Fresh or Frozen Embryo Transfer in The Antagonist 
In Vitro Fertilization Cycles: A Retrospective Cohort Study

Fariba Seyyedoshohadaei, M.D.1, Khaled Rahmani, Ph.D.2, Azra Allahveisii, Ph.D.3, Masoumeh Rezaei, M.D.1, Mohammad Jafar Rezaei, Ph.D.3, Farnaz Zandvakili, M.D.3, Nasrin Soufizadeh, M.D.1, Yasamin Honarbaksh, M.D.1

1. Department of Obstetrics and Gynecology, School of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
2. Liver and Digestive Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran
3. Department of Anatomical Sciences, School of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran

Abstract

Background: Gonadotropin-releasing hormone antagonist (GnRH-ant), widely adopted protocol, is more in line with the physiological processes, and induces a shorter and more cost-effective ovarian stimulation. In order to assess the success rate of embryo transferring (ET) in the antagonist in vitro fertilization (IVF) cycles, we compared the fresh ET with the frozen ET outcomes.

Materials and Methods: In this retrospective cohort study, one hundred five cases of ET of the infertility clinic of the Besat hospital (Kurdistan, Iran) between March 2014 to March 2020 that were treated with antagonist cycle (both fresh and frozen) were analyzed. The difference between the two groups in baseline data and reproductive outcomes were evaluated using Independent sample t test, Mann-Whitney U test, Chi-squared test, and Fisher's exact test in SPSS software (version 22).

Results: Out of 105 cases, 48 and 57 were in the fresh and frozen ET groups, respectively. The participants age was 35.75 ± 4.9 Y. In the fresh ET group, and 33.98 ± 5.1 Y in the frozen ET group. The percentage of chemical pregnancy was 12 (25%) in the fresh ET group and 15 (26.3%) in the frozen ET group (P=0.8); Clinical pregnancy rate was 11 (22.9%) in the fresh ET group and 11 (19.3%) in the frozen ET group (P=0.6); the rate of abortion in the fresh ET group was 3 (6.3%, P=0.2), and in the frozen ET group was 8 (14%, P=0.2); and the live birth rate was 9 (18.8%) in the fresh ET group, in comparison with 7 (12.3%) in the frozen ET group (P=0.3).

Conclusion: Not statistically significant, the percentage of chemical pregnancy and abortion were higher in the frozen ET group. The percentage of clinical pregnancy and live birth were higher in the fresh ET group.

Keywords: Assisted Reproductive Technology, Embryo Transfer, In Vitro Fertilization

Introduction

Infertility can be defined as the failure to achieve a pregnancy within one year of regular unprotected intercourse (1, 2). Infertility is affecting 8-12% of couples worldwide (3). Couples undergo infertility treatments due to male factor, female factors or unexplained infertility (4). Female factor accounts for 33-41% of infertility cases, male factor accounts for 25-39% of the cases and 9-39% are due to a combination of both male and female factors (5). The variability in patient characteristics and response to assisted reproductive technology (ART) dictate the need for proven, personalized diagnostic and therapeutic approaches to optimize efficacy and safety of treatment (6). Under a standard infertility treatment algorithm (SITA), couples who do not become pregnant with ovulation induction, undergo assisted reproductive techniques such as in vitro fertilization and embryo transfer (IVF-ET). Although, a fresh ET is still routine practice in the IVF cycles, elective frozen ET has emerged as an important method that can influence IVF outcomes (7).

After 40 years of development of IVF and ET, many IVF-ET cycles are failing and no signs of embryo implantation or the production of human chorionic gonadotropin (hCG) are achieved (8). One possible cause of the unsuccessful implantation rate is reduced endometrial receptivity despite of high quality transferred embryos (9). Poor endometrial receptivity is a major factor that leads to recurrent implantation failure. However, the traditional method cannot accurately evaluate endometrial receptivity (10). Endometrial receptivity is reduced during ovulation cycles, including in both gonadotropin-releasing hormone [GnRH agonists (GnRH-a) and GnRH antagonist (GnRH-ant) cycles], and is lower in patients who undergo GnRH-ant
Patients of the infertility clinic of the Besat Hospital (Kurdistan, Iran) between March 2014 to March 2020, who received antagonist IVF cycle treatment were invited to this study. They were informed that only the outcome of their clinical process will reanalyze and targeted for research purposes. Then, the records of whom that provided written informed consent used in this study. This study was conducted after approval by the Ethics Committee of the Kurdistan University of Medical Sciences, Kurdistan, Iran (IR.MUK.REC.1399.042).

