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Randomized controlled trial comparing pregnancy outcome using artificial versus letrozole stimulated cycle in cryo preserved embryo transfer

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ABSTRACT

Background: Intracytoplasmic sperm injection (ICSI) is a procedure performed in the IVF embryo laboratory after the eggs have been retrieved from the ovaries and involves placing a single sperm directly inside the egg. It is indicated for cases which involve very low sperm counts or motility. **Aim of the Work:** To compare the outcome of pregnancy using artificial and letrozol stimulated cycle in cryo preserved embryo transfer. **Patients and Methods:** This randomized controlled trial was conducted on 190 patients attending the clinic submitted for embryo transfer of cryo embryos. All cycles were performed at international Islamic Center for population Studies and Research Assisted Reproduction Unit (ART unit IICPSR) at Al-Azhar University and evidence based centers. **Results:** Pregnancy rate was significantly higher in letrozole (53.2%) compared to the artificial group (40.6%). Furthermore, first trimester abortion rate was non-significantly lower in letrozole group. **Conclusion:** ovulation induction with letrozole during endometrial preparation for cryopreserved ET has a significantly higher ongoing pregnancy rate than artificial cycle. The reported ongoing pregnancy rate in letrozole arm was encouraging and may provide an alternative mean to artificial cycles in endometrial preparation in FET.

Key words: Pregnancy outcome, letrozole, cryopreserved embryo transfer

INTRODUCTION

Intracytoplasmic Sperm Injection (ICSI) is a procedure performed in the IVF embryo laboratory after the eggs have been retrieved from the ovaries and involves placing a single sperm directly inside the egg. It was indicated in cases with very low sperm counts or motility [(1)].It may also be considered for cases of previous failed fertilization cycles by conventional IVF, or in eggs of women of late maternal age [(2)].

Frozen–thawed embryo transfer (FET) enables the excess embryos generated by IVF andICSI to be stored and utilized later. This can increase the cumulative pregnancy rate of IVF/ICSI treatment[(3)].In contrast to the complex stimulation protocols employed to stimulate multiple follicular growth for IVF, FET are protocols simpler, with the primary aim limited to adequate preparation of the endometrium to receive the thawed transferred embryo[(4)].

Furthermore, FET results in similar live birth rates to fresh cycles when frozen top quality embryos are transferred [(5)].

Hormone replacement cycle with or without GnRh agonists is usually recommended for older women, woman without ovaries or non-functioning ovaries, women with irregular infrequent menstrual cycles or ovulation[(6)].

Different IVF clinics have different protocols for giving these medications and in some women GnRh agonists may be given in addition to hormonereplacement to suppress any hormone production by the ovaries which may interfere with the treatment[(7)].

Stimulated cycle is where fertility drugs such as gonadotropin injection is usually recommended for women who do not ovulate regularly and did not respond to hormone replacement treatment in a previous cycle. There are insufficient evidences to recommend any one particular protocol for endometrial preparation over another with regard to pregnancy rates after embryo[(8)].

This study designed to determine whether there is a difference in pregnancy outcome between artificial cycle FET and using letrozole.

In artificial cycle FET (AC-FET), estrogen and progesterone were administered in a sequential regimen which aimed to mimic the endocrine exposure of the endometrium in the normal cycle. Initially, estradiol was given in order to cause proliferation of the endometrium, during the development of the dominant follicle [(9)].

The timing of embryo thawing and transfer was planned according to the moment of progesterone supplementation [(10)].

Letrozole, a selective aromatase inhibitor, prevents the conversion of androgens to estrogen in the granulosa cells, thus, releasing the hypothalamo-pituitary axis from the negative feedback of estrogen, resulting in an increase of FSH secretion from the anterior pituitary. In addition, the accumulated androgens in the ovary further increase follicular sensitivity to FSH [(11)].

