TREND ANALYSIS OF FROZEN EMBRYO TRANSFER AT ALHIKMAH FERTILITY CENTER, ADDIS ABABA, ETHIOPIA

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ABSTRACT

BACKGROUND: In recent years, the use of frozen embryo transfers in Artificial reproductive technology has gradually increased, owing to improvements in laboratory techniques such as vitrification and blastocyst culture making pregnancy rates approaching those of fresh transfer cycles.

OBJECTIVE: The objective of this study is to determine the pregnancy rate after frozen embryo transfer, analyze the clinical factors that can potentially influence the result of pregnancy and identify patients who could benefit from this approach.

METHODS: A retrospective cross sectional study design was used to select patients that have undergone frozen embryo transfer from January 1, 2019- March 31, 2022 at Alhikmah fertility center, Addis Ababa, Ethiopia. Baseline data and pregnancy rate was collected, computed with SPSS version 24 for statistical associations and presented with tables. The primary outcome was a positive pregnancy test defined as serum β-HCG level of >10mIU/ml.

RESULT: A total of 180 frozen embryo transfers were done for 128 women over the study period and the pregnancy rate was 26.6%. The mean age of the patients was 31.88(± 5.04). Majority (45.3%) had tubal factor infertility. ≥ 13(median) oocytes were collected for 53.9%. A freeze-all technique was used in 46% of the patients and the rest had extra embryos. Age of the patient <35 and retrieval of oocytes above median had relation with positive pregnancy outcome. More importantly, freezing for risk of hyperstimulation and transfer of day 5embryos had a significant association with pregnancy with p-value <0.05.

CONCLUSION: From this study, we've seen promising pregnancy rate after frozen embryo transfer in patients with age group <35 and those having ≥13 oocyte. In addition, we can conclude that freezing for women at risk of hyperstimulation and transferring day 5embryos leads to a positive pregnancy result. However, since this is a retrospective study, we suggest a prospective comparative study to be done in the future for better recommendation.

KEYWORDS: Frozen embryo transfer, pregnancy rate, outcome predictors, assisted reproductive technology

(The Ethiopian Journal of Reproductive Health; 2023; 15; 47-55)

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INTRODUCTION

Assisted reproductive technology (ART) has evolved rapidly and its utilization has increased over the years. Globally, the number of initiated and frozen embryo transfer (FET) cycles has increased by 4.8% and 46%, respectively in the years 2010 to 2015; the delivery rate after IVF/ICSI remained relatively constant over the five year period (20% vs. 19.2%) whereas delivery rate after FET increased by 16% (20.7% vs., 24.8%).[1, 2].

Similarly, the African registry (ANARA) released reports of ART utilization from 13 countries in 2013 and 18 countries in 2017, but only with 10% increment of initiated cycles. Nevertheless, utilization of FET and PR from FET has increased from 17.2% to 24.2% and 27.6% to 37.9%, from 2013 to 2017, respectively[3, 4].

In Ethiopia, there are currently few private and one public centers providing ART services. The ART cycles has shown an increment of 77% and FET cycles by 94% from 2016-2022 as seen from the report in our center.

Reasons why FET success was lower in the past:

Typically, when FET was performed, the embryos that were frozen were those that were not chosen for the initial fresh ET, since the "best" embryos are already chosen for the initial transfer. As in the case of fresh embryo transfer, embryo quality has a profound effect on the chance for pregnancy.[5, 6]

The other reason is the method to freeze embryos underwent a big change over the years. In the past, embryos were frozen by a slow method. More recently, embryos are frozen by a fast method known as vitrification with better survival rates >90% and PR[5, 6].

So far several studies have shown pregnancy rate after FET of 27.8-66 %[4, 7-11] which is actually comparable to the pregnancy rate after fresh embryo transfer (33-64.6%)[4, 7-11]

Currently, researches are focusing on whether the overall IVF population could benefit from a freeze-all strategy, which yields improved reproductive outcomes especially in women with increased risk of ovarian hyperstimulation syndrome[7, 8, 12, 13, 14, 15].