Participants

The inclusion criteria for this study consisted of infertile women, in their reproductive age, referred to the infertility clinic of the Besat Hospital, admitted from March 2014 to March 2020, and being treated with an antagonistic IVF cycle. Patients with incomplete hospital records, that we were unable to obtain the necessary information, patients with no retrieved oocytes, and also patients who did not complete their antagonist cycle and embryo transfer, were excluded from the study.

Study design

We considered two groups for this study. Fresh ET group, and frozen ET group. Fresh ET group includes patients undergoing antagonist IVF cycle who received fresh embryo(s). The frozen ET group included the frozen embryo(s) transfer. The demographic data and other required clinical and paraclinical data were collected from patients’ records.

ET was performed on the third day of fertilization when the embryos were at the 8 cell stage (cleavage-stage embryos). The embryos were graded into four categories according to their fragmentation index: grade A: equal size blastomeres and less than 10% fragmentation; grade B: slightly unequal blastomeres with up to 20% fragmentation; grade C: unequal sized blastomeres, up to 50% fragmentation and large granules; and grade D: unequal blastomeres with significant fragmentation (>50%) and large granules (24, 25). Due to low implantation potential of human embryos with greater than 25% fragmentation, have a (25), we only transferred embryos grade A and B.

We did not transfer embryos that were arrested in 2 cell stage, 4 cell stage, and 6 cell stage, as these factors can be considered confounding variables. In this study, we only transferred grade A, and B embryos. The criteria we considered for ET included: being equal in size, low fragmentation percent, and the accordance of embryo growth to fetal age. We used vitrification technique. The Kitazato vitrification kit (VT-601, Kitazato, Japan) was used, and we followed Kitazato kit protocol; i.e. fifteen minutes in the equilibration solution (ES), and the last one minute in the vitrification solution (VS).

In the frozen group, all embryos have been frozen, and with an interval of more than 2 months, the embryos were...
transferred in one of the following methods: i. Suppression with the gonadotropin agonist, Diphereline, with half of a 3.75 ampoule, one week before menstruation, and initiation of the Estradiol valerate, the dose of which was determined individually for each patient, ii. Starting the cycle without suppression, starting with Estradiol valerate from the second day of the cycle, iii. Cycle stimulated with Clomiphene or Letrozole, and injection of hCG during follicle maturation, and subsequent embryo transfer, and iv. Patients’ own normal cycle and stimulation with hCG.

After the EMT reached above 8 mm, 100 mg of Progesterone (Fertigest, 50 mg Amp*2, Aburaihan Company, Iran) was given daily for 2 to 4 days, and frozen embryos were transferred according to the patient’s condition. We performed a Beta-HCG laboratory test to assess chemical pregnancy, and ultrasound evaluation of the patients to determine clinical pregnancy. If the pregnancy was confirmed, patients were followed by phone calls, clinic visits, and also obtaining information from their medical records, to record any abortion, or continuation of the pregnancy, or any other possible consequences. We also contacted patients and reviewed their hospital records, to obtain any information regarding unwanted events.

Measurements

Demographic information of the patients, including age, and body mass index (BMI), was collected from patients’ records. We also gathered information regarding the type of infertility, and the reason they were selected for the antagonist IVF cycle. BMI of the patients was divided into five categories: i. Underweight (BMI<18.5), ii. Normal (BMI: 18.5-24.9), iii. Overweight (BMI 25-29.9), iv. Obese (BMI 30-34.9), and v. Extremely obese (BMI>35). The type of infertility was divided into two groups: i. Primary infertility ii. Secondary infertility. The reason for choosing antagonist IVF cycle was categorized into three reasons: i. Polycystic ovarian syndrome (PCOS), ii. Poor responders, and iii. Failure of the previous agonist cycle. During this study, we assessed and compared the number of follicles, number of degenerated oocytes, mature oocytes, immature oocytes, injected oocytes, fertilized oocytes, number of transferred embryos, and quality of transferred embryos, in both groups. After completing the antagonist cycle, we studied cases leading to chemical pregnancy and clinical pregnancy, which were determined using the β-hCG test, and ultrasound results, respectively. Among the pregnant cases, we studied the number of miscarriages, twin, and live birth. In both groups, complications were also recorded and compared based on hospital records and specialist reports.

