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INVESTIGATING FACTORS INFLUENCING CLINICAL PREGNANCY RATES IN HORMONE REPLACEMENT THERAPY FROZEN-THAWED EMBRYO TRANSFER CYCLES: A CROSS-SECTIONAL STUDY

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Approximately 50% of embryo transfer cycles are performed as frozen embryo transfer (FET) cycles; however, research on the factors influencing pregnancy rates in these cycles is limited in northern Iran. The aim of this study was to identify the factors influencing the clinical pregnancy rate in hormone replacement therapy (HRT) FET cycles among infertile women.

This descriptive-analytical observational study analyzed HRT FET cycles of 429 infertile couples whose embryos were obtained by microinjection at two in vitro fertilization (IVF) centers in Sari, northern Iran, from April 2015 to March 2019. Data were analyzed using SPSS software, version 22, with a significance level set at $p < 0.05$.

The mean ± SD age of women and men was 32 ± 2.52 and 36 ± 1.62 years, respectively. The mean ± SD age of women at the time of oocyte collection was 31.06 ± 5.3 years. Among the 429 patients, 171 cases (39.9%) achieved chemical pregnancy and 156 cases (36.3%) achieved clinical pregnancy. Multivariate regression analysis revealed significant differences between the clinically pregnant and non-pregnant groups ($p < 0.05$) in factors such as the woman's age at oocyte retrieval, duration of infertility, occupation, body mass index (BMI), developmental stage of the transferred embryo, type of catheter used for transfer, and embryo grade.

The study concluded that younger maternal age at oocyte retrieval, shorter duration of infertility, optimal BMI, higher embryo grade, and appropriate selection of transfer techniques are key determinants of achieving clinical pregnancy in HRT-FET cycles. These findings can guide specialists in optimizing FET protocols to improve pregnancy outcomes.

Keywords: frozen, embryo transfer, pregnancy, infertility, in vitro fertilization

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INTRODUCTION

In vitro fertilization (IVF) has revolutionized reproductive medicine and brought hope to couples facing infertility. Among the various techniques used in assisted reproductive technology, frozen-thawed embryo transfer (FET) has gained prominence due to its potential benefits, including the flexibility in transfer timing and reduced risk of ovarian hyperstimulation syndrome. Despite advances in cryopreservation techniques, clinical pregnancy rates following FET cycles exhibit significant variability (1, 2).

Numerous factors are thought to influence the success of FET, including embryo quality, endometrial receptivity, timing of transfer, and patient characteristics such as age and hormonal profile. In addition, clinical factors such as the ovarian stimulation protocol used, the experience of the medical team, and laboratory conditions can also play critical role. The interaction of these elements can create complex scenarios that challenge our understanding of the optimal conditions for successful implantation and subsequent pregnancy. Moreover, the increased use of preimplantation genetic testing has introduced additional dimensions to the evaluation of embryo viability and selection, adding layers to the decision-making process in FET cycles (3–5).

Investigating factors that influence clinical pregnancy rates in FET cycles is particularly important given the impact of demographic factors on reproductive health. Variations in age, ethnicity, and body mass index (BMI) can significantly influence fertility treatments and outcomes (6).

Although some studies have been conducted in Iran (7, 8), research specifically addressing factors that influence clinical pregnancy rates in FET cycles remains limited. Furthermore, global studies on this topic have yielded inconclusive results, highlighting the need for more focused research to clarify these relationships. Understanding the influences of associated factors on FET outcomes may lead to the development of targeted strategies that address barriers to successful pregnancy (9–11). This knowledge is essential for improving clinical practice and ensuring equitable access to reproductive technologies for all individuals seeking assistance with infertility.

By examining how these factors interact with clinical variables, this study aims to provide insights that can improve patient care and optimize clinical protocols in different populations. Therefore, this study aims to identify and analyze the specific fac-tors that influence clinical pregnancy rates in frozen-thawed embryo transfer cycles in IVF.

METHODS

This descriptive-analytical study focused on frozen-thawed embryo cycles resulting from microinjection in couples referred to the IVF Center of Imam Khomeini Hospital and the Private Mother IVF Center in Sari, Northern Iran, between 2015 and 2020. The study was approved by the Ethics Committee of Mazandaran University of Medical Sciences (code: IR MAZUMS.IMAMHOSPITAL. REC.1399.042) and adhered to the tenets of the Declaration of Helsinki. Eligible couples were selected by availability sampling, and written informed consent was obtained from all participants to ensure confidentiality and permission to use the data for research purposes.

