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RESEARCH

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Reproductive outcomes in women with advanced endometriosis in fresh versus frozen embryo transfer cycles

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Abstract

Background ART in women with endometriosis is associated with poor quality of the retrieved oocytes and lower fertilization and pregnancy rates, reflecting that endometriosis may influence fertility by altering the quality of both the oocyte and embryo quality and also by impairing the endometrial receptivity. On comparing endometriosis-affected patients to healthy counterparts, many differences were demonstrated at the endometrial level. Thus, choosing the appropriate method of embryo transfer is of utmost importance, particularly for patients with advanced endometriosis.

Objective The aim of the present study was to compare the reproductive outcomes between fresh and frozen embryo transfer cycles in women with advanced endometriosis.

Material and methods A retrospective cohort study was conducted in the period from January 2018 until December 2021 for patients recruited from two IVF centers, Alexandria, Egypt. Careful review of paper and electronic medical records of infertile women (primary, relative, or secondary infertility) aged 18–37 years who were diagnosed with advanced endometriosis by means of laparoscopy and were scheduled for ICSI followed by either fresh embryo transfer (group I) or freeze-all embryos and deferred embryo transfer (group II) of day 5 embryo(s) was included in the study.

Results Two-hundred and eleven women were eligible and included in the study. Women in each study group were matched regarding baseline characteristics. Clinical pregnancy, implantation, and ongoing pregnancy rates were statistically significantly higher in the group of frozen embryo transfer ($p < 0.001$). Miscarriage rate was found to be higher in the group of fresh transfer compared to FET group but without a statistical significance (20.9% vs 9.2%, $p = 0.072$).

Conclusion In women with advanced endometriosis, freeze-all policy seems to be associated with better implantation, ongoing pregnancy rates.

Keywords Endometriosis, Freeze all, Frozen embryo transfer, Ongoing pregnancy rate

Introduction

Endometriosis is defined as a disease characterized by the presence of endometrium resembling epithelium and/or stroma outside the endometrium and myometrium, usually in association with an inflammatory process [1]. It roughly affects 10% (approximately 190 million women worldwide) of girls and women of reproductive age globally.

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Endometriosis-infertility relationship has been a point of debate for decades. The fecundity ranges from 15 to 20% per month in normal couples and declines with age. Patients with endometriosis have a decreased monthly fecundity of about 0.2–1% each month [2, 3]. Moreover, endometriosis is associated with decline in the live birth rate (LBR) [4]. Although ART remain the most effective treatment modality for infertile women with endometriosis, it still yields poor outcomes as ART could not overcome all the substantial effects of endometriosis [5, 6]. Endometriosis-affected women have declined pregnancy and implantation rates in comparison to women without endometriosis [7].

It was noticed that endometriosis has undesirable effect on the pregnancy, miscarriage, and live birth rates. It was found that ART outcomes, as the quality of the retrieved oocytes and fertilization rate, are negatively influenced by the existence of endometriosis, reflecting that endometriosis influence fertility by altering the quality of both the oocyte and embryo quality and by impairing the endometrial receptivity [6]. On comparing endometriosis-affected patients to healthy counterparts, many differences were demonstrated at the endometrial level [8], which define the decline in the receptivity of the endometrium. Thus, choosing the appropriate method of embryo transfer is of utmost importance, particularly for patients with advanced endometriosis.

In the view of the impact of the supraphysiological level of hormones associated with stimulation in fresh embryo transfer on the uterine environment during early peri-implantation, it was demonstrated that flowing of blood in the endometrium and sub-endometrium is reduced in stimulated cycles in comparison to natural cycles as evaluated by three-dimensional power Doppler ultrasonography [9]. Furthermore, histopathologic alterations of the stimulated endometrium were reported, and this includes advancement in the maturation of the endometrium [10] and premature development of channel systems in the nucleolus [11].

