ISSN 2063-5346

MODIFIED NATURAL CYCLE VS HORMONE REPLACEMENT THERAPY IN ENDOMETRIAL PREPARATION FOR FROZEN EMBRYO TRANSFER

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Article History: Received: 02-06-2023 Revised: 22-07-2023 Accepted: 06-08-2023

Abstract

Background: *Frozen embryo transfer has become one of the main therapeutic approaches for infertility. Endometrial preparation is a key process in endometrium receptivity and treatment outcomes; there are two primary approaches for endometrial preparation, i.e., natural cycle and hormone replacement therapy (HRT). In the current study, we investigated modified natural cycle (MNC) with HRT in terms of endometrial thickness, biochemical pregnancy, clinical pregnancy, and ongoing pregnancy rates.*
Material and Methods: *From September 2021 to March 2023, 162 patients aged between 20 to 40 years old were included in this study. Following informed consent, the included patients underwent HRT or MNC for preparing the endometrium for frozen embryo transfer. Following the collection of clinical and laboratory results of included patients, the endometrial thickness, biochemical pregnancy, clinical pregnancy, and ongoing pregnancy rates of the patients were analyzed.*
Results: *Our results have shown that the biochemical pregnancy and ongoing pregnancy rates of patients treated with MNC are substantially higher compared with patients treated with HRT. However, there have been no statistically significant differences in terms of clinical pregnancy rate and endometrial thickness of patients treated with MNC compared to patients treated with HRT.*
Conclusion: *Using the MNC for endometrial preparation for frozen embryo transfer has been associated with higher biochemical pregnancy and ongoing pregnancy rates than HRT.*

Keywords: *Assisted Reproductive Technology, Endometrium, Hormone Replacement Therapy, Modified Natural Cycle.*

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DOI: 10.48047/ecb/2023.12.8.590

1. Introduction

Infertility is defined as the failure to establish pregnancy following unprotected sexual intercourse after 12 months; it is estimated that 12% of couples are affected by infertility worldwide (1). Assisted reproductive technologies (ARTs) have opened a new era in the treatment of infertility. Since the first birth of a newborn conceived with ART in 1981, the use of ARTs have been increased; it has been estimated that ARTs have contributed to 1.8% of infants born in the US in 2016 (2). Consistent with this, it has been reported that 25% of couples suffer from infertility in China and there have been 1,211,303 *in-vitro* fertilization between 2013 to 2016 in China (3, 4).

Based on the reports from US Centers for Disease Control and Prevention, approximately 50% of transferred embryos are cryopreserved in 2014; a similar trend has been reported by the European Society of Human Reproduction and Embryology (5). It has been shown that the outcomes of frozen-thawed embryo transfer are comparable to fresh one and the frozen-thawed embryo is beneficial for women at risk of ovarian hyperstimulation syndrome (6). Kansal Kalra et al. have reported that the perinatal morbidity is considerably lower in the frozen embryo transfer approach compared with fresh embryo transfer (7). A recent meta-analysis has indicated that *in-vitro* fertilization outcomes are substantially improved in frozen embryo transfer compared to fresh embryo transfer (8). Although frozen embryo thawing and transferring is considered one of the safe and cost-effective ART approaches, the optimal endometrial preparation technique has been debated (5). Indeed, the synchronization of endometrium receptivity with the embryo has key roles in the implantation and development of the fetus (9). Endometrial preparation can be generally classified into two categories, i.e., hormone replacement therapy (HRT) and natural cycle. In the HRT approach, estrogen and progesterone are supplied from exogenous sources to prepare the endometrium; however, in the natural cycle, the dominant follicle-produced estrogen and progesterone support the endometrium. Although HRT is considered a flexible and convenient approach, the natural cycle is close to the physiological state (10). It has been indicated that HRT is associated with an increased risk of preterm delivery, very preterm delivery, cesarean delivery, macrosomia, premature rupture of membrane, and hypertensive disorders of pregnancy compared to the natural cycle (11). Therefore, these results have led to the notion of "back-to-nature" in terms of endometrial preparation for frozen embryo transfer.