The pregnancy outcomes after the FET is known to be dependent on multiple clinical factors like age of the patient, FSH level, number of oocytes, reason for freezing, day of embryo[4, 7, 8, 15, 16, 17]

Additionally FET is beneficial for patients with cancer to preserve their fertility, to do PGD test, for women having uterine problem and also for women with high progesterone[24-26].

As previously shown, the success of FET has improved over the years and its utilization has increased worldwide. Thus, the objective of this study is to assess the PR and clinical factors that can influence PR after FET in our center; and to compare our results with available literature. This study is hoped to answer the following questions.

What is the PR after FET?

What are the clinical factors associated with pregnancy after FET?

Which patients can benefit from FET?

METHODS

Study setting, period, and design

This was a retrospective cross sectional study of all patients that have undergone FET from January1, 2019-March 31, 2022. The study is conducted at Alhikmah fertility center located in Addis Ababa, Ethiopia which is the first private IVF center in the country. The center established in 2016, provides advanced ART services and has served >5000 patients till present.

Study variables

Dependent variables: Positive pregnancy test defined as serum β-HCG level of >10mIU/ml determined 10-12 days after FET. The pregnancy rate was determined as a ratio between the number of positive β-HCG tests and the total number of frozen embryo transfers.

Independent variables: age, BMI, FSH, number of oocytes, reason for freezing, day of embryo

Procedures

Ovarian stimulation was achieved using either recombinant FSH and LH (Menopure) or

recombinant FSH (Gonal F). The patients underwent pituitary desensitization using either GnRH agonist or antagonist protocol. After giving HCG trigger, egg collection was conducted. After that, embryos were cryopreserved by vitrification method. At the start of new menstrual cycle, patients received exogenous estrogen therapy for endometrial preparation before the embryo transfer. Endometrial thickness was documented by transvaginal ultrasonography. When endometrial thickness had reached 8mm or more, dyhydrogestrone were commenced. Embryos were transferred using a Labotect or Wallace catheter.

RESULT

1. Baseline characteristics

The present study was conducted on 128 patients who had undergone FET. Table 1 gives an overview of the different characteristics of the patients. Among the patients 87(68%) were below the age of 35. The minimum age was 21 and the maximum was 42 with the mean age being 31.88(± 5.04). A quarter of patients had previous pregnancy of which 14% were with IVF. Majority (45.3%) of patients had tubal factor infertility.

Table1: Baseline characteristic of women undergoing FET at Alhikmah fertility center, Jan 1, 2019- Mar 31, 2022

Characteristics		No	%
Age	<35	87	68
	≥35	41	32
Infertility type	Primary	114	89.1
	Secondary	14	10.9
	<18.3	9	7
BMI(kg/m2)	18.3-24.9	74	57.8
	25-29.9	40	31.3
	30-34.9	5	3.9
	Tubal	58	45.3
Cause of infertility	Male	43	33.6
	PCOS	7	5.5
	Combined	8	6.3
	Unexplained	10	7.8
	POF	2	1.6
FSH level(miu/ml)	<10	114	89.1
	≥10	14	10.9

2. Clinical characteristics

The long protocol was used for 53.6% of patients. 60.2% had a good response with 4-17 oocytes collected. Freeze-all strategy was followed for 59(46%) cases; majority 42(71.1%) was for women at risk of hyperstimulation. 101(78.9%) of embryos were frozen at blastocyst stage.

Table 2: Clinical characteristic of patients at Alhikmah fertility center, Jan 1, 2019- Mar 31, 2022

Characteristics		No	%
Stimulation protocol	Agonist	69	53.9
	Antagonist	59	46.1
	<4	8	6.3
No of oocytes collected	<4	8	6.3
	4-17	77	60.2
	≥18	43	33.6
Reason of freezing	Extra embryos	69	53.9
	Risk of OHSS	42	32.8
	High progesterone	13	10.2
	Endometrial mass	4	3.1
Day of embryos	Day 3	27	21.1
	Day 5	101	78.9
No of embryos transferred	1	23	18
	2	69	53.9
	3	36	28.1

3. Magnitude of positive pregnancy and influencing factors

We also performed forward logistic regression for all clinical and embryological factors that we assumed to have an influence on β-HCG result. Out of the 128 patients for whom FET was done, 34(26.6%) of them had positive pregnancy test. From the different clinical factors, significant associations were found using binary logistic regression tests between pregnancy rate and age of the patient, oocyte above median, freezing for risk of hyperstimulation and Day 5 embryos. But no association was found with BMI, FSH level, cause of infertility, stimulation protocol and number of embryos transferred.