On the other hand, in frozen embryo transfer (FET) cycles, endometrial growth can be more controlled than in fresh cycles [12]. Based on that, the use of frozen embryos avoided the supraphysiological hormone level found during ovarian hyperstimulation in in vitro fertilization (IVF)/ICSI with fresh embryo transfer, which leads to an unfavorable environment for implantation and has been found to have detrimental effects on the endometrial receptivity, irrespective of the amount of retrieved oocytes or levels of progesterone (P) [13–16].

Therefore, the aim of the present study was to compare the reproductive outcomes between fresh and frozen embryo transfer cycles in women with advanced endometriosis.

Study design and setting

A retrospective cohort study was conducted in the period from January 2018 until December 2021 for patients recruited from two IVF centers (Agial Fertility Center & Dar-Elkhosoba Center), Alexandria, Egypt.

Materials and methods

Careful review of paper and electronic medical records of infertile women (primary, relative, or secondary infertility) aged 18–37 years who were diagnosed with advanced endometriosis, stage III/IV r-ASRM classification [17], by means of laparoscopy and were scheduled for intracytoplasmic sperm injection (ICSI) followed by either fresh embryo transfer or freeze-all embryos and deferred embryo transfer of day 5 embryo(s) was included in the study.

Couples with abnormal semen analysis of the male partner, recurrent implantation failure in previous ICSI trials, and women with uterine lesions such as fibroids, adenomyosis, polypi, uterine septum, and women with no available follow up data were excluded from the study. The study protocol was approved from the Ethics Committee of Faculty of Medicine, Alexandria University.

Ovarian stimulation

All the women enrolled in the study underwent controlled ovarian stimulation via the fixed GnRH antagonist protocol. Before ovarian stimulation, women were pretreated with combined oral contraceptive pills for 2–3 weeks. On day 2 of the menstrual cycle (stimulation day 1), patients received a fixed daily dose of recombinant FSH or combination of rec-FSH and human menopausal gonadotropins. Starting on day 5 of stimulation, patients underwent monitoring with transvaginal ultrasound and serial assessment of estradiol every 2–3 days as required. A daily subcutaneous dose of 0.25 mg of GnRH antagonist cetrorelix was initiated on day 6 of ovarian stimulation and continued up to the day of trigger administration. When at least three follicles reached 17 mm in diameter, final oocyte maturation was triggered using 10,000 IU of human chorionic gonadotropin (hCG). Ovum pickup (OPU) was performed 35–36 h of hCG administration. Mature oocytes were inseminated by means of intracytoplasmic sperm injection and cultured to blastocyst stage.

Group I: Women who underwent fresh embryo transfer (ET)

Combined vaginal suppositories (400 mg twice daily) and intramuscular progesterone in oil (50 mg once daily)

injections were started on day of OPU, and ET was performed 5 days after P administration.

Group II: Women who underwent frozen embryo transfer after endometrial preparation through artificial or programmed cycle

After vitrification of the available embryos, in the subsequent cycle, women underwent ovarian suppression by combined pills for 2–3 weeks, and then endometrial preparation was achieved with a daily dose of 8 mg of estradiol valerate for at least 12–14 days. When endometrium thickness was at least 8 mm and E2 level reached at least 200 pg/dl, progesterone as vaginal suppositories (400 mg twice daily) and intramuscular in oil (50 mg once daily) injection was administered, and ET of thawed embryos was performed after 5 days of P therapy.

Outcome variables

The primary outcome of the study was the ongoing pregnancy rate (OPR) defined as pregnancy progressed beyond 14 weeks' gestation. Secondary outcomes include the following: implantation rate, defined as the number of intrauterine gestational sacs observed by transvaginal ultrasound divided by the number of transferred embryos; clinical pregnancy rate (CPR), calculated by considering clinical pregnancy and determined by the visualization of a viable gestational sac within the uterine cavity by ultrasound 3–4 weeks after embryo transfer; and miscarriage rate, defined as the number of cases who aborted (after a confirmed clinical pregnancy) divided by the total number of pregnant cases.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp.). Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR). Significance of the obtained results was judged at the 5% level. The used tests were chi-square test for categorical variables, to compare between different groups; Student *t*-test for normally distributed quantitative variables, to compare between two studied groups; and Mann-Whitney test for abnormally distributed quantitative variables, to compare between two studied groups.