Data analysis

The collected data were analyzed using SPSS software (version 22, SPSS Inc., Chicago, IL, USA). In the data description section, descriptive statistical methods such as mean, standard deviation, frequency, and relative frequency as well as the related tables were used to summarize the results. The difference between the two study groups were evaluated using Independent sample t test, Mann-Whitney U test, Chi-squared test, and Fisher’s exact test. The significance level of the tests was considered 0.05.

Results

According to the number of available records and to increase the accuracy of the study, 105 patients were studied, including 48 patients in the fresh group, 57 patients in the frozen group. The sample size was calculated using alpha error of 0.05, and beta error of 0.20, and assuming 40% difference in outcome indices in the two groups, using R software.

We compared the reason for choosing antagonist IVF cycle and no statistically significant difference was found (Table 1).

| Variables                        | Fresh | Frozen | P value |
|----------------------------------|-------|--------|---------|
| Reason for antagonist cycle      |       |        |         |
| PCOS                             | 14(29.2) | 19(33.3) | 0.3*    |
| Poor responder                   | 20(41.7) | 16(28.1) |         |
| Previous failure of agonist cycle| 14(29.2) | 22(38.6) |         |
| Type of infertility              |       |        |         |
| Primary                          | 39(81.3) | 43(75.4) |         |
| Secondary                        | 9(18.8)  | 14(24.6) |         |

Data are presented as n (%). *: Chi-squared test, IVF: In vitro fertilization, and PCOS: Polycystic ovarian syndrome. P≤0.05 was considered significant.

Using an independent t test, we did not observe a significant difference of age, and BMI, between our groups. Also, no statistically significant difference was found in the other parameters such as Immature GV (Table 2).

The quality of transferred embryos

Considering the quality of transferred embryos in fresh and frozen ET groups, can be concluded that the most common type of embryo transferred in both groups was grade "A". After grade A, the "both grades A & B" group and the "grade B" groups were the most frequent qualities used. Type C embryos were not used in any of the patients in our study. Out of 48 patients in the fresh ET group, 41 (85.4%) received the grade "A" quality embryos, 2 (4.2%) received the grade "B" quality embryos, and in 5 (10.4%) patients, "both grade A and grade B" embryos were transferred. Out of 57 patients in the FET group, 50 (87.7%) received "A" quality embryos, 4 (7%) received "B" quality embryos, and 3 (5.3%) received both grade A and grade B. In this study, no grade "C" embryos were transferred to any of the patient groups (Table 2).
Table 2: Comparing age, BMI, embryogenic factors, and quality of transferred embryos in our groups

| Variable                  | Fresh (n=48) | Frozen (n=57) | P value |
|---------------------------|--------------|---------------|---------|
| Age (Y)                   | 35.75±4.9    | 33.98±5.1     | 0.07    |
| BMI (kg/m²)               | 26.71±3.8    | 27.52±4.3     | 0.6    |
| Follicles/oocytes         | 6.63±4.83    | 7.58±6.02     | 0.3    |
| Degenerated oocytes       | 0.56±0.82    | 0.56±1.01     | 0.9    |
| Immature GV               | 0.27±0.70    | 0.40±1.05     | 0.4    |
| Immature M1               | 0.48±0.82    | 0.51±0.98     | 0.8    |
| Mature M2                 | 5.29±3.74    | 6.18±5.68     | 0.3    |
| Injected oocytes          | 5.65±4.02    | 6.68±5.69     | 0.2    |
| Fertilized oocytes 2PN    | 4.75±3.16    | 5.37±4.45     | 0.4    |
| Embryos                   | 4.65±3.21    | 5.35±4.48     | 0.3    |
| Grade of transferred embryo |            |               |         |
| A                         | 41 (85.4)    | 50 (87.7)     | 0.5    |
| B                         | 2 (4.2)      | 4 (7)         |         |
| A and B                   | 5(10.4)      | 3 (5.3)       |         |
| C                         | 0            | 0             |         |

Comparing the frequency of chemical pregnancies, a positive serum β-HCG in the fresh ET group with the frozen ET group, was non significantly lower (Table 3). Comparing the frequency of clinical pregnancies detected by a first trimester ultrasonography, in the fresh ET group with the frozen ET group reveals that the percentage of clinical pregnancy is higher in the group of fresh ET, but this difference is not statistically significant. The abortion frequency in the fresh ET group in comparison with the frozen ET group, was non significantly higher in the fresh ET group. Comparison of the frequency of twins in the fresh ET group with the frozen ET group, confirms that the rate of twins in the group of fresh ET is non significantly higher. The live birth frequency in the fresh ET group in comparison with the frozen ET group shows the nonsignificant higher rate (Table 3).