Letrozole has a relatively short half-life (±2 days) compared with CC (±2 weeks), so estrogen target tissues (e.g., endometrium and cervix) spared anti-estrogenic adverse effects. Because of these

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mechanisms, it was postulated that Letrozole may have superior ovulation induction properties in terms of follicular growth and endometrium development, which is important for embryo implantation [(12)].

AIM OF THE WORK

To compare the outcome of pregnancy using artificial and letrozol stimulated cycle in cryo preserved embryo transfer.

PATIENTS AND METHODS

Study design: Randomized controlled trial

Study setting: All cycles were performed at international Islamic Center for population Studies and Research Assisted Reproduction Unit(ART unit IICPSR) at AL- AZHAR University and evidence based centers. **The study was approved by the Ethics Board of Al-Azhar University.**

Study population: The study included190 patients attending the clinic submitted for embryo transfer of cryoembryos.

Ethical points: A verbal and written consent was obtained from each patient. All patients' information was kept private. The study included 190 patients attending the clinic submitted for embryo transfer of cryo embryos.

Patients were selected to be included in the study according to the following criteria:

Inclusion criteria: Any ICSI case except PCO patients. Included women were followed up after randomization into 2 groups.

Group 1: Artificial cycle (96 patients), artificial hormonal preparation with estradiol valerate and progesterone was used.

Group 2: Letrozole cycle (94 patients), letrozole was used to stimulate ovulation and hence prepare the endometrium.

Study design

This study was performed as a clinical trial in which all participants were subjected to the followings: Explanation of procedure and informed consent for all women participating in the study. History: Menstrual history with determination ofmenarche, regularity of the cycle, history of endocrine diseases, abdominal or CNS operations. Physical examination: (General, Abdominal, Local) Radiological evaluation which includes the following: **H.S.G**. **Ultrasonography** Using a high-frequency 6.5 MHz trans vaginal transducer.

Endometrial thickness: The endometrial thickness was measured in the sagittal plane. The distance from the hyper- echogenic interface between the endometrium and the myometrium to the opposite interface including the Medline echo (endometrial interface)

Protocol of ovulation induction:

Group 1: Artificial cycle: Ultrasound evaluation of the patients was performed to confirm ovarian quiescence then estradiol valerate (propagnova) 2 mg three times daily was started from day 2 or 3 of the for endometrial preparation. The patients were evaluated after 9–10 days, by TVS, when the endometrium reached a minimum thickness of 7 mm, progesterone was started vaginally. Vitrified–warmed ET was scheduled 3–5 days after starting progesterone. If the endometrium had not reached a thickness of 7 mm, the estradiol valerate was continued for another 5–7 days with increasing the dose to 4 mg twice daily. If the endometrium did not reach 7 mm thickness after 21 days of estradiol supplementation the cycle was canceled. The same doses of estrogen and progesterone were continued until 14 days after vitrified–warmed ET when a serum hCG level was measured. If the pregnancy test was positive, the estradiol was continued till 8 weeks and progesterone till the end of first trimester.

Group 2: Letrozole cycle: Letrozole was used to induce follicular growth and, hence, endometrial preparation by natural hormones derived from the growing follicles. Following initial TVS (to exclude ovarian cysts). Letrozole (Femara) 2.5 mg/day was started from day 3 of the menstrual cycle and continued for five consecutive days. Ultrasonography (TVS) folliculometry and endometrial evaluation was started on the 8thday of the cycle and follow up was scheduled according to the response. When the follicle reached 17 mm and the endometrium 7 mm, hCG was given in a dose of 5.000 IU to trigger ovulation. Progesterone was started. Vitrified ET was scheduled 3–5 days after starting progesterone (according to the day of embryos vitrification). Vaginal progesterone supplementation was continued until 14 days after vitrified–warmed ET when a serum hCG level was measured. If the pregnancy test was positive, progesterone supplementation was continued till the end of first trimester.

Outcome measures: The outcome is the pregnancy rate.

Statistical analysis:

Methods of randomization: Randomization was performed using a list of computer generated programs.

