Is Late Preparation with Gonadotropin-releasing Hormone (GnRh) Agonist Injection on Day-1 of Hormonal Replacement Cycle improves pregnancy rate after Frozen-thawed Embryo Transfer?

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Abstract
Objectives: To evaluate effect of changing time of gonadotropin-releasing hormone agonist (GnRH-a) injection on outcome of infertile women with polycystic ovary syndrome (PCOS) and scheduled for HRT frozen embryo transfer (HRT-FET) cycles.

Patients & Methods: Out of PCOS women who underwent ICSI and embryo cryopreservation, to guard against ovarian hyperstimulation syndrome, 164 women underwent HRT-FET. These women were randomly divided according to timing of GnRH-a triptoreline (Decapeptyl depot; 3.75 mg) subcutaneous injection into Control received injection on day-21 of menstrual cycle preceding ET cycle or Study women receive injection of day-1 of menses of ET cycle. All women received estradiol valerate (2 mg/day) on day-2 of menses of ET cycle with incremental increase till endometrial thickness was 8 mm. Intravaginal progesterone was given for 2-days before ET and continued thereafter. Chemical pregnancy was diagnosed 14 day after ET and clinical pregnancy was assured by TVU 2-weeks later. In case of pregnancy, progesterone was continued till week 10. Study outcomes included the clinical pregnancy rate (CPR) and the 12-wk pregnancy loss rate.

Results: Collectively, 90 women got pregnant for a CPR of 54.9% per woman; 35 control and 55 study women for CPR of 42.7% versus 67.1% per woman with a significantly higher CPR per woman (p=0.00013) in study group. The 12-wk pregnancy loss rate was non-significantly (p=0.647) higher among control women. The frequency of women completed their 12-wk follow-up without actual or threatened abortion was significantly (p=0.005) higher in study group.

Conclusion: Injecting depot GnRH-a with HRT is appropriate protocol for FET in infertile PCOS women with irregular menstruation, giving collective CPR of 54.9%. Injection of depot GnRH-a on day-1 of menses of transfer cycle improved outcome and increased the CPR by >50% than injection on day-21 of previous menses.

Keyword: PCOS infertility, GnRH agonist, Time of injection, Clinical pregnancy rate

Introduction
Polycystic ovary syndrome (PCOS) is a complex disease characterized by ovarian dysfunction and polycystic ovarian morphology (1) in association with various endocrine disorders that are the potential cause of anovulation and hyperandrogenism (2). The underlying pathogenesis for PCOS is still a matter of debate; recently, it was suggested that adropin may have a probable role in the pathophysiology of PCOS (3) and single nucleotide polymorphisms in the fat mass and obesity-associated gene are found to be associated with PCOS susceptibility with women having risk alleles have less ovulation numbers (4).

Systematic review and meta-analysis of protocols used to prepare endometrium for frozen embryo transfer (FET) cycles including true or modified natural cycle (5), artificial cycle with or without suppression (6) and mild ovarian stimulation with gonadotropin (7) or aromatase inhibitor (AI) showed no statistically significant difference for both clinical pregnancy rate (CPR) and live birth (8).

Ovarian hyperstimulation syndrome (OHSS) is one of the most important complications of ovarian stimulation (9) with considerable morbidity and a small risk of mortality (10) and is still a threat to every patient undergoing ovulation induction (9). PCOS causes a significantly increased risk of OHSS (11). High ovarian response manifested by high number of large follicles, high estradiol concentration or high
number of retrieved oocytes is the best method of predicting the occurrence of OHSS (12).

Currently, there is no good test or method to identify patients susceptible to OHSS, so progress has been made in its prevention (13). There is high-quality evidence that replacing human chorionic gonadotropin (hCG) by gonadotropin-releasing hormone (GnRH) agonists triggering after GnRH antagonists reduce the occurrence of OHSS (14) but did not eliminate it completely (15).