Inclusion criteria consisted of patients undergoing a frozen embryo transfer cycle with at least one grade A or B embryo designated for transfer and who also consented to participate in the study. In addition, only patients undergoing hormone replacement therapy (HRT) cycles were included in this study. Exclusion criteria included participants in donation or surrogacy cycles, individuals with uterine abnormalities or severe endometriosis, the presence of hydrosalpinx detected by hysterosalpingography or ultrasound, a history of difficult uterine embryo transfers, azoospermia requiring testicular sperm extraction (TESE), and individuals with uncontrolled endocrine disorders such as diabetes, hypothyroidism, or hyperthyroidism.

A total of 30 variables related to individual patient factors, treatment cycle characteristics, and embryo factors were evaluated. These variables included the woman's age at the time of embryo transfer and oocyte retrieval, the spouse's age, both partners' occupations, smoking and alcohol consumption habits, duration of infertility, type of infertility (primary or secondary), cause of infertility, reason for embryo freezing (extra embryos, risk of ovarian hyperstimulation syndrome, or inappropriate endometrium), endometrial thickness on the day of embryo transfer, and grade of transferred embryos (A, B, or C), time interval between embryo thawing and transfer, developmental stage of the transferred embryo (cleavage, morula, or blastocyst), embryo grading before freezing, grading after thawing, endometrial pattern on the day of transfer (triple-line, hyperechoic, or isoechoic), type of catheter used for transfer (with or without obturator), presence of blood at the catheter tip after transfer, ease of transfer (easy, forced, difficult, requiring tenaculum or anesthesia), performance of hysteroscopy or laparoscopy prior to the transfer cycle, season of transfer, number of previous failed cycles, use of oral contraceptives prior to the transfer cycle, treatment protocol during the ovarian stimulation cycle (agonist or antagonist), suppression with or without GnRH (gonadotropin-releasing hormone) agonist in the transfer cycle, body mass index (BMI), timing of the transfer within the cycle, and duration of embryo freezing. In addition, we recorded the outcomes of clinical and chemical pregnancy rates, early and late spontaneous abortion rates, ectopic pregnancy rates, and multiple pregnancy rate.

Endometrial preparation with hormonal drugs followed a specific protocol. All patients were referred to the clinic on the second or third day of their menstrual cycle for a vaginal ultrasound to evaluate the uterus and ovaries. If the ultrasound results were normal, patients were prescribed estradiol valerate tablets (2 mg estradiol tablets manufactured by Aburaihan Pharmaceutical Company, Iran), starting on the third day of the menstrual cycle, with a daily dosage ranging from 2 to 4 mg. Periodic vaginal ultrasounds were performed to measure endometrial thickness, and the estradiol dose was adjusted as needed. The maximum prescribed dose of estradiol tablets was 8 mg per day. Progesterone supplementation began when the endometrial thickness reached 8 mm, with patients receiving either vaginal progesterone suppositories (Fertigest® 400 mg, Aburaihan Pharmaceutical Company, Iran) every 12 hours at 400 mg or daily intramuscular injections of 100 mg progesterone (50 mg vial, Iran Hormon Pharmaceutical Company) for 3 to 5 days prior to embryo transfer. Estradiol supplementation was continued along with progesterone supplementation until the day of the embryo transfer.

The timing of embryo thawing and transfer was planned based on the age of the frozen embryo and the decision to transfer the embryo at the blastocyst stage or another stage of development. In some cases, patients were prescribed oral contraceptive pills for one month prior to starting estradiol treatment to synchronize cycles or in cases where the endometrium required suppression. In addition, a daily subcutaneous injection of a GnRH agonist (Sinafact 5 mg vial, Sinagen Pharmaceuticals, buserelin) at a dose of 0.5 mg in the middle of the luteal cycle was used to suppress the hypothalamic-pituitary axis and increase endometrial receptivity. The GnRH agonist dose was reduced to 0.25 mg per day with the onset of menses and continued until progesterone treatment was initiated.