Results

Over the 4-year study period, there were 237 women with advanced endometriosis who underwent either fresh or frozen embryo transfer cycles; out of them, 211 women were eligible and included in the study, and Fig. 1 demonstrates the flow chart of the study. The study included 211 patients divided into two groups as follows: Group I includes 103 patients who underwent fresh embryo transfer, and Group II includes 108 patients who underwent frozen embryo transfer.

Regarding the baseline characteristics of both groups (Table 1), patients in the two study groups were matched regarding mean age, infertility duration, type of infertility (primary or secondary), body mass index, and AMH level.

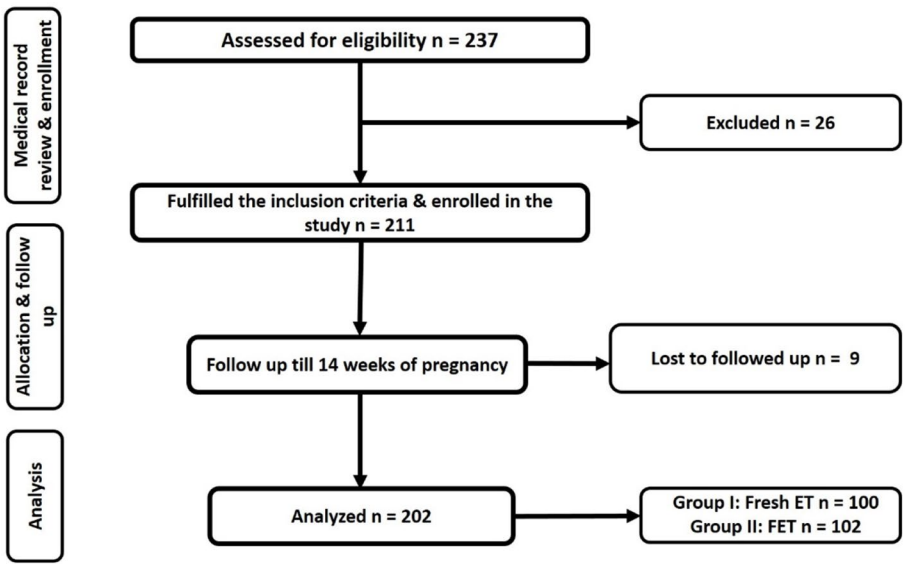

Fig. 1 Study flowchart

Table 1 Comparison between the two studied groups according to the baseline characteristics

Baseline characteristics	Fresh ET ($n=103$)		Frozen ET ($n=108$)		Test of sig	P
Mean age (years)	31.22 ± 4.94		30.87 ± 4.45		$t=0.546$	0.586
Median infertility duration (IQR) years	4.0 (3.0–7.0)		4.25 (3.0–8.0)		$U=5266.50$	0.502
Mean BMI (kg/m^2)	25.83 ± 3.26		25.01 ± 3.04		$t=1.903$	0.058
Infertility type	No	%	No	%	$\chi^2=3.987$	0.136
Primary	76	73.8	71	65.7		
Secondary	17	16.5	16	14.8		
Relative	10	9.7	21	19.4		
Mean AMH	1.91 ± 1.11		2.21 ± 1.31		$t=1.813$	0.071
AFC					$U=5064.5$	0.258
Mean	7.74 ± 2.93		8.18 ± 2.96			
Median (IQR)	7.0 (6.0–10.0)		8.0 (6.0–10.0)			
Chocolate cyst before OI					$\chi^2=0.021$	0.884
Absent	87 (84.5%)		92 (85.2%)			
Present	16 (15.5%)		16 (14.8%)			

Table 2 Comparison between the two groups regarding the number of oocyte retrieved, mature oocytes, and available embryos number at day 5