Since the referred patients to the Reproductive Clinic of Alzahra Hospital have low socioeconomic status and this center is governmental, we aimed to decrease the frequency of related office visits and the expenditure of commuting and laboratory tests; therefore, we used the modified natural cycle (MNC) approach. Given the importance of endometrial preparation for embryo transfer, there is no study investigating the pregnancy outcomes of HRT and modified natural cycle (MNC) in Iran. The current study aimed to investigate the biochemical pregnancy, clinical pregnancy, and ongoing pregnancy rates of patients treated with HRT and MNC. The results of this study can guide clinicians to select optimal endometrial preparation for frozen embryo transfer.

2. Material and Methods

Data Source

The included patients were all from the Reproductive Clinic of Alzahra Hospital, the teaching hospital affiliated with Tabriz University of Medical Sciences. The protocol of this study was approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1401.10.81). Informed consent was obtained from the patients before including in the study. From September 2021 to March 2023, patients aged between 20 to 40 years old with infertility underwent MNC or HRT. The identification code for the current study is IRCT20130603013566N11. The included patients had one/two times futile frozen embryo transfer and the grade of the frozen embryo was A or B. All the included patients had the endometrial thickness of at least 7.5 mm at the expected time. None of the included patients had endometriosis, uterus anomaly, and uterine myoma. Finally, 79 patients treated with MNC and 83 patients treated with HRT were included in our study (Fig. 1).

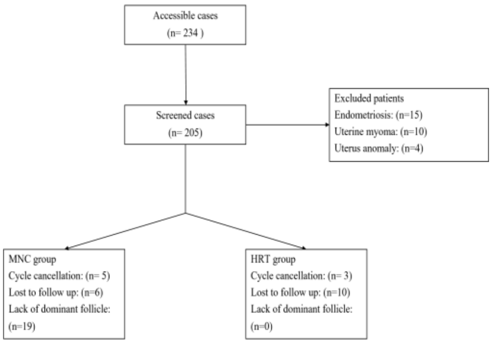

Figure 1: Case Screening Flow Chart

Study Procedure

The patients' ovaries were examined using vaginal ultrasonography on the first to third day of the cycle. In case of lack of ovarian cyst or antral ovarian follicle > 12 mm, they received HRT or MNC for endometrial preparation.

In the HRT group, the endometrial preparation was done using estrogen and progesterone hormones; 2 mg estradiol tablets were started on the second day of the menstrual cycle every 8 hours. The frequency of estradiol was 3 times per day up to the day of frozen embryo transfer and it would continue after frozen embryo transfer. After 10 to 12 days of estradiol administration, the endometrial thickness was examined using vaginal ultrasonography. After reaching an endometrial thickness above 7.5 mm, intramuscular progesterone was prescribed for the patients for 4 days, 50 mg at the first day and then 100 mg a day, on the fourth day of progesterone administration, 2 or 3 embryos in cleavage stage and grade A or B, were transferred to the uterus, and the luteal phase was supported by rectal 400 mg progesterone twice a day and 50 mg progesterone injection every other day. The estradiol was continued with the previous dose up to the eighth week and it was tapered off until the twelfth week. The progesterone dosage remained unchanged up to the twelfth week.

If the endometrial thickness did not reach 7.5 mm, estradiol tablets would be continued and the endometrial thickness would be studied using vaginal ultrasonography. If the endometrial thickness did not reach 7.5 mm after 20 days, the cycle would be canceled.

In the MNC, transvaginal ultrasonography was performed on the tenth or twelfth days of the cycle. In case of the presence of a follicle above 17 mm, endometrial thickness above 7.5 mm, and serum progesterone level below 1.5 ng/mL, 5000 units HCG would be administrated. When there was no follicle above 17 mm or endometrial thickness was less than 7.5 mm, vaginal ultrasonography would be performed after 2 days. Two days after HCG administration 50 mg progesterone was administrated intramuscularly to the patients every day. On the fourth day of progesterone administration, 2 or 3 embryos in cleavage stage and grade A or B were transferred to the uterus and the luteal phase was supported by rectal 400 mg progesterone. The progesterone dosage would remain the same up to the twelfth week.