The frozen embryos were created by microinjection of oocytes obtained during the ovarian stimulation cycle, using sperm obtained from the spouses' ejaculations. These embryos were frozen between days 3 and 5 after microinjection, from the 8-cell stage to the blastocyst stage. Embryos were graded based on blastomere morphology and cytoplasmic fragmentation, both before freezing and after thawing. Cleavage stage embryos were graded as follows: grade A indicated the absence of fragmentation with 6–8 equally sized blastomeres; grade B had fragmentation of less than 25% with blastomeres that may or may not be equally sized; grade C had fragmentation between 25–50% or blastomeres that were not equally sized; and grade D had fragmentation greater than 50%. Blastocyst grading followed the Gardner scoring system, with grade D embryos typically not frozen (12, 13). Embryos were frozen using Kitazato embryo vitrification media freezing kits from Tokyo, Japan.

Prior to transfer, the embryos were thawed and placed in embryo culture medium (Life Global, single-step media, CooperSurgical, US) using Kitazato embryo thawing kits. Embryos with less than 50% fragmentation after thawing were considered suitable for transfer, which was performed under abdominal ultrasound guidance by one or two fertility specialists. Two types of catheters were used for embryo transfer: one without an obturator (CCD catheter, Paris, France) and one with an obturator, provided by either a CCD catheter (TDT SET, Paris, France) or a Wallace obturator (Sure-Pro Ultra, PEB623, CooperSurgical, Denmark). The report documented the method of embryo transfer, classified it as difficult or easy, and noted the use of tenaculum or anesthesia, along with any bleeding from the catheter after transfer. After the embryo transfer, patients rested in the supine position for approximately 30 minutes before being discharged. The administration of estradiol tablets and vaginal or injectable progesterone continued until two weeks after embryo transfer, at which time serum β-HCG levels were measured. A serum β-HCG level greater than10 milliunits per milliliter indicated a positive chemical pregnancy and prompted reassessment two days later. If the pregnancy progressed, estradiol and progesterone therapy was continued until 10 weeks' gestation, with clinical pregnancy confirmed by ultrasound observation of the gestational sac and fetal pole with heartbeat at 7 weeks' gestation (14).

Statistical analysis

Data were analyzed using SPSS software, version 22, and the Kolmogorov-Smirnov test was used to test the assumption of normality. Frequencies and percentages were used to describe qualitative variables, and means and standard deviations were used for quantitative variables. The sample size for this study was determined to be 429 infertile couples based on a similar study, considering a clinical pregnancy rate of 29.2%, a significance level of 0.05, a test power of 0.80, and an effect size of 6.15%, selected by convenience sampling.
Chi-square tests, Fisher's exact tests, and independent samples t-tests were used to compare demographic and clinical data between clinically pregnant and non-pregnant women undergoing frozen embryo transfer (FET) cycles. For variables with non-normal distributions, the non-parametric Mann-Whitney test was used to compare the two groups. To investigate the effect of different factors on clinical pregnancy, individual variables were first entered into a simple logistic regression model (crude). Those with a p-value < 0.2 were then entered into a multiple logistic regression model (adjusted). Effective demographic and clinical variables affecting the success of frozen-thawed embryo transfer cycles were identified based on a significance level of $p < 0.05$, and the results were expressed as odds ratios (OR) with a 95% confidence interval. The validity of the regression models was confirmed using the Hosmer-Lemeshow test and the omnibus test. A significance level of $p > 0.05$ was considered for all tests.

RESULTS

In this study, 429 frozen embryo transfer (FET) cycles involving 429 patients were analyzed. The mean age of the women at the time of transfer was 32 ± 2.52 years, while the mean age of their spouses was 36 ± 1.62 years. The mean age of the women at the time of oocyte retrieval was 31.06 ± 5.3 years. Among the participants, 171 cases (39.9%) resulted in chemical pregnancies, and 156 cases (36.3%) resulted in clinical pregnancies. In the group of women with clinical pregnancies, there were 127 singleton pregnancies, 28 twin pregnancies, and one triplet pregnancy. The mean body mass index (BMI) of the women was 25.06 ± 4 kg/m^2. Of the women, 58.7% were homemakers, and 11% were employed. Demographic characteristics and treatment cycle outcomes for the pregnant and non-pregnant groups are detailed in Tables 1 and 2. The results show significant differences in certain variables between the pregnant and non-pregnant groups, including the woman's age at oocyte retrieval, BMI, duration of infertility, occupation, embryo grade before freezing and after thawing, quality and developmental stage of the transferred embryo, type of catheter used for transfer, and ease of transfer ($p < 0.05$). Other variables did not reach statistical significance (Table 1).
When looking at embryo quality and women's age, the study found that clinical pregnancy rates were higher in women under 37 years of age who received two A-A or A-B embryos compared to those who received two B-B embryos. In addition, pregnancies were more successful in women over 37 years of age when two A-A embryos were transferred (Figure 1A).

