phase protocol in this study with a downregulated GnRH receptor status in the pituitary and no possible LH effect. Data in humans for such an effect is limited. Reports have suggested that GnRHa can enhance embryo development *in vitro*. This was demonstrated by adding GnRHa to the culture media *in vitro* (Nam et al., 2005; Raga et al., 1999). Although molecular studies have suggested a direct effect of GnRHa on endometrial function, research has failed to show any clinically relevant effect (Fauser and Devroey, 2003).

A possible explanation for the promotion of implantation in the GnRHa arm might be the direct effect of GnRHa as the regulator of embryo–endometrial interactions and the facilitator of embryonic development (Fujii et al., 2001).

It was repeatedly documented that luteal phase is inefficient after embryo transfer in cycle stimulated by the long GnRHa protocol as well as GnRH antagonist protocol (Beckers et al., 2003; Macklon and Fauser, 2000). This is possibly due to the luteolytic effect of GnRHa in humans during ovarian stimulation (Casper and Yen, 1979; Lemay et al., 1992; Sheehan et al., 1982).

The meta-analaysis, conducted by Kyrou et al. (2011) showed a positive effect of GnRHa on improving clinical pregnancy rate and live birth rate. The study by Fujii et al. (2001), however, was included, which was quasi-randomized. Also, when live birth rate was calculated, a total analysis was carried out for both agonist and antagonist protocols, mixing two different regimens.

The fact that not a single centre out of the 10 centres surveyed was using GnRHa for support of luteal phase suggests a lack of confidence that administration of GnRHa in the luteal phase improves the pregnancy rate after IVF.

In conclusion, continuous daily administration of GnRHa in the luteal phase in IVF cycles did not improve pregnancy rate in long GnRHa protocol. Large studies and a

meta-analysis are required to establish the role of GnRHa in the luteal phase.

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