Table3. The univariate statistical analysis of clinical factors influencing the positive pregnancy in frozen embryo transfer at Alhikmah fertility center, Jan 1, 2019- Mar 31, 2022

Characteristics	Negative (n=94)	Positive(n=34)	P-value
Age mean	32.47(± 5.12)	30.24(±4.49)	0.027*
Age			
<35	59(62.8%)	28(82.4%)	0.036*
>35	35(37.2%)	6(17.6%)	
BMI(kg/m2)			
<18.3	6(6.4%)	3(8.8%)	0.739
18.4-24.9	57(60.6%)	17(50%)	
25-29.9	27(28.7%)	13(38.2%)	
30-34.9	4(4.3%)	1(2.9%)	
FSH			
<10miu/ml	82(87.2%)	32(94.1%)	0.274
≥10miu/ml	12(12.8%)	2(5.9%)	
Number of oocytes retrieved, median	11.0(2.0,20.0)	16.0(9.0,25.0)	0.023*
Stimulation protocol			
Agonist	51(54.3%)	18(52.9%)	0.896
Antagonist	43(45.7%)	16(47.1%)	
Reason of freezing			
Extra embryo	58(61.7%)	11(32.4%)	0.031*
Risk of OHSS	24(25.5%)	18(52.9%)	
High progesterone	10(10.6%)	3(8.8%)	
Endometrial mass	2(2.1%)	2(5.9%)	
No of embryo transferred			
1	21(22.3%)	2(5.9%)	0.101
2	48(51%)	21(61.8%)	
3	25(26.5%)	11(32.4%)	
Day of embryos			
Day 3	24(25.5%)	3(8.8%)	0.014*
Day 5	70(74.5%)	31(91.2%)	

Note: *P<0.05

4. Multivariable regression of factors associated with pregnancy result

A standard multiple regression was performed to assess variables associated with pregnancy to control confounders. It was found out that women for whom FET was done risk of hyperstimulation was 15 times more likely to get pregnant than women who had FET for other reason (AOR; 15.45 95% CI 1.4-170.56, P-0.025). Patients who had Day 5 embryos transferred were 4.54 times more likely to get pregnant compared to those with day 3 embryo transfer (AOR 4.54 95% CI 0.93- 22.09, p-0.03).

Table 4: Multivariable logistic regression test for associated factors with pregnancy at Alhikmah fertility center, Jan 1, 2019- Mar 31, 2022

Characteristics	AOR	P-value	95% CI
Age			
<35	0.43	0.138	0.148
≥35			1.302
Number of oocytes retrieved, median	1.17	0.788	0.372 3.688
Reason of freezing Extra embryo Risk of OHSS	15.45	0.025	1.400 170.562
High progesterone	3.83	0.3	0.29- 50.34
Endometrial mass	8.42	0.11	0.59- 118.6
Day of embryos			
Day 3			
Day 5	4.54	0.038	0.936 22.095

The present retrospective study was carried out to provide a better understanding of the clinical factors in predicting the pregnancy rate of frozen-thawed embryo transfers using data from frozen embryo transfer cycles performed at Alhikmah fertility center, Addis Ababa, Ethiopia. In this review, we've found the overall PR from FET is 26.6%.

Our PR shows comparable outcome as in 27.8%[11], 25.8%[18], 28.1%[20] and 27.6%[23] but a lower value when compared with studies who had PR of 37.9% [4], 66%[7], 64%[8], 39%[9], 62.3%[10], 44%[21], 63%[15]. This could be due to the low number of subjects in our study group.

