Letrozole versus HRT in frozen-thawed embryo transfer cycles

Original Article

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ABSTRACT

Background: A progressive increase in FET cycles was observed in recent years. Different endometrial preparation strategies have been described, however, there is still some controversy as to the ideal endometrial preparation protocol.
Objective: To evaluate the efficacy of letrozole in the endometrial preparation for frozen thawed embryo transfer cycles and to compare it to the HRT with estrogen and progesterone.
The Study Design : Retrospective study.
Patients and Methods: 320 cycles of endometrial preparation for frozen thawed embryo transfer were analyzed from 1st of January 2015 to 31st of June 2017, the recruited patients were divided into two groups: Group1 (n =160): Induction of ovulation group (letrozole group) and Group 2 (n = 160): Hormonal replacement therapy group (HRT group).
Results: the biochemical pregnancy rate was not significantly higher in the letrozole group 90 (56.3%) than in the HRT group 78 (48.8%) ; while the implantation rate, the clinical pregnancy rate and ongoing pregnancy rate were significantly higher in the induction group 22 (27.766), 81 (50.6%), 71 (44.4%) compared to HRT group 16.72 (24.096), 62 (38.8%), 44 (27.5%), respectively. First trimester abortion rate was significantly higher in the HRT group 19 (11.9%) compared to induction group 10 (6.3%).
Conclusion: The use of letrozole in patients undergoing FET was associated with significantly higher implantation rate, clinical pregnancy rate, ongoing pregnancy rate and a lower 1st trimesteric abortion rate, than use of HRT in frozen thawed embryo transfer cycles.

Key Words: Endometrial, frozen-thawed, HRT, letrozole

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INTRODUCTION

Kuwayama was the first to describe embryos vitrification[1]. The year 1983 showed the first successful pregnancy from a frozen-thawed embryo transfer (FET)[2].

The cumulative pregnancy rate of IVF/ICSI treatment is believed to be increased by the transfer of frozen thawed embryos that enables the excess embryos generated by IVF and ICSI to be stored and utilized at a later date[3].

A progressive increase in FET cycles was observed in recent years, due to limitations on the number of embryos to be transferred, also the use of agonist trigger in antagonist protocol and "freeze all" strategy in patients with high risk to develop OHSS, and transfer the embryos in subsequent FET cycle. FET is offered also in cases with late follicular progesterone rise, embryo-endometrial asynchrony, recently its use is extended to involve cycles with PGS or PGD.

Similar live birth rates in FET has been observed when comparing to fresh cycles when frozen top quality embryos are transferred[4].

In order to optimize pregnancy rates, adequate synchronization between the development of embryo and endometrium should be present. This can be achieved in various ways. Different endometrial preparation strategies have been described, however, there is still some controversy as to the ideal endometrial preparation protocol. They include a purely natural cycle (NC) with LH detection in blood or urine; a natural modified cycle (NMC) in which hCG is administered to schedule embryo transfer instead of measuring LH; hormone replacement therapy (HRT) or artificial cycle with estradiol (E2) and progesterone (P4), with or without using gonadotropin-releasing hormone (GnRH) analogs, and finally induction of ovulation cycle using Letrozole or low dose gonadotropins; Letrozole is a selective aromatase inhibitor, having a relatively short half-life +/-2 days compared to CC +/-2 weeks, so estrogen target tissues eg. Endometrium and cervix are spared antiestrogenic adverse effects. For this reason, letrozole is much more preferred over CC in induction of ovulation in endometrial preparation for FET, in addition letrozole results in mono ovulation and hence a reduced effect of the superphysiolosical levels of estrogen of multiple growing follicles on the endometrium and embryos[5]. Our

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objective was to compare the reproductive outcome of two protocols in endometrial preparation for frozen thawed embryo transfer (FET), the induction of ovulation protocol using Letrozole and HRT protocol using estrogen and progesterone.

PATIENTS AND METHODS

The present study was conducted in Kasr Alainy Hospital and private center in the period between the 1st of January 2015 and 31st June 2017. This study was approved by ethical committee and all patients signed an informed consent before the procedure.