	Fresh ($n=103$)	Frozen ($n=108$)	U	p
Oocyte retrieved				
Min.–max	2.0–10.0	3.0–12.0	3710.5*	< 0.001*
Mean ± SD	5.29 ± 2.08	6.56 ± 1.93		
Median (IQR)	5.0 (4.0–7.0)	6.0 (6.0–8.0)		
MII oocytes				
Min.–max	1.0–7.0	2.0–9.0	3546.5*	< 0.001*
Mean ± SD	3.78 ± 1.62	4.87 ± 1.46		
Median (IQR)	4.0 (2.0–5.0)	5.0 (4.0–6.0)		
Embryo no. on day 5				
Min.–max	1.0–5.0	1.0–7.0	3612.0*	< 0.001*
Mean ± SD	2.55 ± 0.96	3.22 ± 1.06		
Median (IQR)	2.0 (2.0–3.0)	3.0 (2.0–4.0)		

* : statistically significant

Table 2 shows that the number of retrieved oocytes, MII (metaphase II) oocyte number, and the number of available blastocysts for transfer on day 5 were significantly higher in the group of frozen embryo transfer ($p<0.001$).

As shown in Table 3, the number of embryos transferred was either a single embryo or two embryos, and there was no statistical significant difference between the two groups (p 0.473); for the quality of the embryos transferred that were either high-quality embryos alone or low -quality embryos alone or both of them together, both groups showed no statistically significant difference (p 0.325).

The clinical pregnancy rate was significantly higher in the frozen embryo transfer group than the fresh group (82 patients (75.9%) vs 46 patients (44.7%) ($p<0.001$)); again, the FET group showed a significantly higher

Table 3 Comparison between the two studied groups according to the embryos transferred

Embryos transferred	Fresh ET ($n=103$)		Frozen ET ($n=108$)		χ^2	P
	No	%	No	%		
Number						
Single embryo	47	45.6	44	40.7	0.514	0.473
Two embryos	56	54.4	64	59.3		
Quality						
Low	26	25.2	22	20.4	2.246	0.325
High	73	70.9	77	71.3		
High + low	4	3.9	9	8.3		

Table 4 Comparison between the two studied groups according clinical pregnancy and implantation rates

	Fresh ET ($n=103$)		Frozen ET ($n=108$)		χ^2	p
	No	%	No	%		
Clinical pregnancy rate	46	44.7	82	75.9	21.597*	< 0.001*
Number of GS seen by TVU (after 3–5 weeks)	49		93		**< 0.001***	
Number of transferred embryos	159		172			
Implantation rate	30.8%		54.1%			

* : statistically significant

Table 5 Comparison between the two studied groups according to the ongoing pregnancy

	Fresh ET No. (%)	Frozen ET No. (%)	χ^2	p
Ongoing pregnancy	**($n=100$)**	**($n=102$)**	22.876*	< 0.001*
	34 (34.0%)	69 (67.6%)		

* : statistically significant

implantation rate in comparison to the fresh group as illustrated in Table 4 (30.8% vs 54.1% ($p<0.001$)).

Out of the 128 pregnant females in the study, 3 pregnant patients dropped out from the fresh group, and 6 pregnant patients dropped out from the frozen group; those 9 patients were excluded from our statistical results while estimating the miscarriage rate. The miscarriage rate was higher in the fresh ET group than in the frozen ET group; however, the difference did not reach statistical significance (9 (20.9%) vs 7 (9.2%) ($p=0.072$).

As for the primary outcome of the study, the ongoing pregnancy rate, out of the 211 patients included in our study, there was no available data for 9 pregnant patients, 3 patients in group 1, and 6 patients in group 2; those 9 patients were excluded from our statistical analysis while estimating the ongoing pregnancy rate. Sixty-nine patients in the frozen embryo transfer group (69/102 = 67.6%) continued their pregnancy beyond 14 weeks, while 33 patients (34/100 = 34%) of the fresh embryo transfer group continued their pregnancy beyond 14 weeks, and the OPR was significantly higher in the frozen embryo transfer group ($p<0.001$) (Table 5).