However, Atkinson et al. (16) documented that GnRH agonist trigger and a freeze-all approach prevents OHSS with a good pregnancy rate. Moreover, Zech et al. (17) reported that elective cryopreservation and consecutive frozen-thawed embryo transfer (FET) for a woman at risk for OHSS is safe and shows excellent cumulative live birth rates.

In HRT-FET cycles, the administration of estrogen and progesterone could not guarantee pituitary suppression sufficient to prevent development of a dominant follicle that may undergo spontaneous luteinization thus exposing the endometrium to progesterone earlier than required and so inducing bias between endometrial exposure to progesterone and ET (18). On contrary, GnRH-agonist co-administration may assure full pituitary down-regulation and prevention of follicular growth thus obviating such bias (19).

Hypothesis
The hypothesis of the current study was improvement of PCOS women scheduled for HRT-FET cycles on changing time of GnRH-a (Decapeptyl depot, 3.75) injection to day-1 of menses of replacement cycle versus injection on the 21st day of menstrual cycle preceding the replacement cycle.

Setting
Benha and Tanta University and Insurance hospitals, Egypt

Design
Multi-center randomized controlled clinical trial

Patients & Methods
The study protocol was approved by the Local Ethical Committees and all patients signed written fully informed consent prior to study inclusion. The study intended to include >150 infertile PCOS women with irregular and anovulatory menstrual cycle who underwent ICSI and embryo cryopreservation to guard against ovarian hyperstimulation syndrome and will be subjected to HRT-FET. Inclusion criteria included PCOS women underwent oocyte retrieval and IVF for preparing frozen embryos. Exclusion criteria included women had regular and ovulatory menstrual cycle, underwent fresh cycles, presence of less than 9 embryos freeze in a state of 2PN, refusal of study participation or to sign written consent to undergo three transfer cycles.

Randomization & Grouping
Randomization into study groups was conducted using sealed envelops containing cards prepared by a blinded assistant, named either Study or Control and cards were chosen by patient herself. All patients received the same protocol apart from the timing of injection of GnRH-a triptoreline (Decapeptyl, Ferring Pharmaceuticals Ltd., Wittland, Germany; 3.75 mg, subcutaneous injection). GnRH-a injection was received on the day-21 of the menstrual cycle preceding the replacement cycle in control group or day-1 of menses of the replacement cycle in study group.
Study protocol

The classic Testart slow freezing and rapid thawing protocol \(^{20,21}\) was applied using a programmable freezer (Planer; Middlesex, UK) and embryo freezing and thawing kits (Irvine Scientific, Santa Ana, CA, USA) were used. After thawing, all embryos were transferred to culture in vitro for 2 days. Embryos were assessed for cell number and morphology and presence of cellular debris \(^{22}\). On the third day, embryos in G1 and G2 grade were defined as good quality embryos and 1-3 embryos of good quality were transferred, while poorer grade embryos were discarded \(^{23}\).

In both groups, estradiol valerate (Progynova, 2 mg, Bayer Schering Pharma, UK) was started on day-2 of menses of replacement cycle as a daily dose of 2 mg and incremental doses were provided till endometrial thickness was 8 mm. Endometrial thickness was measured in the midsagittal plane using transvaginal ultrasound (Sonoline Prima 7.5 MHz, Siemens). Measurements were made from the outer edge of the endometrial/myometrial interface to the outer edge in the widest part of the endometrium.

Then, intravaginal progesterone (Crinone 8%, progesterone vaginal gel, Merck serono, UK, once daily) was given for two days before embryos transfer and continued thereafter. Chemical pregnancy was diagnosed by measurement of β-human chorionic gonadotropin level on the 14th day after ET and was confirmed 2-weeks later by TVU to assure clinical pregnancy. In case of pregnancy, the progesterone treatment was continued up to pregnancy week 10. All women were follow-up till the 12th wk gestation and the frequency of pregnancy loss was determined.

Study outcome

1. Primary outcome was defined as the clinical pregnancy rate (CPR).
2. Secondary outcome was the 12-wk pregnancy loss rate.