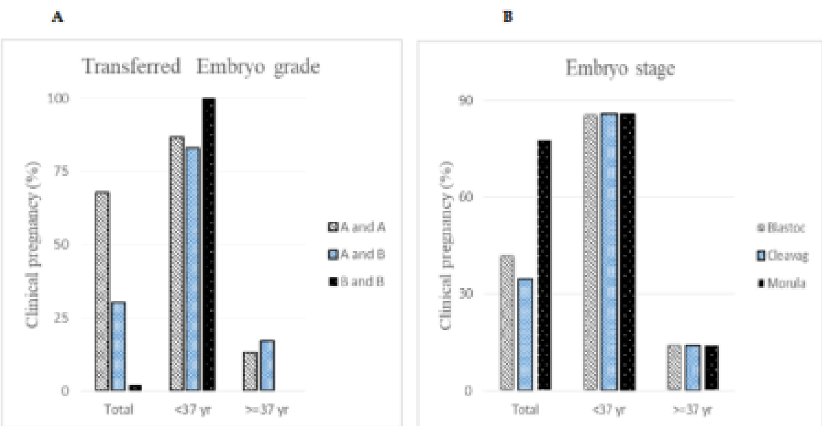

Figure 1. *A. Clinical pregnancy (%) for each embryo grade in females aged under 37 and > = 37, B. Clinical pregnancy (%) for each embryo stage in females aged under 37 and > = 37*

Table 1. *Demographic and clinical characteristics in two groups of pregnant and non-pregnant women in frozen-thawed embryo transfer cycles*

Variables	Clinical pregnancy		Total (n = 429)	P-value
	No (n = 273)	Yes (n =156)		
Female age on transfer day				
< 37 years	219 (80.2%)	134 (85.9%)	353 (82.3%)	0.138
≥ 37 years	54 (19.8%)	22 (14.1%)	76 (17.7%)	
Spouse's age				
< 37 years	153 (56%)	96 (61.5%)	249 (58.0%)	0.268
≥ 37 years	120 (44%)	60 (38.5%)	180 (42.0%)	
Female age on oocyte pick-up day				
< 37 years	233 (85.3%)	146 (93.6%)	379 (88.3%)	0.010
≥ 37 years	40 (14.7%)	10 (6.4%)	50 (11.7%)	
Female occupation				
Employed	23 (8.4%)	24 (15.4%)	47 (11.0%)	0.020
Housekeeper	170 (62.3%)	82 (52.6%)	252 (58.7%)	
Self-employment	8 (2.9%)	11 (7.1%)	19 (4.4%)	
Other	72 (26.4%)	39 (25.0%)	111(25.9%)	
Spouse's occupation				
Employed	62 (22.7%)	44 (28.2%)	106 (24.7%)	0.439
Self-employment	105 (38.5%)	57 (36.5%)	162 (37.8%)	
Other	106 (38.8%)	55 (35.3%)	161 (37.5%)	
Cigarette smoking				
No	252 (92.3%)	138 (88.5%)	390 (90.9%)	0.183
Yes	21 (7.7%)	18 (11.5%)	39 (9.1%)	
Alcohol				
No	263 (96.3%)	151 (96.8%)	414 (96.5%)	0.804
Yes	10 (3.7%)	5 (3.2%)	15 (3.5%)	
Infertility duration				
< 5 years	147 (53.8%)	101 (64.7%)	248 (57.8%)	0.028
≥ 5 years	126 (46.2%)	55 (35.3%)	181 (42.2%)	
Infertility type				
Primary	172 (63.0%)	93 (59.6%)	265 (61.8%)	0.487
Secondary	101 (37.0%)	63 (40.4%)	164 (38.2%)	
Infertility cause				
Anovulation	39 (14.3%)	16 (10.3%)	55 (12.8%)	0.555
Endometriosis	21 (7.7%)	11 (7.1%)	32 (7.5%)	
Male factor	112 (41.0%)	72 (46.2%)	184 (42.9%)	
Multiple female	10 (3.7%)	4 (2.6%)	14 (3.3%)	
Both male and female	53 (19.4%)	30 (19.2%)	83 (19.3%)	
Tubal factor	37 (13.6%)	20 (12.8%)	57 (13.3%)	
Unexplained	1 (0.4%)	3 (1.9%)	4 (0.9%)	
Embryo grade before freezing				
A - A	139 (50.9%)	106 (67.9%)	245 (57.1%)	0.001
A - B	115 (42.1%)	47 (30.1%)	162 (37.8%)	
B - B	19 (7.0%)	3 (1.9%)	22 (5.1%)	
Embryo grade after thawing				