Sample size justification: The required sample has been calculated using IBM Statistical Package for Social Science software (SPSS statistics version 19.0). Descriptive statistical analyses were performed for each variable; quantitative results were presented as the mean ± SD.

Proportions for the two groups were compared using the x2 test. $P < 0.05$ level was considered to indicate the statistical significant difference between the variables.

The results were considered: Non-significant when the probability of error is more than 5% ($p > 0.05$). Significant when the probability of error is less than 5% ($p \leq 0.05$). Highly significant when the probability of error is less than 0.1% ($p \leq 0.001$). The smaller the p-value obtained, the more significant difference between the results.

RESULTS

This work was a randomized controlled study comparing the results of two different protocols of endometrial preparation for embryo transfer in cryopreserved cycles.

In the first protocol (96 patients), artificial hormonal preparation with estradiol valerate and progesterone was used while in the second one (94 patients), letrozole was used to stimulate ovulation and hence prepare the endometrium.

As shown in Table 1, there were no significant differences in the demographic and clinical characteristics (as age, type and cause and duration of infertility) of both groups.

Table (1): Patients clinical characteristics.

Characteristics of patients	Artificial (n = 96)	Letrozole (n = 94)	P value
Age (Y)	27.1 ± 4.2	27.7 ± 4.2	0.31
Type of infertility			
Primary	82 (85.4%)	84 (89.4%)	0.41
Secondary	14 (14.6%)	10 (10.6%)	
Duration of infertility (Y)	5.7 ± 3.9	6.2 ± 3.9	0.38
Cause of infertility			
Male	59 (61.5%)	67 (71.3%)	0.15
Female	15 (15.6%)	12 (12.8%)	0.57
Male and female	13 (13.5%)	11 (11.7%)	0.7
Unexplained	9 (9.4%)	4 (4.3%)	0.16

Higher endometrial thickness was reported in the artificial group (9.9 mm ± 1.7) compared to letrozole group (9.1 mm ± 1.6), otherwise, no significant differences between both groups were found regarding the number and quality of embryos at vitrification, warming or embryo transfer.

Table (3): Characteristics of cryo preserved cycle.

Characteristics of cryopreservedcycle	Artificial (n = 96)	Letrozole (n = 94)	P value
No of vitrified embryos	4.1 ± 2.2	4.1 ± 1.7	0.86
No of good quality embryos at vitrification	3.6 ± 2.4	3.5 ± 1.7	0.83
No of fair quality embryos at vitrification	1.8 ± 1.7	1.7 ± 0.8	0.69
No of good quality embryos at warming	2.5 ± 1.1	2.3 ± 0.9	0.18
No of fair quality embryos at warming	1.6 ± 0.9	1.6 ± 0.8	0.81
No of transferred embryos	2.4 ± 0.7	2.4 ± 0.8	0.98
No of good quality embryos transferred	2.1 ± 0.8	2.1 ± 0.8	0.82
No of fair quality embryos transferred	1.3 ± 0.7	1.5 ± 0.7	0.27
Endometrial thickness (mm)	9.9 ± 1.7	9.1 ± 1.6	0.001

Pregnancy rate was significantly higher in letrozole (53.2%) compared to artificial group (40.6%). Furthermore, first trimester abortion rate was non-significantly lower in letrozole group (Table 3).

Table (4): Clinical outcome following cryo preserved ET.

Outcome of v–w cycles	Artificial (n = 96)	Letrozole (n = 94)	P value
Clinical pregnancy	39 (40.6%)	50 (53.2%)	0.08
Ongoing pregnancy	31 (32.3%)	45 (47.9%)	0.021
Abortions (first trimester)	8 (8.3%)	5 (5.3%)	0.41

However, the ongoing pregnancy rate was significantly higher in the Letrozole group (47.9%) in comparison with the artificial group (32.3%) (P value 0.02) (Table 4).