Outcomes

The clinical and laboratory data of the included patients were collected. The primary outcomes were to investigate and compare the clinical pregnancy rate, ongoing pregnancy, and biochemical pregnancy rate in MNC and HRT groups. The biochemical pregnancy was defined as the b-HCG serum level of at least 10 IU per liter at 15 days after embryo transfer. The clinical pregnancy rate was defined as the presence of intrauterine gestation sacs at 35 days following embryo transfer. The ongoing pregnancy rate was defined as the presence of viable fetal heartbeat at 11 weeks of gestation. These definitions were in line with the previous study (10).

Statistical Analysis

GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA) was utilized to analyze the obtained data. The student t-test and chi-squared test were used to study the differences between the groups. The Kolmogorov–Smirnov test was used to study the normality of data. P-values less than 0.05 were regarded as statistically significant.

3. Results

The Basic Clinical and Laboratory Characteristics of Included Patients

The basic clinical and laboratory characteristics of included patients were matched in two groups in terms of age, BMI, infertility duration, sperm count, sperm morphology, sperm motility, history of frozen embryo transfer, number of retrieved oocytes, M2 oocyte, M1 oocyte, and GV oocyte, number of in vitro embryo, and number of the transferred embryos (all P-values > 0.05) (Table. 1). Also, our results have indicated the included patients in HRT group are matched with MNC group in terms of familial marriage and type of infertility, i.e., being primary or secondary (data are not shown).

Table 1: The Basic Clinical and Laboratory Characteristics of Included Patients

	HRT (mean±Std)	**MNC (mean±Std)**	**P-value**
Age (year)	29.99 ± 5.707	28.97 ± 5.328	0.1834
BMI (Kg/m2)	27.72 ± 4.733	26.91 ± 3.933	0.1304
Infertility duration (year)	6.750 ± 4.184	5.947 ± 4.218	0.1993
Sperm count	56.41 ± 22.33	53.03 ± 26.09	0.4392
Sperm morphology (%)	5.468 ± 4.558	6.687 ± 6.347	0.5319
Sperm motility (%)	39.53 ± 19.23	42.61 ± 21.22	0.3002
History of frozen embryo transfer	1.862 ± 1.044	1.615 ± 0.6045	0.1009
Number of retrieved oocytes	12.21 ± 6.750	12.22 ± 6.658	0.8311
M2 oocyte	8.364 ± 4.595	7.160 ± 5.178	0.3536
M1 oocyte	2.245 ± 2.146	1.983 ± 1.613	0.7477
GV oocyte	2.304 ± 2.536	1.955 ± 2.026	0.5083
Number of *in-vitro* embryo	7.456 ± 4.107	7.581 ± 4.632	0.9847
Number of transferred embryo	2.105 ± 0.3494	2.110 ± 0.5154	0.9995

The Endometrial Thickness, Biochemical Pregnancy, Clinical Pregnancy, and Ongoing Pregnancy Rates in MNC and HRT Groups

Endometrial thickness in two groups were similar (P-value = 0.6403). Results have shown that the biochemical pregnancy and ongoing pregnancy rates in MNC group are significantly higher than HRT group (P-value = 0.0102, and P-value = 0.0105, respectively). However, there have been no statistically significant differences between the clinical pregnancy rate of patients treated with MNC compared to patients treated with HRT (P-value = 0.0676) (Table. 2).

Table 2: The Endometrium and Pregnancy Outcome of MNC and HRT Groups

	HRT	**MNC**	**P-value**
Endometrial thickness (mm) (mean±Std)	8.406 ± 1.011	8.495 ± 1.685	0.6403
Biochemical pregnancy rate	25/83	40/79	*0.0102*
Clinical pregnancy rate	15/83	25/79	0.0676
Ongoing pregnancy rate	10/83	23/79	*0.0105*

4. Discussion

Although there have been remarkable advances in ARTs, there is a pressing need to develop novel approaches to increase the efficacy of the available therapeutic approaches. Given the significance of frozen embryo transfer and endometrial preparation for optimal receptivity, the current study investigated the HRT and MNC approaches in terms of endometrial thickness, biochemical pregnancy rate, clinical pregnancy rate, and ongoing pregnancy. Our results have shown that the endometrial thickness and clinical pregnancy rate of patients treated with MNC are comparable with the HRT group; however, patients treated with the MNC approach have substantially higher biochemical pregnancy and ongoing pregnancy rates than patients treated with HRT.