It is well known that age is a significant factor in the success of IVF whether in fresh or frozen transfer cycles.[16, 19, 22, 23] In our study, the mean age of the patients was 31.88(± 5.04) comparable with most other studies[16, 18, 19, 20, 29]. Furthermore, 82.4% of our clients below the age 35 were pregnant compared to 73.5%, Dyer et al[4], 66%, Chen et a[17] and 57.7% Eftekhar et al[16].

The PR in those above 40 years was 2.9% in this study, compared to the 15.4% Liu et al[27] 7.7% [20], 7.5%[19] and nil[23]. In conclusion age is a significant factor associated with positive pregnancy with p-value<0.05 as shown in this study as well in others[4, 7, 16, 18, 19, 23].

FSH is one of the tests that predict ovarian response to stimulation, and thus increase the chance of pregnancy. In those women with FSH of > 10miu/ml, PR was 56.3% Eftekhar et al[16], 17.3% Roque et al[28], 15.4%, Liu et al[27], and 5.9%, in our study. In a study done by Ahmed et al[29] the clinical pregnancy was significantly higher in women with FSH levels less than 9 IU/L compared with those with an FSH level of 9 IU/L or more. In another investigation, Eftekher et al[16] reported inverse correlation between basal serum FSH levels before fresh IVF/ICSI cycle with pregnancy outcome in FET cycles unlike this study where no association was found. This could again be due to the smaller sample size than the other studies and also the different laboratory calibrations might have an impact on the Regarding the relationship between the type of protocol used and pregnancy, no significant association was found in this study as well in others.[16, 21] but Mahnaz et al[20] found that the implantation rate in women stimulated with gonadotrophin releasing hormone agonist long protocol was higher than that in the patients stimulated with antagonist protocol ($p < 0.05$).

The other clinical factor influencing PR is the number of oocytes retrieved. A number of studies showed that a higher number of oocytes, all above 11 oocytes, are significantly associated with positive pregnancy.[8, 9, 15] In this study, the median number of oocytes found was 13, with PR in the group above the median 70.5% compared to 39% and 87.5%, Aflatoonian et al[9], Shapiro et al[8], 63.1%[15] respectively.

However in one study, Mahnaz et al[20] found out that women with a lower number of retrieved oocytes had more chance to have a positive β-HCG result, meaning that with increasing number of oocytes, the chance of pregnancy decreases. This

suggests that with increasing number of oocytes, the quality may reduce. But this was shown in any other researches.

In < 4 oocytes collected, PR was 17.3%, Roque et al [28], 9.6% Liu et al [27] and 2.9% in this study. The number oocytes collected is significantly associated with positive outcome with p-value of <0.05.

There are a number of reasons for freezing of embryos, the commonest being presence of extra embryos and risk of hyperstimulation; PR was highest, 52.9%, recorded in those for whom embryo was frozen for risk of hyperstimulation with p-value of 0.03 even after controlling for possible confounders with multiple regression analysis. This was also shown in one study where PR was 63.1% in women with risk of hyperstimulation after FET, Absalan et al[15]. However, in one retrospective study Eftekhar et al[16], PR didn't differ between FET done for risk of hyperstimulation and extra embryos, 54.5% vs. 50.2%, respectively. Nevertheless, the increased PR rate was basically seen in many studies that led to the conclusion that freezing should be reserved for this category of patients.[7, 8, 11, 14 and 15].

There is a controversy among studies in the relationship between the number of embryos transferred and pregnancy outcome. Some researchers have reported a higher pregnancy rate with greater number of embryos transferred, and other studies fail this relationship. Salumets et al [18] indicated that the pregnancy (positive hCG) and clinical pregnancy rates were significantly higher after Double Frozen embryo transfer than they were after Single Frozen embryo transfer. However this association was not seen, in this study as well in others[16, 20, 23].

Regarding the day of embryos for transfer, many researches showed a positive outcome with transfer of blastocysts rather than day 3 embryos.[5, 6] In this study, with 78.9% of embryos transferred on day 5 the PR was 91.2% which was comparable to 85.7%, Shapiro et al[8] which showed significant association with p-value of <0.05. This shows that there is better PR with transfer of embryos at blastocyst stage.