Statistical analysis

Obtained data were presented as numbers and percentages. Results were analyzed using Chi-square test (\(X^2\) test). Statistical analysis was conducted using the IBM SPSS (Version 23, 2015) for Windows statistical package. P value >0.05 was considered statistically significant.

Results

The study included 191 PCOS women; 27 were excluded and 164 women were equally divided into the two study groups. The included women previously gave a mean number of retrieved oocytes of 17.9 oocytes. ICSI was performed for all retrieved mature oocytes and resulted in 2736 frozen embryos. At time of transfer, 2401 embryos survived and were transferred for a rate of 14.6 frozen embryos/woman. There were 1809 (75.3%) G1 embryos and 592 (24.7%) were G2 embryo.

All women underwent the 1st cycle and 46 women developed clinical pregnancy for a total CPR for 1st cycle of 28%; only 16 women of control group had clinical pregnancy for a CPR of 19.5%, while 30 women of the study group had clinical pregnancy for a CPR of 36.6% with significantly higher CPR in favor of study group. One hundred and eighteen women underwent the 2nd cycle and 31 women had clinical pregnancy for a total CPR for the 2nd cycle of 26.3%. Fourteen of control group women had clinical pregnancy for CPR of 21.2%, while 17 of study group women had clinical pregnancy for CPR of 32.7% of women underwent the 2nd cycle. Eighty-seven women had a third cycle, but only 13 women got pregnant for a
total CPR of 14.9%; 5 control and 8 study women got pregnant for CPR of 9.6% and 22.9%, respectively.

Collectively, 90 women got pregnant for a CPR of 54.9% per woman; 35 control women got pregnant for a CPR of 42.7% per woman and 55 study women got pregnant group for a CPR of 67.1% per woman with a significantly higher CPR per patient (p=0.00013) in study women compared to control women (Fig 2).

Throughout 12-wk follow up period 22 women had pregnancy loss; 12 control and 10 study women for a collective rate of 13.4% per women participated in the study and of 24.4% per women got pregnant with non-significantly (p=0.647) higher pregnancy loss rate among women of control group versus those of study group. On the other hand, 68 women completed their 12-wk follow-up period without actual or threatened abortion; 23 control and 45 study women with significantly (p=0.005) higher frequency of women completed pregnancy beyond 12-wk duration in favor of study group.

Fig. (2): Clinical pregnancy rate (CPR) for women of both groups at the end of the third cycle

Fig. (1): Study outcome flow chart
Discussion

The current selective study included only infertile PCOS women for being frequently encountered in infertility clinics and for their ability to give high number of oocytes on controlled ovarian hyperstimulation (COH), so could accommodate the target of the trial. The enrolled women gave a mean oocyte number of 17.9 oocyte per woman resulted in a mean number of frozen embryos of 14.6 per woman. In line with these data, Timur et al. (24) and Zhu et al. (25) reported a mean number of retrieved oocytes of about 14 and 13 oocytes per PCOS woman, respectively. Thereafter, Lai et al. (26) detected higher number of retrieved oocytes in PCOS versus tubal factor infertility women and Tannus et al. (27) found infertile PCOS women higher number of retrieved oocytes (16.6 vs. 10.4) and cycles with embryo cryopreservation (47 vs. 22.9%) compared to women with tubal factor infertility.

Moreover, inclusion of PCOS women with anovulatory cycles allowed the artificial endometrial preparation and excluding women had ovulatory cycles depending on the previously documented by Abo Ragab (28) that FET natural cycle is an appropriate protocol for management of infertile women free of endocrinial underlying cause and having a regular and ovulatory menstrual cycle. Furthermore, depending on these previous results (28) women asked for fresh cycle ET were excluded and all women had FET to guard against development of ovarian hyperstimulation syndrome (OHSS) and to get benefit of using the high number of retrieved oocytes in multiple cycles of transport.

In support of this policy, Li et al. (29) reported higher rates of withholding fresh ET for risk of OHSS that was accounted for 11.5 % in PCOS versus 4.9% in controls. Also, Evans et al. (30) documented that FET reduces the risk of OHSS and improves outcomes for both the mother and baby especially in women with reduced endometrial receptivity or vulnerable to development of OHSS.