A - A	139 (50.9%)	106 (67.9%)	245 (57.1%)	0.001
A - B	115 (42.1%)	47 (30.1%)	162 (37.8%)	
B - B	19 (7.0%)	3 (1.9%)	22 (5.1%)	
Embryo grade on transfer day				
A - A	138 (50.5%)	107 (68.6%)	245 (57.1%)	0.001
A - B	116 (42.5%)	46 (29.5%)	162 (37.8%)	
B - B	19 (7.0%)	3 (1.9%)	22 (5.1%)	
Interval between thawing and transfer				
0 day	73 (26.7%)	32 (20.5%)	105 (24.5%)	0.352
1 day	197 (72.2%)	122 (78.2%)	319 (74.4%)	
2 day	3 (1.1%)	2 (1.3%)	5 (1.2%)	
Endometrial line thickness on transfer day				
< 9 mm	17 (6.2%)	9 (5.8%)	26 (6.1%)	0.848
≥ 9 mm	256 (93.8%)	147 (94.2%)	403 (93.9%)	
BMI (mean ± SD)	25.17±3.44	25.96±4.02	25.45±3.68	0.031*
Protocol for ovarian stimulation cycles				
Agonist	23 (8.4%)	11 (7.1%)	34 (7.9%)	0.380
Antagonist	241 (88.3%)	143 (91.7%)	384 (89.5%)	
None	9 (3.3%)	2 (1.3%)	11 (2.6%)	
Protocol for suppression in transfer cycles				
With Agonist	88 (32.2%)	46 (29.5%)	134 (31.2%)	0.555
Without Agonist	185 (67.8%)	110 (70.5%)	295 (68.8%)	
Embryo growth stage on transfer day				
Blastocyst	29 (10.6%)	21 (13.5%)	50 (11.7%)	0.020
Cleavage	242 (88.6%)	128 (82.1%)	370 (86.2%)	
Morula	2 (0.7%)	7 (4.5%)	9 (2.1%)	
History of laparoscopy-hysteroscopy				
No	133 (48.7%)	75 (48.1%)	208 (48.5%)	0.898
Yes	140 (51.3%)	81 (51.9%)	221 (51.5%)	
Season on transfer cycles				
Autumn	64 (23.4%)	36 (23.1%)	100 (23.3%)	0.339
Spring	64 (23.4%)	39 (25.0%)	103 (24.0%)	
Summer	79 (28.9%)	34 (21.8%)	113 (26.3%)	
Winter	66 (24.2%)	47 (30.1%)	113 (26.3%)	
Prior transfer cycles—number				
0	165 (60.4%)	88 (56.4%)	253 (59.0%)	0.577
1	64 (23.4%)	46 (29.5%)	110 (25.6%)	
2	27 (9.9%)	13 (8.3%)	40 (9.3%)	
≥ 3	17 (6.2%)	9 (5.8%)	26 (6.1%)	
Pre-treatment use of contraceptive pills				
No	11 (2.5%)	12 (2.7%)	23 (5.3%)	0.132
Yes	271 (63.1%)	135 (31.4%)	406 (94.7%)	
Cause of freezing				
Endometrial insufficiency	1 (0.4%)	0 (0.0%)	1 (0.2%)	0.313
OHSS risk	0 (0.0%)	1 (0.6%)	1 (0.2%)	
Surplus Embryos	272 (99.6%)	155 (99.4%)	427 (99.5%)	
Endometrial pattern				
Hyperechoic	2 (0.7%)	0 (0.0%)	2 (0.5%)	0.556
Isoechoic	3 (1.1%)	2 (1.3%)	5 (1.2%)	
Trilaminar	268 (98.2%)	154 (98.7%)	422 (98.4%)	
The day of transfer in the cycle (mean± SD)	17.89±1.56	17.83±1.20	17.79±1.37	0.722*
Embryo catheter type				