Furthermore, a subgroup analysis was performed to compare the pregnancy rates between women who underwent single embryo transfer (SET) and those to whom two embryos were transferred (Table 6). It was observed that the clinical and ongoing pregnancy rates in women who underwent SET and who have underwent double embryo transfer are not significantly different (in the group of fresh ET, the group of FET, and for the total sample).

Table 6 Relation between numbers of embryos transferred with clinical and ongoing pregnancy rates

	Number		χ^2	p
	1	2		
Clinical pregnancy rate				
Fresh ($n=103$)	**($n=47$)**	**($n=56$)**		
Negative	30 (63.8%)	27 (48.2%)	2.521	0.112
Positive	17 (36.2%)	29 (51.8%)		
Frozen ($n=108$)	**($n=44$)**	**($n=64$)**		
Negative	9 (20.5%)	17 (26.6%)	0.532	0.466
Positive	35 (79.5%)	47 (73.4%)		
Total ($n=211$)	**($n=91$)**	**($n=120$)**		
Negative	39 (42.9%)	44 (36.7%)	0.831	0.362
Positive	52 (57.1%)	76 (63.3%)		
Ongoing pregnancy rate				
Fresh ($n=100$)[a]	**($n=47$)**	**($n=53$)**		
Negative/miscarriage	35 (74.5%)	31 (58.5%)	2.834	0.092
Positive	12 (25.5%)	22 (41.5%)		
Frozen ($n=102$)[a]	**($n=43$)**	**($n=59$)**		
Negative/miscarriage	14 (32.6%)	19 (32.2%)	0.001	0.970
Positive	29 (67.4%)	40 (67.8%)		
Total ($n=202$)[a]	**($n=90$)**	**($n=112$)**		
Negative/miscarriage	49 (54.4%)	50 (44.6%)	1.918	0.166
Positive	41 (45.6%)	62 (55.4%)		

[a] Not available cases were excluded

Discussion

The present study showed that OPR in women with advanced endometriosis is significantly higher in the group of frozen embryo transfer compared to the fresh embryo transfer group (67.6% vs. 34%, respectively). Regarding the secondary outcomes, there was a statistically significant higher implantation and clinical pregnancy rates also in the frozen embryo transfer group. To the best of our knowledge, only few studies have addressed the issue of freeze-all policy in women with advanced endometriosis.

In agreement with the findings of the current study, Wu et al. [18] conducted a retrospective study that

encompassed 1651 women with advanced stages of endometriosis performing ICSI. After matching, 506 women and 255 women were eligible in the freeze-all group and the fresh group, respectively. In their matched cohort study, the implantation, CPR, and LBR were significantly higher in the FET group in comparison to the fresh groups. Those results coincide with the current study in all perspectives except for the LBR as it was not included in the outcomes for the study.

However, the present study differs from Wu et al., where in their study the development of the embryo was accessed on day 3, and the embryos selected to be transferred were high-quality cleavage-stage embryos only (at least six blastomeres with ≤ 20% fragmentation based on the Cummins' criteria). In the fresh embryo transfer group, patients were arranged for a day 3 fresh embryo transfer and vitrification of the extra embryos. For the FET group, vitrification on day 3 of the entire cohort of good quality embryos. While in the present study, embryo development was assessed on day 5, and patients performing fresh transfer were scheduled for a day-5 ET, while in the frozen group, the embryos were vitrified on day 5, and embryo transfer was not exclusively for high-quality embryos, but in some cases, average or low-quality embryos were transferred either alone or in combination with a high-quality embryo.

Again, the results of the present study coincided with a matched cohort prospective study conducted by Bourdon et al. [19], and they compared the results of FET to fresh ET in women having endometriosis, where the FET group involved 135 women and the fresh group involved 424 matched women. CPR was of higher significance toward the FET group in comparison to the fresh group; also, the ongoing pregnancy rate showed greater statistical significance in the FET group (34.8%) in comparison to the fresh-ET group (17.8%) ($p = 0.005$), and the live birth rate as well was of higher significance in FET in comparison to fresh embryo transfer group.