DISCUSSION

The results of the present study showed better vitrified–warmed ET outcomes in letrozole group, even though the women in this group had a significantly lower endometrial thickness than those in artificial one.

As most confounding variables (women's baseline characteristics) were not significantly different between both groups, differences in the outcome could be attributed to the difference in endometrial preparation's protocol.

We reported a significantly lower endometrial thickness in letrozole group (9.1 ± 1.6 mm) compared to artificial group (9.9 ± 1.7 mm). This can be attributed to direct aromatase antagonistic action of letrozole which blocks the conversion of androgen to estrogen with a subsequent reduced serum estrogen and endometrial thickness.

However, this reduction of endometrial thickness did not adversely affect pregnancy or ongoing pregnancy rates. This can possibly explained by better endometrial receptivity in letrozole group compared to artificial one.

The improved receptivity in letrozole group probably attributed to increased endometrial VEGF with subsequent improved vascularization.

Additionally, there have been reports that letrozole increases integrins expression in surface epithelium and glandular epithelium. The localization of β1 and β4 integrins to apical pole of surface epithelium has role in initial embryo and endometrium interaction .

Letrozole induces moderate ovarian stimulation with E2 levels similar to that of normal cycles. However, higher midluteal progesterone level was reported due to the developing corpora lutea. Interestingly, endometrial morphology during the implantation window of letrozole treated cycles was characterized by in-phase histological dating according to Noyes criteria.

The results of this study are in agreement with those of **Son and colleagues** [(13)]who reported that endometrial thickness alone is not an effective predictor of pregnancy outcome in frozen-thawed embryo transfer cycles.

In the current study, there were clinically, but not statistically, higher pregnancy rate (53.2% vs. 40.6%) and a lower miscarriage rate (5.3% vs. 8.3%) in letrozole group compared to artificial group. However, there was a statistically higher ongoing pregnancy rate in letrozole group compared to artificial group (47.9% vs. 32.3%).

This can justify by better endometrial receptivity in letrozole group (mentioned above). In addition, letrozole ovarian stimulation would result in the development of corpus luteum (may be absent in artificial cycle due to hypothalamo-pituitary downregulation by E2 and P) which supports luteal phase and first trimester of pregnancy. This, further, can clarify a non-significant lower abortion rate in letrozole arm reported in our study.

On the other hand, in artificial cycles, the administration of estrogen and progesterone does not guarantee complete pituitary suppression, and a dominant follicle may therefore occur. Should the follicle undergo spontaneous luteinization then the endometrium may be exposed to progesterone earlier, risking incorrect timing of thawing and transferring and subsequent implantation and pregnancy rates.

Holzer et al. [(11)]reported that a new era in ovulation induction showed that ovulation induction with letrozole is associated with an ovulation rate of 70%–84% and a pregnancy rate of 20%–27% per cycle. In a study, it was found that ovulation and pregnancy rates with letrozole seemed to be higher than those of anastrazole. In super ovulation, letrozole is associated with few developing follicles and thick endometrium. The use of letrozole for superovulation is associated with a pregnancy rate higher than with the use of clomiphene citrate (CC) (16.7% vs. 5.6%). The addition of letrozole to FSH treatment led to a decreased FSH requirement. The pregnancy rate for treatment with letrozole and FSH was similar to that for FSH alone.

The present results showed that Aromatase inhibitors was as effective as or superior to CC in ovulation induction and in superovulation. Unlike CC, they do not carry an antiestrogenic effect on the endometrium. Given the advantages of aromatase inhibitors, they can be used to replace CC as ovulation-inducing drugs.

As study was carried out by ***Mitwally et al.***[(14)] to report the outcome of pregnancies achieved after ovarian stimulation, including the use of the aromatase inhibitor, letrozole, for ovarian stimulation.

A cohort study comparing the outcome of pregnancies achieved after letrozole and other ovarian stimulation treatments with a control group of pregnancies spontaneously conceived without ovarian stimulation.