Considering the high number of retrieved oocyte, the current study followed the 'Freeze all' method for all enrolled women for the possibility of having high number of retrieved oocytes (31), this allowed getting high number of frozen embryos with subsequent repeating ET cycles for a total cycles of 369 cycles giving a rate of 2.25 cycles per woman. In support of this policy, Toftager et al. (32) documented that the more retrieved oocytes, the significantly higher cumulative live birth rates, irrespective of the triggering protocol.

The hypothesis of the current study was improvement of HRT-FET outcome as judged by CPR on changing time of GnRH-a (Decapeptyl depot, 3.75) injection to day-1 of menses of replacement cycle (Study group) versus injection on the 21th day of menstrual cycle proceeding the replacement cycle (Control group). The obtained results considerably approved this hypothesis, where 55 study and 35 control women got pregnant for CPR of 67.1% and 42.7% that was significantly higher in study versus control women.

Multiple trials used GnRH-a for LPS mostly on the 6th to 7th day after fresh ET and reported improved outcome; wherein, Kung et al. (33) reported a significantly higher CPR and live birth rate with GnRH-a (single dose of 0.1 mg decapeptyl) given 6 days after ICSI as additional LPS compared to control group. Another trial was conducted by Yıldız et al. (34) who tried leuprolide acetate (1 mg s.c. injection) on day-3 or on days-3 and 6 after ET versus control group and reported higher CPR, despite showing non-significant difference, of 36%, 42.9% and 27.4%, in the three groups respectively. On contrary, Martins et al. (35) searched for randomized controlled trials comparing luteal phase injection of GnRH-a versus the standard LPS
and concluded that there is evidence of very low quality that luteal phase GnRH-a improves the likelihood of ongoing pregnancy.

On the other hand, the reported CPR of the study group (67.1%) superseded that of the control group and of these studies; such outcome could be attributed to the timing of GnRH-a injection on the 1st day of menses taking the advantages of its pharmacokinetics and dynamics, where Han et al. (36) experimentally tried to characterize the pharmacokinetics and pharmacodynamics of slow-release 28-day form of triptorelin and documented that triptorelin acts as a potent inhibitor of gonadotropin secretion, initially producing a transient flare-up in LH and FSH within 5 days after injection followed by desensitization of pituitary LH-releasing hormone receptors and inhibits LH and FSH secretion, resulting in chemical castration till the next dose.

Thus, this transient rise of LH and FSH in women of study group coincided with the duration of menses and thereafter inhibition of LH release was started and continued for 3-4 weeks. Such prolonged LH inhibition with early E2 support gave a miniature of physiological ovulation with maintained action of corpus luteum, then the scenario was completed by progesterone LPS, thus giving the endometrium the full chance to be prepared for the oncoming pregnancy improving its receptivity with subsequent improved implantation of the transferred embryo.

In support of this assumption, Yang et al. (37) found hormonally controlled endometrial preparation with prior GnRH-a suppression may increase endometrial receptivity in women who had experienced repeated failures of IVF treatment despite having morphologically optimal embryos. Also, Zhou et al. (38) detected significantly higher expression amount of pinopodes in women received GnRH-a once on day-7 after ovulation compared to before treatment and to placebo-controlled group and the percentage of maturation of pinopodes was significantly higher than in control women, and so concluded that GnRH-a in luteal phase may promote the growth of pinopodes, and improve the endometrial receptivity.

**Conclusion**

The applied protocol of injecting depot GnRH-a with HRT, irrespective of timing of injection, is appropriate protocol for FET in infertile PCOS women with irregular menstruation giving a collective CPR of 54.9%. Injection of depot GnRH-a on the day-1 of menses of the transfer cycle improved outcome of ART in infertile PCOS women and increased the chance of getting clinical pregnancy by >50% to approach 67.1% versus 42.9% with injection of day-21 of the previous menses. However, wider scale studies are mandatory to establish the outcome of this protocol.

**References**