Table 3: Comparing the final results between fresh vs. frozen embryo transfer groups

| Variable                  | Treatment group | Yes | No  | P value |
|---------------------------|-----------------|-----|-----|---------|
| Chemical pregnancy        | Fresh           | 12 (25) | 36 (75) | 0.8* |
|                           | Frozen          | 15 (26.3) | 42 (73.7) |       |
| Clinical pregnancy        | Fresh           | 11 (22.9) | 37 (77.1) | 0.6* |
|                           | Frozen          | 11 (19.3) | 46 (80.7) |       |
| Abortion                  | Fresh           | 3 (6.3)  | 45 (93.8) | 0.2* |
|                           | Frozen          | 8 (14)   | 49 (86)   |       |
| Twin                      | Fresh           | 2 (4.2)  | 46 (95.8) | 0.5* |
|                           | Frozen          | 1 (1.8)  | 56 (98.2) |       |
| Live birth                | Fresh           | 9 (18.8) | 39 (81.3) | 0.3* |
|                           | Frozen          | 7 (12.3) | 50 (87.7) |       |

Unwanted side effects

In this study, three types of unwanted side effects were observed and recorded during the treatment period in our groups. These unwanted events included: 1. Ovarian hyperstimulation syndrome, 2. Ectopic pregnancy, and 3. Loss of a fetus in a twin pregnancy (Table 4).

Table 4: Comparing the unwanted adverse events between the two groups

| Type of adverse event                        | Fresh | Frozen | P value |
|----------------------------------------------|-------|--------|---------|
| Severe ovarian hyper stimulation syndrome    | 4 (8.3) | 3 (5.3) | 0.48*  |
| Ectopic pregnancy                            | 0 (0) | 1 (1.8) |         |
| Loss of one embryo in twin pregnancy         | 1 (2.1) | 0 (0) |         |
| No adverse events                            | 43 (89.6) | 53 (93) |       |

Overall, adverse events happened in 5 patients (10.4%) in the fresh group, and 4 patients (7.1%) in the frozen group (P=0.48). Fortunately, 43 patients (89.6%) in the fresh group, and 53 patients (93%) in the frozen group did not experience any type of adverse events (P=0.48, Table 4).

Effect of quality of transferred embryos on final results

Using fisher’s exact test, and chi-squared test, we assess the different quality of embryos that we transferred in both groups and their effect on our results. We observed that the highest rate of chemical and clinical pregnancy, in both groups, was in “grade A” embryo transfer, but this difference was not statistically significant. And also, the highest percentage of abortions was seen in the frozen ET group to which “grade A” embryos were transferred, but this difference was not statistically significant. Three cases of twins were observed, all cases from “grade A” embryo group, 2 cases in the fresh group, and 1 case in the FET group. It was seen that the most live births belonged to the group that received “grade A” embryos, but this difference was not statistically significant.

Discussion

The Gonadotropin Releasing Hormone antagonist (GnRH-ant) protocol is widely used as a convenient and cost-effective treatment for patients undergoing IVF (26). Currently, there is no consensus whether fresh ET versus frozen one, could improve IVF outcomes in GnRH-ant cycles. In this retrospective cohort study, we reviewed the treatment process and analyzed data from one hundred five patients treated with antagonist IVF cycles in two groups of fresh and frozen ET.

Impaired endometrial receptivity has been suggested as an etiology of reduced pregnancy rates in the fresh embryos transferred ARTs (27). Endometrial receptivity can affect implantation rate, and decrease the chance of the embryo to implant (28). Frozen ET cycles are performed in a physiological uterine environment, and this may be the reason that some studies observed better IVF outcomes following the frozen ET than after fresh
Fresh ET vs. FET in Antagonist IVF

ET (19, 27, 29). In a systematic review and meta-analysis performed by Roque et al. (30), they compared the outcomes in the fresh ET versus frozen ET in IVF cycles. They concluded that IVF outcomes may be improved by performing frozen ET (FET) compared with fresh ET.