With obturator	142 (52.0%)	103 (66.0%)	245 (57.1%)	0.005
Without obturator	131 (48.0%)	53 (34.0%)	184 (42.9%)	
Bloody catheter after transfer				
No	268 (98.2%)	152 (97.4%)	420 (97.9%)	0.611
Yes	5 (1.8%)	4 (2.6%)	9 (2.1%)	
Freeze duration by months (mean± SD)	6.65±8.40	7.04±10.33	6.80±9.14	0.675*
Ease of doing transfer				
Easy under anesthesia	3 (1.1%)	0 (0.0%)	3 (0.7%)	0.033
Easy (without instrument)	249 (91.2%)	147 (94.2%)	396 (92.3%)	
Enforcement	0 (0.0%)	2 (1.3%)	2 (0.5%)	
Difficult and need anesthesia	21 (7.7%)	6 (3.8%)	27 (6.3%)	
Difficult and need to use tenaculum	0 (0.0%)	1 (0.6%)	1 (0.2%)	

Chi-square *Independent sample t-tests

Table 2. *The result of frozen-thawed embryo transfer cycles in patients*

Variables	Results	
B-HCG	Positive	171 (39.9%)
	Negative	258 (60.1%)
Clinical pregnancy (embryo sac number)	(1)	127 (81.4%)
	(2)	28 (17.9%)
	(3)	1 (0.6%)
Abortion	Early	15 (8.7%)
	Late	9 (5.2%)
EP	0	
Multiple pregnancy	29 (18.5%)	

* Frequency; B-HCG: Beta-Human Chorionic Gonadotropin; EP: Ectopic pregnancy

Regarding embryo development stage and women's age, morula-stage embryo transfers resulted in higher clinical pregnancy rates compared to blastocyst-stage and cleavage-stage transfers. However, morula-stage embryo transfers were performed in only 2.1% of all FET cycles (nine out of 429 patients). The small sample size of morula-stage transfers limits the ability to draw definitive conclusions about their efficacy compared to other embryo stages (Figure 1B).

Table 3 shows the relationship between factors affecting clinical pregnancy rates and the results of the multiple logistic regression model.

The adjusted model shows that patients under 37 years of age at oocyte retrieval had nearly four times the odds of achieving clinical pregnancy compared with those over 37 years of age. Conversely, patients with infertility of less than five years had 1.66 times the odds of clinical pregnancy compared to those with infertility of more than five years. In addition, the analysis revealed that homemakers had a 0.69 lower chance of clinical pregnancy compared to employed individuals.

Table 3. *The results of logistic regression test in predicting success in frozen-thawed embryo transfer cycles in patients (based on the occurrence of clinical pregnancy)*

Variable	Crude		Adjusted	
	OR (95%CI)	P-value	OR (95%CI)	P-value
Female age on transfer day (reference: ≥ 37)				
< 37 years	1.51 (0.85, 2.58)	0.140	0.74 (0.32, 1.75)	0.490
Female age on oocyte pick-up day (reference: ≥ 37years)				
< 37	2.51 (1.22, 3.28)	0.013	4.17 (1.41, 12.81)	0.011
Infertility duration (reference: ≥ 5)				
< 5 year	1.57 (1.05, 2.36)	0.028	1.66 (1.06, 2.61)	0.026
Cigarette smoking (reference: yes)				
No	0.64 (0.33, 1.24)	0.185	0.64 (0.30, 1.33)	0.225
Female occupation (reference: employed)				
Housekeeper	0.46 (0.25, 0.87)	0.016	0.31 (0.15, 0.63)	0.001
Self-employment	1.32 (0.45, 3.86)	0.615	0.79 (0.25, 2.59)	0.698
Other	0.52 (0.26, 1.04)	0.063	0.38 (0.18, 0.81)	0.012
Transferred embryo grade (ref: A - A)				
A - B	0.51 (0.33, 0.78)	0.002	0.54 (0.33, 0.84)	0.007
B - B	0.20 (0.06, 0.71)	0.012	0.26 (0.06, 0.81)	0.037
BMI	1.06 (1.01, 1.12)	0.032	1.06 (1.01, 1.13)	0.044
Embryo stage (ref: morula)				
Blastocyst	0.21 (0.04, 1.10)	0.064	0.14 (0.02, 0.78)	0.039
Cleavage	0.15 (0.03, 0.74)	0.020	0.15 (0.02, 0.77)	0.038