The study included 320 patients with the following inclusion criteria, age (20-40), no more than previous two failed ICSI cycle, with ovulatory disorders, if after failed fresh cycle, she should have at least two menstrual cycles after OPU, endometrial thickness ≥ 8 but not more than 14 mm. Exclusion criteria , poor responders according to Bolognia criteria, stage 3,4 endometriosis, severe male factor, uterine factors (intrauterine synechiae), immunological disorders (antiphospholipid syndrome), failed dominant follicle development in induction of ovulation group, development of dominant follicle in HRT group and echogenic endometrium with thickness less than 8 mm and equals or more than 14 mm . The recruited patients were divided into two groups: Group 1: Induction of ovulation group (letrozole group). Group 2: Hormonal replacement treatment group (HRT group).

Endometrial preparation was done in the induction group as follow: letrozole 2.55-mg/day was given from D3 to D7 cycle, U/S started from D10 to monitor follicular development and endometrial thickness, when a DF 1822-mm is seen and endometrial thickness is ≥ 8mm (not > 14 mm) , 5000 IU HCG IM was given, the day of HCG corresponds to the day of HCG of the source cycle so progesterone vag.supp 400 mgw twice daily 24 hrs was started after HCG and FET was scheduled according to the day embryos where freezed. If freezed on D3, transfer was done 4 days after HCG, if freezed on D5 transfer was done 6 days after HCG. Luteal phase support was done by progesterone vag.supp 400 mg/twice daily and progesterone 100 mg IM injection daily and continued till 8 weeks of gestation if pregnancy occurs.

Endometrial preparation in the hormonal therapy group (HRT group) as follow: estrogen in the form of white tablets of cycloprogenova (estradiol valerate) was used for endometrial preparation in a dose of 2 mg/day from day 1 to day 5 cycle, 4 mg /day from day 6 to day10, 6 mg/day from day 11 to day15 cycle, GnRh is not given prior to preparation (as was classically described in Bourne hall protocol) consider increasing dose of gonadotrophin injections, U/S was done on D13 to evaluate the endometrial thickness once it is ≥8mm (not >14mm) start progesterone, consider increasing the dose, if less than 8 mm, progesterone was given in the form of vag.supp.400 mg twice per day, the 1st day of progesterone is considered the day of OPU of source cycle and accordingly FET was carried as follow if embryos freezed on D3, FET was carried on D4 of progesterone, if freezed on D5, FET was carried on D6 of progesterone. Luteal phase support by E and P must be continued if pregnancy occurs till 12 weeks gestation.

N.B: Cases with amenorrhea started after withdrawal bleeding using OCP or cycloprogenova or progesterone.

Warming of vitrified embryos was done on day of transfer (if freezed on day 5) or one day before transfer (if freezed on day 3). Embryos were transferred if at least more than 50% of their blastomeres were survived. Cancellation was done if there is no embryo survival after thawing.

Embryo transfer: embryo transfer was performed using a Cook catheter (K-SOFT-5000 Soft-Trans; Cook Ob/Gyn, Cork, Ireland) with transabdominal ultrasound guidance. Our institution protocol of the standardization of procedures was followed. Serum hCG assessment to detect pregnancy is performed at 14 days after embryo transfer if positive(biochemical pregnancy), women underwent trans-vaginal ultrasonography 2 weeks after, to confirm fetal pulsations as well as number of gestational sacs (clinical pregnancy), follow up of the gestational sac till 12 weeks' gestation was continued (ongoing pregnancy rate and 1st trimesteric abortion rate).

In this study, the primary outcome was the ongoing pregnancy rate, defined as at least one viable fetus beyond gestation week 12 by ultrasound. Secondary outcomes were implantation rate, biochemical pregnancy rate (defined as a positive pregnancy test), clinical pregnancy rate (defined as the presence of a gestational sac with an embryo with a positive heartbeat), and abortion rate (pregnancy loss until week 12 of gestation).

Sample size: sample size calculation was done using the comparison of ongoing pregnancy rate between cases treated with letrozole and those treated with HRT in endometrial preparation in frozen thawed embryo transfer. Calculation was done based on comparing 2 proportions from independent samples in a prospective study using Chi test, the α-error level was fixed at 0.05, the power was set at 80% and the intervention groups (case: control) ratio was set at 1. As previously published (Letrozole versus artificial hormonal endometrial preparation for vitrified-warmed embryos transfer cycles; https://doi.org/10.1016/j.mefs.2015.09.002), women treated with letrozole achieved on-going pregnancy rate of 47.9% while HRT treated women achieved 32.3% on-going pregnancy rate. Accordingly, the minimum optimum sample size should be 154 participants in each arm. Sample size calculation was done using PS Power and Sample Size Calculations software, version 3.0.11 for MS Windows (William D. Dupont and Walton D. Vanderbilt, USA).