The progress in embryo cryopreservation techniques has made freeze-all strategy more acceptable. Freeze all strategy has its advantages and disadvantages. No clinical data supports the use of freeze-all strategy for all patients (31). Dieamant et al. (32) conducted a meta-analysis to evaluate whether the freeze-all strategy can improve the outcomes when compared to the fresh ET in patients undergoing an ART cycle in accordance with the mean number of oocytes collected. They concluded that the freeze-all strategy could be favorable when high numbers of oocytes are collected, signaling an association between higher ovarian stimulation and consequent impairment of endometrial receptivity. However, when the mean number of oocytes collected is <15, the freeze-all strategy does not appear to be advantageous. In our study, the mean number of collected oocytes was 6.6 in the fresh ET group, and 7.5 in the frozen ET group, and the ART outcome was not significantly different between the two groups, and therefore, the results matched with "freeze-all strategy" study.

Similar results have been reported in other studies. Basirat et al. (33) observed in their study population that there was no significant difference in the pregnancy rate following ICSI treatment between fresh ET and frozen ET groups. Seyedoshohadai et al. (34) reported that fresh ET versus frozen ET in their patients who underwent intrauterine sperm injection (ICSI) had no significant effect on the final ART outcomes. Although, they did not study antagonist cycles specifically, they concluded that no statistically significant difference was found in the chemical and clinical pregnancy between frozen ET and fresh ET methods. In the current study, we could not find a significant difference in the chemical and clinical pregnancy between the two groups as well.

However, some other investigations have reported different results. Roque et al. compared IVF outcomes between fresh ET and frozen ET (the "freeze-all" policy) (35). Five hundred thirty patients underwent a gonadotropin-releasing hormone-antagonist protocol, and cleavage-stage, day-3 ET. The ART outcomes were significantly better in the freeze-all group in comparison with the fresh ET. Their results suggested that endometrial receptivity may have been impaired by COS, and outcomes may be improved by using the freeze-all policy, which is different from the results obtained in our study. Liu et al. (36) conducted a retrospective cohort study to compare frozen ET versus fresh ET in GnRH antagonist cycle in women with 3-10 oocytes retrieved. They concluded that the pregnancy rate was significantly higher in the frozen ET group than the fresh ET group (63.70% vs. 54.50%, P<0.001), which is different with the results in our study.

Pregnancies following ART are at higher risk of antenatal complications, and poor neonatal outcomes. This can result from not only a higher incidence of multiple pregnancy, but also the manipulation involved in ART processes (37). The high twinning rate is directly linked to the number of embryos transferred (38). Particularly at risk are young women who have good quality embryos. Single embryo transfer (SET) can decrease the incidence of multiple pregnancies, including twin pregnancies, after assisted reproduction. Among our study population, we had 2 twin pregnancies (4.2%) in the fresh ET group and 1 twin pregnancy (1.8%) in the frozen ET group. In a recent study, Stormlund et al. (39) compared the ongoing pregnancy rate (OPR) between a freeze-all strategy and a fresh transfer strategy in ART treatment in women with regular menstrual cycles. They had 223 patients in the freeze-all group and 230 in the fresh transfer group, no twin pregnancies occurred in either of the groups in their study, that is lower than the twin rate in our study, probably due to fewer number of embryos transferred. In another study performed by Ashrafi et al. (40), the factors affecting the outcome of a frozen ET cycle were assessed. The number of singletons in their study was 45 (78.9%), and multiple pregnancies were observed in 21.1% (17.6% twins and 3.5% triplets), twin percentage was higher compared to our study, this can be explained by different number of embryos transferred.

Application of a proper embryo scoring system has many potential benefits such as; i. Accurate selection of embryos prior to transfer, ii. Reduction of the risk of multiple pregnancies, iii. Assessment of different culture media, and iv. Comparison of embryo quality between patient cycles. Quality assessment of cleavage stage embryos is a common method in embryology assessment accepted by numerous embryologists. For this aim, some morphological features have been suggested. The most important qualities to consider are: fragmentation rate (Fr), blastomeres irregularities, multinucleation and blastomere number (25).