Moreover, patients with A-A grade embryos had approximately 0.46 higher odds of clinical pregnancy than those with A-B grade embryos, while patients with B-B grade embryos had approximately 0.74 lower odds of clinical pregnancy than those with A-A grade embryos. The results showed that for each unit increase in BMI, the chance of clinical pregnancy increased by 6%. The odds of clinical pregnancy for blastocyst embryos were 0.14 lower than for morula stage embryos, and the odds of cleavage stage embryos were 0.15 lower than for morula stage embryos. In addition, the odds of pregnancy were 1.67 times higher for catheters with an obturator compared to those without (Table 3, Figure 1).

DISCUSSION

This aim of this study was to identify the factors influencing the clinical pregnancy rate of FET cycles in infertile women referred to infertility centres between 2015 and 2020. The analysis revealed significant factors influencing the success of FET cycles, distinguishing between pregnancy and non-pregnancy groups. Key variables included the woman's age at egg retrieval, duration of infertility, occupation, body mass index (BMI), developmental stage of the transferred embryo, type of catheter used, and embryo grade. These variables have been identified as influential in the success or failure of FET cycles.

A woman's age is an important determinant of pregnancy success in fresh transfer cycles, as declining oocyte quality in women over 35 years of age can affect success rates (15, 16). However, in this study, neither the age of the woman at transfer nor the age of the man correlated with clinical pregnancy outcomes. Notably, women under 37 years of age at oocyte retrieval had improved odds of achieving a successful clinical pregnancy. This is consistent with the results of another study, which showed that age did not influence the outcome of cryopreservation in women younger than 35 years (17). Moreover, research has demonstrated comparable pregnancy rates in FET cycles between women under and over 40 years of age when high quality embryos are used, underscoring the importance of a woman's age at egg retrieval as a critical determinant of cycle success (8). Thus, the age at oocyte retrieval may be more important than the age at transfer in predicting the outcome of a transfer cycle.

Our results also showed a significant relationship between morula stage embryo transfer and clinical pregnancy rates.

Previous research has highlighted blastocyst embryo transfer as a primary pre-dictor of live birth rates, with lower abortion rates associated with grade A blastocyst embryos compared to grade C (18, 19). In our study, the odds of clinical pregnancy were 0.14 lower for blastocyst embryos compared to morula stage embryos, and the odds were 0.15 lower for cleavage stage embryos compared to morula stage embryos. However, morulastage embryo transfers were performed in only 2.1% (9/429) of all FET cycles. This discrepancy results from clinical practice decisions, as blastocyst stage transfers are generally prioritized in most studies due to their association with higher implantation rates. It is also important to note that progression to the blastocyst stage depends on the quality of the culture environment and laboratory conditions. Progressing embryos to the blastocyst stage could reduce the number of viable embryos available for transfer due to the risk of destruction during development and cell division.

The clinical outcomes of morula-stage versus blastocyst-stage embryo transfer have been evaluated in other studies with varying results. Bavishi et al. (20) performed a retrospective analysis comparing morula and blastocyst transfers in fresh IVF-ICSI cycles. Their results showed that although the implantation rate, clinical pregnancy rate (CPR), and live birth rate (LBR) were slightly higher for blastocyst transfers (37.79%, 51.35%, and 45.6%, respectively) than for morula transfers (34.54%, 45.28%, and 37.73%, respectively), these differences were not statistically significant. The study concluded that morula transfer can serve as an effective alternative to blastocyst transfer in selected cases without compromising outcomes. Similarly, Korkmaz et al. (21) evaluated the clinical pregnancy and live birth outcomes of vitrified and thawed embryos transferred at the cleavage, morula, and blastocyst stages. They found that embryos frozen on day 4 (morula stage) and transferred on day 5 had significantly higher clinical pregnancy and live birth rates compared to other stages. This highlights the potential advantages of morula stage transfers in certain scenarios. In addition, Tao et al. (22) evaluated the survival and viability of frozen-thawed morula embryos. They demonstrated that good quality morula embryos (grade 3) had significantly higher post-thaw survival, pregnancy rates, and implantation rates compared to lower quality morula embryos. Their results support the feasibility of morula cryopreservation and indicate that careful selection of morula embryos can lead to favourable outcomes.