CONCLUSION

There a number of factors affecting the success of IVF/FET. From this study, we've seen promising pregnancy rate after frozen embryo transfer in patients with age group <35and those having ≥13 oocyte. In addition we can conclude that freezing for women at risk of hyperstimulation and transferring day 5 embryo leads to a positive pregnancy. However, since this is a retrospective study, we suggest a prospective comparative study to be done in the future for better recommendation.

DECELERATIONS

Strength and limitation of the study

This is the first study done in Ethiopia for the objective of assessing the PR after FET which can serve as a baseline study to lay the foundation for further researches. The study has some limitations. The first is the small sample size which makes it difficult to create stronger associations. Additionally, the study being a retrospective cross sectional study, it is hard to generalize the result to the whole ART population.

Acknowledgement

We would like to thank individuals who were involved in tracing and retrieving the charts and for those who helped us in each step of the way.

Abbreviation

ANARA- African Network and Registry for Assisted Reproductive Technology
ART- Assisted Reproductive Technology
BMI- Body Mass Index
ET-Embryo Transfer
FET- Frozen Embryo Transfer
FSH- Follicular Stimulating Hormone
GnRH- Gonadotropin Releasing Hormone
HCG- Human Chorionic Gonadotropin
ICMART-International Committee for Monitoring Assisted Reproductive Technologies
ICSI- Intracytoplasmic Sperm Injection
IVF- In vitro Fertilization
OHSS- Ovarian Hyper stimulation Syndrome

PCOS- Polycystic Ovarian Syndrome
POF- Premature Ovarian Failure
PR- Pregnancy rate

Funding: The authors did not receive any funding for this study.