Also, there were studies in the past, which evaluated the ART outcome of fresh ET and frozen ET, but present study focused on the patients who received an antagonist IVF cycle. As these patients are usually poor responders, older in age, or polycystic ovary syndrome (PCOS) cases, and therefore a much harder group to achieve pregnancy. This study had its limitations. It was a single-center research project with limited study population; therefore, we suggest performing same studies on a larger study population, prospective, or multi centric.

Conclusion

In order to have a better chance of ET with higher success rates, we studied the fertility rate and ART outcome of fresh ET and frozen ET in antagonist IVF cycles. Currently, there is no consensus whether fresh ET versus frozen one, could improve IVF outcomes in GnRH-ant cycles. GnRH antagonists have been widely used recently as a convenient and cost-effective treatment for patients undergoing IVF, and has many advantages including: prevention of premature luteinizing hormone
References

1. Carson SA, Kallen AN. Diagnosis and management of infertility: a Review. JAMA. 2021; 326(1): 65-76.

2. Vonder Bergh M, Warth C. Fertility and infertility: definition and epidemiology. Clin Biochem. 2018; 62: 2-10.

3. Macearenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. A systematic analysis of 277 health surveys. PLoS Med. 2012; 9(12): e1001356.

4. Gluvovsky D, Pesce R, Suelo C, Quinteiro Retamar AM, Hart RJ, Ciapponi A. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. Cochrane Database Syst Rev. 2010; (1): CD006359.

5. Wasilewski T, Łukaszewicz-Zając M, Wasilewska J, Mroczko B. Biochemistry of infertility. Clinica Chimica Acta. 2020; 508: 185-190.

6. Fauser BC, Dietrich K, Devroey P. Predictors of ovarian response: progress towards individualized treatment in ovulation induction and ovarian stimulation. Hum Reprod Update. 2008; 14(1): 1-14.

7. Roque M, Vallee M, Guimarães F, Sampaio M, Geber S. Freeze-all cycle: a new approach to ovarian hyperstimulation. Reprod Biol Endocrinol. 2012; 10: 26.

8. Gerber RS, Fazzari M, Kappy M, Cohen A, Galperin S, Lieberman H, et al. Differential impact of controlled ovarian hyperstimulation on live birth rate in fresh versus frozen embryo transfer cycles: a Society for Assisted Reproductive Technology Clinic Outcome System study. Fertil Steril. 2020; 114(6): 1225-1231.

9. Kliman HJ, Frankfurter D. Clinical approach to recurrent implantation failure: evidence-based evaluation of the endometrium. Fertil Steril. 2010; 93(11): 168-169.

10. Zhao Y, He D, Zeng H, Luo Y, Yang S, Chen J, et al. Expression and significance of miR-30d-5p and SOCS1 in patients with recurrent implantation failure during implantation window. Reprod Biol Endocrinol. 2021; 19(1): 138.

11. Zhang D, Han M, Zhou M, Liu M, Li Y, Xu B, et al. Down-regulation of S100P induces apoptosis in endometrial epithelium during GnRH agonist protocol and GnRH antagonist protocol. Int J Fertil Steril. 2019; 13(12): 1294-1296.

12. Yeh JS, Steward RG, Dude AM, Shah AA, Goldfarb JM, Muasher SJ. Pregnancy rates in donor oocyte cycles compared to similar autologous in vitro fertilization cycles: an analysis of 26,457 fresh cycles from the Society for Assisted Reproductive Technology. Fertil Steril. 2014; 102(2): 399-404.

13. Orvieto R, Meltzer S, Rabinson J, Zohav A, Antony EY, Nahum R. GnRH agonist versus GnRH antagonist in ovarian stimulation: the role of endometrial receptivity. Fertil Steril. 2008; 89(4): 1294-1296.

14. Chan JM, Sukumar AI, Ramalingam M, Ranbir Singh SS, Abdul MF. The impact of endometrial thickness (EMT) on the day of human chorionic gonadotropin (hCG) administration on pregnancy outcomes: a 5-year retrospective cohort analysis in Malaysia. Fertil Res Pract. 2018; 4: 5.

15. Basir GS, O WS, So WW, Ng EH, Ho PC. Evaluation of cycle-to-cycle variation of endometrial responsiveness using transvaginal sonography in women undergoing assisted reproduction. Ultrasound Obstet Gynecol. 2002; 19(5): 484-489.