Endometrial thickness has a pivotal role in the outcome of *in-vitro* fertilization (12); thin endometrium is associated with an increased risk of obstetric complications, like ectopic pregnancy, low birth weight, spontaneous abortion, and placenta previa development (13-16). However, endometrial thickness above 7-8 mm is associated with improved outcomes of *in-vitro* fertilization (17). Consistent with these, it has been reported that endometrial thickness below 6 mm is associated with decreased live birth rate in the frozen embryo transfer approach (18). Shaodi et al. have reported that there are positive associations between endometrial thickness with implantation rate, clinical pregnancy rate, and live birth rate in patients who underwent HRT treatment (19). Pang et al. have shown that the endometrial thickness of patients treated with the natural cycle is considerably higher than the endometrial thickness of patients treated with HRT for frozen embryo transplantation (20). Also, Levron et al. have shown that the natural cycle leads to increased endometrial thickness compared to HRT for frozen embryo transfer (21). However, Hancke et al. have shown that there is no statistically significant difference in terms of endometrial thickness in patients treated with spontaneous cycle and patients treated with artificial cycle (22). Based on our results, the endometrial thickness of patients treated with MNC has been comparable with the endometrial thickness of patients treated with HRT.

Besides endometrial thickness, the synchronization of endometrium receptivity has a crucial role in the outcomes of *in-vitro* fertilization. For this reason, two strategies have been proposed to optimize endometrium receptivity, i.e., HRT and natural cycle-based treatments (5). Ma et al. have reported that the duration of the uterine receptivity window is negatively associated with estrogen levels in animal models (23). In line with this, the estradiol level of patients with ongoing pregnancy/live birth has been considerably lower than the patients without ongoing pregnancy/live birth. In patients treated with frozen embryo transfer, there has been a negative correlation between estradiol levels with ongoing pregnancy/live birth (24). In this regard, it has been reported that the level of E2 is higher in HRT compared to the natural cycle (21). Overall, natural cycle-based treatments are generally considered safer and more natural than the HRT approach (25). Our results have indicated that the biochemical pregnancy and ongoing pregnancy rates are substantially higher in the MNC group compared to the HRT group. Although there has been a trend in which the clinical pregnancy rate of the MNC group has been higher than the patients treated with HRT, this trend has not been statistically significant. Pang et al. have found that there are no differences in terms of clinical pregnancy rate in patients treated with HRT and natural cycle (20). Similar results regarding clinical pregnancy rate have been noted in the study by Pan et al. (10). Levron et al. have shown that the ongoing pregnancy, clinical pregnancy, and implantation rates are higher in the patients treated with natural cycle compared to patients treated with HRT (21). Guan et al. have shown that the natural cycle regimen results in higher implantation and live birth rates compared to the artificial cycle regimen in patients treated for endometrial preparation (26). Given the beneficial effect of the corpus luteum for maternal cardiovascular system adaptation to pregnancy, HRT treatments also lead to the atrophy of the corpus luteum (27). It has been demonstrated that HRT treatment is associated with an increased risk of hypertensive disorders of pregnancy compared with natural cycle treatment (11). Saito et al. have indicated that the HRT increases the risk of hypertensive disorders of pregnancy and placenta accrete development compared to patients treated with natural cycle (28). Also, patients treated with HRT have an increased risk for post-term delivery development and cesarean section compared to patients treated with natural cycle (29). Consistent with this, it has been found that patients treated with HRT have an increased risk of cesarean section than patients treated with natural cycle (10).

The current study has several strengths and limitations. First, this was among the first studies conducted on Iranian patients for evaluating MNC and HRT approaches. Second, we had strict inclusion and exclusion criteria and the included patients had been matched based on various factors. However, this study suffers from limitations as well. One of the main limitations of this study was we were unable to assess the common obstetrics complications in the patients treated with these approaches. Overall, our study provided valuable information regarding the beneficial effect of the MNC approach over HRT in terms of preparing the endometrium for frozen embryo transfer.

5. Conclusion

Our study has indicated MNC results in higher biochemical pregnancy and ongoing pregnancy rates than HRT. However, there have been no considerable differences in terms of clinical pregnancy rate and endometrial thickness between these approaches for endometrial preparation for frozen embryo transfer.

Acknowledgments

This study was supported by the Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran (grant number: 71054).

Conflicts of Interest

The authors declare no conflict of interest.

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