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REFERENCES

1. S Dyer, G M Chambers, J de Mouzon, K G Nygren and et al. International Committee for Monitoring Assisted Reproductive Technologies world report: Assisted Reproductive Technology 2008, 2009 and 2010. Hum Reprod. 2016; 31(7):1588-609.
2. ICMART PRELIMINARY WORLD REPORT 2015
3. S. Dyer, Paversan Archary, Jacques de Mouzon, Moise Fiadjoe, Oladapo Ashiru. Assisted reproductive technologies in Africa: first results from the African Network and Registry for Assisted Reproductive Technology, 2013. RBMO 2019; 38(2).
4. S. Dyer, Paversan Archary, Liezel Potgieter, et al. Assisted reproductive technology in Africa: a 5-year trend analysis from the African Network and Registry for ART. Reproductive Bio Medicine Online. 2020; 41(4):604-615.
5. Vajta G, Nagy ZP, Cobo A, Conceicao J, Yovich J. Vitrification in assisted reproduction: myths, mistakes, disbeliefs and confusion. Reprod Biomed 2009; 19:1.
6. Kai Mee Wong, Sebastiaan Mastenbroek, and Sjoerd Repping. Cryopreservation of human embryos and its contribution to in vitro fertilization success rates. Fertility and Sterility.2014; 102:19–26.
7. Chen ZJ, Shi Y, Sun Y, et al. Fresh versus frozen embryos for infertility in the polycystic ovary syndrome. N Engl J Med 2016; 375:523–33.
8. Shapiro BS, ST Daneshmand, FC Garner and et al. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfers in high responders. Fertil Steril 2011; 96:516–8.
9. Abbas Aflatoonian, Homa Oskouian and Leila Oskouian. Can fresh embryo transfers be replaced by cryopreserved- thawed embryo transfers in assisted reproductive cycles? A randomized controlled trial. J Assist Reprod Genet. 2013; 30(9): 1245.
10. Y. Shi, Y. Sun, C. Hao, H. Zhang, D. Wei. Transfer of Fresh versus Frozen Embryos in Ovulatory Women. N Engl J Med 2018; 378: 126-36.
11. Sacha Stormlund, Negjyp Sopa, Anne Zedeler, Jeanette Bogstad and et al. Freeze-all versus fresh blastocyst transfer strategy during in vitro fertilization in women with regular menstrual cycles: multicenter randomized controlled trial. BMJ 2020; 370.
12. Roque M, Valle M, Kostolias A, Sampaio M, Geber S. Freeze-all cycle in reproductive medicine: current perspectives. JBRA Assist Reprod. 2017; 21(1):49-53.
13. Dahan MH, Tannus S, Seyhan A, Tan SL, Ata B. Combined modalities for the prevention of ovarian hyperstimulation syndrome following an excessive response to stimulation. Gynecol Endocrinol 2018; 34:252.
14. Practice Committee of American Society for Reproductive Medicine, Ovarian hyperstimulation syndrome, Fertil Steril 2008; 90:188.
15. Forouzan Absalan, Alireza Ghannadi, and Marjaneh Kazerooni. Reproductive outcome following thawed embryo transfer in management of ovarian hyperstimulation syndrome. J Reprod Infertil. 2013; 14(3):133-137.
16. Maryan Eftekhar, Elham Rahmani, Soheila Pourmasumi. Evaluation of clinical factors influencing pregnancy rate in frozen embryo transfer. Iran J Reprod Med 2014; 12(7): 513-518.
17. Matheus Roque, Marcello Valle, Fernando Guimarães, Marcos Sampaio, Selmo Geber. Freeze-all cycle for all normal responders? J Assist Reprod Genet.2017; 34(2):179-185.
18. Andres Salumets, Anne-Maria Suikkari, Sirpa Mäkinen , Helle Karro , Anu Roos and Timo Tuuri. Frozen embryo transfers: implications of clinical and embryological factors on the pregnancy outcome. Human Reproduction 2006; 21(9): 2368–2374.
19. J. X. Wang, Y. Y. Yap and C. D. Matthews. Frozen-thawed embryo transfer: influence of clinical factors on implantation rate and risk of multiple conception. Human Reproduction 2001; 16(11): 2316-2319.
20. Mahnaz Ashrafi, Nadia Jahangiri, Fatemeh Hassani, Mohammad Reza Akhoond, Tahereh Madani. The factors affecting the outcome of frozen-thawed embryo transfer cycle. Taiwanese Journal of Obstetrics & Gynecology 2011; (50): 159-164.
21. Maryam Eftekhar, Razieh Dehghani Firouzabadi, Hesamoddin Karimi, Elham Rahmani. Outcome of cryopreserved-thawed embryo transfer in the GnRH agonist versus antagonist protocol. Iran J Reprod Med 2012; 10(4): 297-302.
22. Preutthipan S, Amso N, Curtis P, Shaw RW. Effect of maternal age on clinical outcome in women undergoing in vitro fertilization and embryo transfer (IVF-ET). J Med Assoc Thai 1996; 79: 347-352.
23. Mary E. Schalkoff, Selwyn P. Oskowitz, R. Douglas Powers. A multifactorial analysis of the pregnancy outcome in a successful embryo cryopreservation program. Fertil Steril1993;59:1070-4
24. Oktay K, Rodriguez-Wallberg K, Schover L, Preservation of fertility in patients with cancer, N Engl J Med 2009; 360:268.
25. Practice Committees of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology, The use of preimplantation genetic testing for aneuploidy (PGT-A): a committee opinion. Fertil Steril 2018; 109:429.

26. Labarta E, Martínez-Conejero fnameJA, Alamá P and et al. Endometrial receptivity is affected in women with high circulating progesterone levels at the end of follicular phase: a functional genomics analysis. Hum Reprod. 2011; 26:1813-5.
27. Conghui Liu, Yu Li, Hong Jiang, Yingchun Liu1, Xiaomin Song. The clinical outcomes of fresh versus frozen embryos transfer in women ≥40 years with poor ovarian response. Obstet Gynecol Sci. 2021; 64(3):284-292.
28. M Roque, M Valle, M Sampaio, S Geber. Does freeze-all policy affect IVF outcome in poor ovarian responders? 2018; 52(4):530-534.
29. Ahmed Kassab, Luca Sabatini, Amanda Tozer, Ariel Zosmer, Magdy Mostafa, and Talha Al-Shawaf. The correlation between basal serum follicle stimulating hormone levels before embryo cryopreservation and the clinical outcome of frozen embryo transfers. Fertil Steril 2009; 92:1269–75.