16. Dessolle L, Darai E, Cornet D, Rouzier R, Coutant C, Mandelbaum J, et al. Determinants of pregnancy rate in the donor oocyte model: a multivariate analysis of 450 frozen-thawed embryo transfers. Hum Reprod. 2009; 24(12): 3082-3089.

17. Yang J, Zhang X, Ding X, Wang Y, Huang G, Ye H. Cumulative live birth rates between GnRH-agonist long and GnRH-antagonist protocol in one ART cycle when all embryos transferred: a randomized controlled study. Fertil Steril. 2021; 19(1): 124.

18. Pereira N, Neri QV, Lekovich JP, Palermo GD, Rosenwaks Z. The role of in-vivo and in-vitro maturation time on ooplasmic dysmaturity. Reprod Biol Endocrinol. 2016; 32(4): 401-406.

19. Yang M, Lin L, Sha C, Li T, Gao W, Chen L, et al. Which is better for mothers and babies: fresh or frozen-thawed blastocyst transfer? BMC Pregnancy Childbirth. 2020; 20: 559.

20. Luo X, Pei L, Li F, Li C, Huang G, Ye H. Fixed versus flexible agonist protocol in women with predicted high ovarian response except PCOS: a randomized controlled trial. BMC Pregnancy Childbirth. 2021; 21(1): 348.

21. Xia M, Zheng J. Comparison of clinical outcomes between the depot gonadotropin-releasing hormone agonist protocol and gonadotrophin-releasing hormone antagonist protocol in normal ovarian responders. BMC Pregnancy Childbirth. 2021; 21(1): 372.

22. Depalo R, Jayakrishan K, Garruli G, Toto R, Pannarino M, Giorgino F, et al. GnRH agonist versus GnRH antagonist in in vitro fertilization and embryo transfer (IVF/ET). Reprod Biol Endocrinol. 2012; 10: 26.

23. Liu Y, Su R, Wu Y. Cumulative live birth rate and cost-effectiveness analysis of gonadotropin releasing hormone-agonist protocol and multiple minimal ovarian stimulation in poor responders. Front Endocrin (Lausanne). 2020; 11: 605939.

24. Halvaei I, Khalili MA, Esfandiari N, Safari S, Talebi AR, Miglietta S, et al. Ultrasound of cystic ovarian syndrome, and poor responders are harder groups of patients to achieve pregnancy. Ultrasound Obstet Gynecol. 2002; 19(5): 484-489.
Fresh ET vs. FET in Antagonist IVF

Pasha H, et al. Comparison of pregnancy rate between fresh embryo transfers and frozen-thawed embryo transfers following ICSI treatment. Int J Reprod Biomed. 2016; 14(1): 39-46.

Seyedoshohadai F, Rezaei M, Allahveisi A, Rahmani K, Amirkhani Z. Effect of fresh and frozen embryo transfer method on fertility success in assisted reproduction: a comparative study. J Postgrad Med Inst. 2019; 33(2).

Roque M, Valle M, Guimarães F, Sampaio M, Geber S. Freeze-all policy: fresh vs. frozen-thawed embryo transfer. Fertil Steril. 2015; 103(5): 1190-1193.

Liu X, Bai H, Shi W, Shi J. Frozen-thawed embryo transfer is better than fresh embryo transfer in GnRH antagonist cycle in women with 3-10 oocytes retrieved: a retrospective cohort study. Arch Gynecol Obstet. 2019; 300(6): 1791-1796.

34. Zhu L, Zhang Y, Liu Y, Zhang R, Wu Y, Huang Y, et al. Maternal and live-birth outcomes of pregnancies following assisted reproductive technology: a retrospective cohort study. Sci Rep. 2016; 6: 35141.

35. Karlström PO, Bergh C. Reducing the number of embryos transferred in Sweden-impact on delivery and multiple birth rates. Hum Reprod. 2007; 22(8): 2202-2207.

36. Stormlund S, Sopa N, Zedeler A, Bogstad J, Praetorius L, Nielsen HS, et al. Freeze-all versus fresh blastocyst transfer strategy during in vitro fertilisation in women with regular menstrual cycles: multicentre randomised controlled trial. BMJ. 2020; 370.

37. Ashrafi M, Jahangiri N, Hassani F, Akhoond MR, Madani T. The factors affecting the outcome of frozen–thawed embryo transfer cycle. Taiwan J Obstet Gynecol. 2011; 50(2): 159-164.