Statistical analysis:

Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using one way analysis of variance (ANOVA) test with posthoc multiple 2-group comparisons. For comparing categorical data, Chi-square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5. Correlation between various variables was done using Pearson moment correlation equation for linear relation of normally distributed variables and Spearman rank correlation equation for non-normal variables/non-linear monotonic relation. *P* values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows.

RESULTS

As shown in table 1, there were no significant differences in the demographic and the clinical characteristics (as age, duration and cause of infertility) of both groups. However, significant difference was reported as regards the type of infertility, 1ry infertility in induction group was reported 94 (58.8%) compared to 1ry infertility in HRT group 113 (70.6%) and 2ry infertility in induction group was reported 66 (41.3%) compared to 2ry infertility in HRT group 47 (29.4%).

Table 2 shows the characteristics of the frozen thawed embryo transfer cycle, a significantly higher number of frozen embryos at vitrification was reported in the induction group 3.22 (0.750) compared to the HRT group 2.99 (0.761), furthermore, as significantly higher endometrial preparation days were reported in HRT group 20.03 (1.735) compared to induction group 19.27(2.384), however, a significantly higher implantation rate was reported in induction group 22.76 (27.766) compared to HRT group 16.72 (24.096), otherwise, no significant difference was reported between both groups as regards the number and quality of embryos in thawing, cancelled cycles (embryos non survival after thawing) and number of transferred embryos.

Table 3 shows the clinical outcome following the frozen thawed embryo transfer, the biochemical pregnancy rate is higher in the induction group 90 (56.3%) compared to HRT group 78 (48.8%) but not statistically significant, the clinical pregnancy rate, ongoing pregnancy rate were significantly higher in the induction group 81 (50.6%), 71 (44.4%) compared to HRT group 62 (38.8), 44 (27.5%), respectively. Furthermore, first trimester abortion rate was significantly higher in the HRT group 19 (11.9%) compared to induction group 10 (6.3%).

Table 1 : patients clinical characteristics

Characteristics of the patients	1st group (induction group) (n 160)	2nd group (HRT group) (n 160)	*P.value*
Age	31.14 (6.017)	31.71 (5.919)	0.400
Type of infertility			
1ry	94 (58.8%)	113 (70.6%)	0.026
2ry	66 (41.3%)	47 (29.4%)	
Duration of infertility	6.18 (2.658)	6.25 (3.118)	0.817
Cause of infertility			
Male	28 (17.5%)	33 (20.6%)	
Tubal	24 (15%)	30 (18.8%)	
PCO	75 (46.9%)	57 (35.6%)	0.131
Endometriosis	15 (9.4%)	9 (5.6%)	
Combined	13 (8.1%)	23 (14.4%)	
Unexplained	5 (3.1%)	8 (5.0%)	

*HRT: hormonal replacement treatment
*PCO:Polycystic ovarian syndrome

Table 2 : Characteristics of frozen thawed cycle

Characteristics of frozen thawed cycle	1st group (induction group) (n 160)	2nd group (HRT group) (n 160)	*P.value*
Number of F.E at vitrification	3.22 (0.750)	2.99 (0.761)	0.007
Day of freezing:			
Day 3	69 (43.1%)	79 (49.4%)	
Day 5	91 (56.9%)	81 (50.6%)	0.262
Number & quality of embryos at thawing:			
Grade1 (good embryos)	2.23 (0.768)	2.04 (0.886)	0.051
Grade 2 (fair embryos)	0.62 (0.581)	0.57 (0.509)	0.414
Grade3 (bad embryos)	0.38 (0.547)	0.34 (0.548)	0.54
Number of patients with non-survived embryos at thawing	5 (3.1%)	7 (4.4%)	0.556
Number of transferred embryos	2.71 (0.821)	2.54 (0.903)	0.081
Endometrial thickness at time of transfer (mm)	10.512 (1.508)	10.611 (1.599)	0.57
Days of endometrial preparation	19.27 (2.384)	20.03 (1.735)	0.001
Implantation rate	22.76 (27.766)	16.72 (24.096)	0.038