Despite these promising findings, it is important to note that other studies and meta-analyses, such as those by Perlman et al. (23) and Glujovsky et al. (24), have consistently demonstrated the superior outcomes of blastocyst stage transfers compared to earlier stages of development, including morula. These studies highlight the higher implantation and live birth rates associated with blastocyst transfer, which remains the standard of care in clinical practice. Given the small number of morula-stage transfers in our study and the conflicting evidence in the literature, we caution against overgeneralizing our findings. Further research, including larger, well-controlled prospective studies, is needed to fully evaluate the role of morula-stage transfers in clinical practice and to determine their potential benefits in specific patient populations".

There is conflicting evidence regarding the effect of BMI on FET outcomes. One study found no significant differences in implantation and pregnancy rates between women with a BMI greater than 25 kg/m^2 and those with a BMI less than 25 kg/m^2 when high-quality embryos were available for transfer (25). Conversely, another study reported higher rates of unsuccessful IVF treatments and increased miscarriage rates in patients with a BMI below 40 kg/m^2 compared with normal weight patients (26). The discrepancy between these findings and our results may be due to the mean BMI of 25.06 kg/m^2 among our participants.

Consistent with previous research (27), our study found no significant relationship between endometrial thickness and the success rate of FET cycles. In contrast, other studies have identified endometrial thickness as a critical predictor of FET success, particularly with a threshold of 8.9 mm, noting that patients with thickness greater than 9 mm had higher clinical pregnancy rates (17). In our study, embryo transfers were not performed in cycles where endometrial thickness fell below the minimum requirement of 8 mm. However, the categorization of endometrial thickness into groups above and below 9 mm may have influenced the lack of significance and predictive ability observed in our fertility results.

While some studies have suggested a decrease in pregnancy rates following the use of gonadotropin-releasing hormone antagonist therapy compared to agonist therapy, our results are consistent with previous research indicating that the ovarian stimulation protocol (antagonist or agonist) does not significantly affect the success of frozen-thawed embryo transfer (17, 27).

We have identified the duration of infertility as an important predictor of embryo transfer success. Although the adverse effects of long-term infertility on pregnancy outcomes remain unclear, prolonged waiting for primary infertility treatment may hinder the effectiveness of assisted reproductive methods (17, 28). One study identified a cut-off point of 4.5 years for the duration of infertility (17).

Our results are consistent with a systematic review that identified the type of catheter used for embryo transfer as a potential predictor of favourable outcomes (29). However, another study emphasized that the skill of the embryo transfer operator plays a more important role in determining the success of the transfer cycle (30). We found that employed women were 0.69 times more likely to become pregnant than homemakers. This suggests that employment may influence health literacy, which in turn influences psychological factors related to fertility (31). Additionally, social stigma and cultural influences that divert attention away from infertility may contribute to this disparity. In support of this notion, studies have highlighted the impact of psychological factors, particularly chronic stress, on the outcomes of embryo transfer cycles (32, 33).

In this multicenter study, we used a larger sample size and advanced statistical analysis models to identify variables that affect FET cycles in infertile women. However, several limitations should be noted. The small number of morula-stage embryo transfers (2.1% of total transfers) limits the strength and generalizability of the conclusions regarding their clinical pregnancy outcomes. In addition, we did not assess final pregnancy outcomes or live birth rates. Moreover, only one method of endometrial preparation was used for all patients, and alternative methods were not examined as independent variables. Future studies could investigate factors affecting the success of FET cycles using donor embryos, embryos obtained by microinjection of oocytes with sperm from TESE, or other methods of endometrial preparation and transfer within a standard cycle. Due to the nature of this study, the results may not be generalizable to all infertile couples.

The study concluded that younger maternal age at oocyte retrieval, shorter duration of infertility, optimal BMI, higher embryo grade, and appropriate selection of transfer techniques are key determinants of achieving clinical pregnancy in HRT-FET cycles. These findings can guide specialists in optimizing FET protocols to improve pregnancy outcomes.

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Competing Interest

The authors declare no relevant conflicts of interest.

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