Another interesting study conducted by Mohamed et al. [20] is a retrospective, database-searched cohort study. The study included two groups: the first group had freshly transferred embryos, while the second group performed frozen embryo transfer. The primary outcome for the study was the live birth rate, while the secondary outcomes included the clinical pregnancy rate and the miscarriage rate. Out of the total number of cases, 415 (5.7%) had infertility attributed to endometriosis, in whom frozen ET cycles were associated with a relatively similar clinical pregnancy rate and live birth rate, in comparison to the clinical pregnancy rate and live birth rate of fresh ET cycles, showing no significant difference. Nevertheless, the study of Mohamed et al. differs from the present study as they considered other causes of infertility rather than endometriosis alone as a sole factor of infertility, and it also concluded that there was no significant difference between FET over the fresh ET in cases of endometriosis concerning CPR (18.2 vs. 20.2%, respectively) and LBR (16.9% vs. 15.5%, respectively).

In accordance, a recent systematic review and meta-analysis conducted by Chang et al. [21] aimed at evaluating if FET has the ability to reimpose optimal receptivity targeting better ART results in patients with endometriosis. A total of six studies with moderate methodological quality were included in the meta-analysis. Three-thousand and ten patients with endometriosis who underwent ICSI were included in the studies: 1777 performed FET, and 1233 performed fresh ET. LBR was significantly higher in the FET group in comparison to the fresh group. Although that CPR was similar between the two study groups, there was a significantly higher miscarriage rate in the fresh group.

The endometrium of endometriosis-affected women is different from that of healthy, unaffected women [8], which could be the main reason for the decline in endometrial receptivity quality. Thus, it is crucial to select the right embryo transfer technique, particularly for women who have severe endometriosis. The effects of ovarian stimulation in fresh ET cycles on the early peri-implantation uterine milieu have been documented in a number of studies, and three-dimensional power Doppler ultrasonography measurements have revealed that stimulated cycles have reduced endometrial and subendometrial blood flow in comparison to normal cycles [9]. Furthermore, certain pathologic alterations of the stimulated endometrium have been verified, such as the progression of endometrial maturation [10], as well as the early establishment of nucleolar channel systems [11].

Additionally, a number of researchers have demonstrated that during fresh embryo transfer cycles, there are abnormalities in the transcriptional activity of genes related to endometrial receptivity [22–24]. The alterations indicated above are linked to the hyperestrogenic environment created during fresh IVF, which might subsequently hinder early embryonic adhesion [18, 25] and, consequently, the embryos' capacity to implant. It follows that the current study's findings regarding implantation, clinical pregnancy, and ongoing pregnancy are better in FET cycles.

Our study has the advantage of being one of the few studies that addressed the role of FET in endometriosis-affected women and was conducted for a fair number of patients; moreover, this study focused on advanced endometriosis being the sole factor of infertility in the studied patients, excluding any other infertility-related factors; however, the main study limitation was being a retrospective analysis depending on the availability of

complete medical records, and the follow-up stopped at 14 weeks of gestation.

Conclusions

The findings of the present study suggest that frozen embryo transfer policy in cases of advanced endometriosis would result in better reproductive outcomes in comparison to fresh embryo transfer in terms of clinical and ongoing pregnancy rates. Further, randomized controlled studies are needed for confirmation of such findings.

Abbreviations

ART	Assisted reproductive technologies
LBR	Live birth rate
FET	Frozen embryo transfer
ASRM	American Society of Reproductive Medicine
CPR	Clinical pregnancy rate
OPR	Ongoing pregnancy rate
IVF	In vitro fertilization
ICSI	Intracytoplasmic sperm injection
GnRH	Gonadotropin-releasing hormone
AMH	Anti-Mullerian hormone

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Authors' contributions

HA and *AS* were the main authors responsible of the research and writing the paper. *MK* was responsible for the data collection, and *WEG* was responsible for reviewing and revising the paper. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate

The study protocol was approved by the ethical committee of the Faculty of Medicine, Alexandria University, Egypt. No informed consent was required due to the retrospective design of the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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