Table 3 : Clinical outcome following frozen thawed embryo transfer

Clinical outcome of FET	1st group (induction group) (n 160)	2nd group (HRT group) (n 160)	*P.value*
Biochemical pregnancy	90 (56.3%)	78 (48.8%)	0.176
Clinical pregnancy	81 (50.6%)	62 (38.8%)	0.033
Ongoing pregnancy	71 (44.4%)	44 (27.5%)	0.002
Abortions (1st trimester)	10 (6.3%)	19 (11.9%)	0.08

DISCUSSION

In this study, we compared ovulation induction with Letrozole or HRT in patients undergoing endometrial preparation for FET. Our study shows that clinical outcomes in patients using Letrozole for induction of ovulation are superior to that of patients using HRT for endometrial preparation for FET.

Letrozole acts by inhibiting aromatase enzyme activity, blocking the in vivo conversion of androgen to estrogen, reducing in vivo estrogen levels. This relieves the negative feedback inhibition of estrogen on the hypothalamus, increasing gonadotropin secretion and enhancing follicular development[6]. Furthermore, letrozole induces single follicular development, due to elevated estrogen levels during follicular growth inducing negative feedback inhibition on hypothalamus, suppressing growth of non-dominant follicles[7]. Letrozole is considered to be safe and effective, following oral administration, it is rapidly absorbed and after one hour it reaches maximum blood drug concentration, has high bioavailability (99.9%) and short half-life (45h), resulting in complete removal of letrozole during the embryo implantation period[8]. Letrozole is believed to improve endometrial receptivity, this can be clarified by the increase in endometrial VEGF (vascular endothelial growth factor) with subsequent improved endometrial vascularity[9, 10]. In addition, it has been demonstrated that letrozole increased expression of integrins in surface epithelium and glandular epithelium[9]. This plays an important role in the initial embryo and endometrium interaction[11], this can explain the higher implantation rate and clinical pregnancy rate in the induction group. In addition, endometrial damage and variances in the implantation window are also believed to occur due to non-physiologically high estradiol levels in patients receiving HRT for endometrial preparation[12]. Moreover, the corpus luteum development following induction of ovulation with letrozole use supports the luteal phase and 1st trimester pregnancy, which does not occur with HRT group due to down regulation of hypothalamo-pituitary axis by E and P, this can explain the higher ongoing pregnancy rate and the lower 1st trimester abortion rate in induction group.

The results of this study are in agreement with Li *et al*.[13] that reported significantly higher clinical pregnancy (53.2% vs 44.4%) and lower abortion rates (12.0% vs 21.0%) in letrozole group compared to HRT group. Our findings are consistent also with prior study held by H. Sibai *et al*.[14] that reported significantly higher ongoing pregnancy rate (47.9% vs 32.3%) and lower abortions rates (5.3% vs 8.3%) in letrozole group compared to HRT group. However, compared to our study, their results regarding the clinical pregnancy rate were statistically non-significant (53.6% vs 40.2%), in letrozole group compared to HRT group. This could be attributed to smaller number of cycles analyzed in this study. Hu *et al.* also demonstrated that use of letrozole in FET significantly increased the clinical pregnancy rate (65.0%) and decreased the first trimesteric abortion rate (5.0%) compared with HRT (40.8% for clinical pregnancy and 3.9% for abortion) in 120 patients with polycystic ovary syndrome[15].

A major drawback of the current study is that a retrospective non randomized, larger randomized controlled studies would be needed to confirm the validity of our results.

However , the better clinical outcome as regards the higher clinical pregnancy rate, ongoing pregnancy rate and the lower abortion rate in the induction group compared to HRT group is encouraging for the use of induction protocol using letrozole as an alternative to HRT protocol for endometrial preparation for FET.

CONCLUSION

The use of letrozole in patients undergoing FET was associated with significantly higher implantation rate, clinical pregnancy rate, ongoing pregnancy rate, and a lower 1st trimesteric yabortion rate, than use of HRT. Based on our results, further prospective studies, especially randomized controlled trials, are needed to examine the differences in pregnancy outcome including live birth rate and neonatal outcomes of FET with letrozole compared to other methods.

CONFLICT OF INTEREST

There are no conflicts of interest.

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