In 3 tertiary referral centers, there were 394 pregnancy cycles in 345 infertile couples (63 pregnancies with 2.5 mg of letrozole alone or with gonadotropins, 70 pregnancies with 5.0 mg of letrozole, 113 pregnancies with clomiphene alone or

with gonadotropins, 110 pregnancies with gonadotropins alone, and 38 pregnancies achieved without ovarian stimulation). Pregnancies conceived after letrozole treatments were associated with similar miscarriage and ectopic pregnancy rates compared with all other groups. In addition, letrozole use was associated with a significantly lower rate of multiple gestations compared with clomiphene citrate.

The favorable pregnancy outcome and low multiple gestation rates of aromatase inhibitors for ovarian stimulation were encouraging for the development of these agents as first-line ovulation induction agents.

Givens *et al.*[15]compared the outcomes for patients with frozen embryos who had frozen–thawed embryo transfer (FET) timed to their natural ovulation cycle versus cycles in which endometrial timing was programmed with estrogen and progesterone. A total of 1205 patients undergoing 1677 FET cycles between 1 January 2000 and 31 December 2006 were analysed. Comparisons were made for patients undergoing modified natural versus programmed FET cycles, as well as between patients using their own eggs for frozen embryos versus those using donor-egg-derived embryos. Clinical pregnancy (gestational sac on 7 week ultrasound) rates (CPR), as well as miscarriage rates, were significantly higher in programmed FET cycles in patients using their own eggs (106/262, 40.5% per embryo transfer, P = 0.015) However, there was not a difference in delivered pregnancies between cycle types in own egg patients (natural cycle delivery rate 245/862, 28.4%; programmed cycle delivery rate 77/262, 29.4%). Furthermore, CPR were not different in natural (38/129, 29.5%) versus programmed cycles (144/424, 34.0%) for ovum donor recipients, nor were delivered pregnancy rates different in natural (33/129, 25.6%) versus programmed cycles (114/424, 26.9%) for ovum donor recipients. In conclusion, there is no significant difference in delivery rates for FET in natural (278/991, 28.1%) versus programmed (191/686, 27.8%) cycles using both own embryos and donor-egg-derived embryos.

***Weissman et al.*[16]**on spontaneous ovulation during a natural menstrual cycle represents a simple and efficient method for synchronization between frozen embryos and the endometrium. The objective was to compare serial monitoring until documentation of ovulation, with human chorionic gonadotrophin (HCG) triggering, for timing frozen embryo transfer (FET) in natural cycles (NC). In a retrospective study, 112 women with regular menstrual cycles undergoing 132 NC–FET cycles were divided into two groups: group A (n = 61) patients had FET in an NC after ovulation triggering with HCG; group B (n = 71) patients had FET in an NC after spontaneous ovulation was detected. The main outcome measure was the number of monitoring visits at the clinic. Patients in both groups were similar in terms of demographic characteristics and reproductive history. Clinical and laboratory characteristics of fresh and frozen cycles were also found comparable for both groups, as were pregnancy and delivery rates. The number of monitoring visits in group A (3.46 ± 1.8) was significantly lower than in group B (4.35 ± 1.4) (P < 0.0001). In patients undergoing NC–FET, triggering ovulation by HCG can significantly reduce the number of visits necessary for cycle monitoring without an adverse effect on cycle outcome. Ovulation triggering can increase both patient convenience and cycle cost-effectiveness.

CONCLUSION

In the presented study, we have found that ovulation induction with letrozole during endometrial preparation for cryopreserved ET has a significantly higher ongoing pregnancy rate than artificial cycle. A major advantage of this study is that it is a randomized study whiles all previous studies were retrospective studies.

RECOMMENDATIONS

Larger Randomized controlled trials would be needed to confirm or refute the validity of findings collected in this study. However, the reported ongoing pregnancy rate in letrozole arm is encouraging and may provide an alternative mean to artificial cycles in endometrial preparation in FET.

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