

Pregnancy Outcome after Difficult Embryo Transfer in IVF-ET Cycles

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ABSTRACT

Background: Embryo transfer (ET) is one of the most crucial steps in IVF/ICSI treatment. Although it, apparently, seems simple, it is an integral part of IVF/ICSI and can affect the outcome of the whole treatment cycle. The majority of couples (approximately 80%) who undergo IVF reach the ET stage, yet few pregnancies occur. The pregnancy rate after ET is dependent on multiple factors including embryo quality, endometrial receptivity, and the technique of the ET. **Aim of the Work:** The aim of the current study is to investigate the clinical and ongoing clinical pregnancy rates in women who experience difficult embryo transfer (ET) after IVF/ICSI cycle. **Patients and Methods:** This prospective cohort study was conducted at Dar Al-Teb Infertility and IVF Center between January 2017 and January 2018. The study included 417 women planned to undergo ET. **Results:** The biochemical, clinical and ongoing clinical pregnancy rates were significantly lower in women who had difficult ET when compared to women who had easy ET [ORs 0.5, 95% CI (0.31 to 0.83); 0.48, 95% CI (0.29 to 0.79); 0.36, 95% CI (0.21 to 0.62); respectively]. **Conclusion:** Difficult ET is associated with significantly reduced biochemical, clinical and ongoing clinical pregnancy rates. **Recommendations:** ET should be smooth with easy passage of the transfer catheter. Since any uterine manipulation during ET adversely affects IVF results, therefore precaution should be taken to identify possibly difficult ET cases in advance. **Keywords:** pregnancy outcome, embryo transfer, IVF-ET cycles.

INTRODUCTION

In vitro fertilization (IVF) has been used over decades as treatment for infertility with different etiologies. Despite the developing technology and advances in the field, implantation failure remains a major problem. Many factors have been proposed including endometrial receptivity, embryo quality and the procedure of embryo transfer (ET) ⁽¹⁾. Embryo transfer (ET) is a short, yet the most crucial and critical step that significantly affects the success of the whole process of IVF ⁽²⁾. Most ETs are easy and unforced. Difficulties are, however, encountered in up to 15-20% of cases. Reasons for difficult ET include acute version of flexion angles between the vagina, cervix and the uterine corpus; stenosed internal os; or previous false tract ⁽³⁾.

Several strategies have been proposed to overcome difficulty during ET procedure, including performing ET under ultrasound-guidance, instructing the patient to have a full bladder at the time of the procedure, and use of stylet with soft catheter ⁽⁴⁾. Nevertheless, for a small group of women, ET procedure remains difficult and requires further steps, which may induce uterine contractions or provoke endocervical or endometrial bleeding; which both might have an adverse impact on successful implantation and the whole IVF outcome ⁽⁵⁾. More interestingly, several prospective and retrospective studies have observed that even women who had successful implantation following difficult ETs might have remote bad outcome, in terms of miscarriage ^(1,4,7,8, 9). In addition to the evident association between difficult ET and negative pregnancy

outcome, an association between difficult ET and first trimester miscarriage, among women who had initially a positive clinical pregnancy outcome, has been practically and clinically observed (unpublished observational study).

AIM OF THE WORK

The aim of the current study is to investigate the clinical and ongoing clinical pregnancy rates in women who experience difficult embryo transfer (ET) after IVF/ICSI cycle.

PATIENTS AND METHODS

Study Setting

Dar Al-Teb Infertility and IVF Center.

Study Duration

The period between January 2017 and January 2018.

Study Design

Prospective cohort study.

Study Population

The study included women planned to undergo ET according the following eligibility criteria:

Inclusion criteria

1. Age: 20 – 35 years.
2. Women planned to go their first IVF/ICSI cycle after controlled ovarian hyperstimulation (COH) using the long GnRH agonist protocol.
3. Transfer of fresh good quality blastocyst-stage embryos.

Exclusion criteria

1. Women planned to undergo thawed-frozen embryo transfer.

2. Women who had prior IVF/ICSI failure.
3. Women who had poor response (defined as ≤ 5 oocytes retrieved).
4. Women who had known endometrial or uterine factor that might adversely affect implantation.
5. Women who had cleavage-stage embryo transfer.
6. Women who had retained embryos in the transfer catheter and needed reload and re-transfer.

Study Groups

Included eligible woman were divided into two groups:

1. **Group I:** including women who had difficult ET.
2. **Group II:** including women who had easy ET.

Difficult ET was defined as an ET procedure that is associated by one of the following added steps:

1. Use of the stylet for introducing the ET catheter into the uterine cavity.
2. Grasping and traction of the anterior cervical lip to straighten the cervico-uterine angle.
3. Presence of excessive blood on tip of the ET catheter.

Controlled Ovarian Hyperstimulation and IVF/ICSI

- All included women underwent COH following luteal pituitary downregulation using triptorelin 0.1 mg (Decapeptyl®, Ferring Pharmaceuticals) once subcutaneously per day till day 2 of next menstrual cycle.
- When pituitary desensitization was confirmed biochemically (serum LH < 2 IU/ml and serum estradiol < 50 ng/ml) and sonographically (no follicular activity on either ovary as well as endometrial thickness < 6 mm), controlled ovarian stimulation was started using human menopausal gonadotropin [hMG] 75 IU (Merional®, IBSA, Switzerland), according to the patient's characteristics.
- When at least ≥ 3 follicles reach a dimension of ≥ 17 mm, ovulation is triggered using human chorionic gonadotropin [hCG] (Choriomon®, IBSA, Switzerland) 10,000 IU intramuscularly. Oocyte retrieval was performed 35-37 hours after ovulation triggering.
- If the number of oocytes ≥ 6 and the husband's semen profile is normal, the retrieved oocytes were split at ratio of 1:1 into IVF and ICSI. Otherwise, oocytes are subjected to ICSI.
- Oocytes planned to ICSI were denuded. Only MII oocytes were injected.
- Injected oocytes and oocytes subjected to IVF were examined next day (for confirmation of fertilization) and on day 2 (for confirmation of cleavage).

Embryo Transfer Procedure

- Full urinary bladder was ensured before ET procedure.
- In most of cases, ET was performed without anesthesia, unless there was extreme difficulty, patient's intolerance or patient's request.

- All ET procedures were performed using the Wallace® catheter under abdominal ultrasound guidance by the same operator.
- Two or three embryos of the blastocyst-stage with grades of 2AA, 3AA or 4AA are loaded and transferred.
- Cusco's or Pederson's speculum was inserted into the vagina.
- The cervix was cleaned using normal saline.
- Cervical mucus, if present, was gently removed using a cotton-tipped swab.
- The ET catheter was then gently advanced into the cervical canal towards the mid-uterine cavity. Embryos were placed 1-1.5 cm below the uterine fundus.
- When there was a difficulty in advancing the catheter into the cervical canal, the Wallace® stylet was used after adequate curving of its tip to fit into the curvature of the cervico-uterine angle.
- If introduction of the Wallace® style failed, the anterior cervical lip was grasped using a multi-toothed vulsellum. Gentle traction was then performed along with trial of gentle advancement of the ET catheter.
- After placing the embryos into the uterine cavity, the ET catheter was flushed and checked for retained embryos. Women who required reloading and retransfer were excluded from the study.

Outcomes

- The primary outcome was ongoing clinical pregnancy rate. Ongoing clinical pregnancy is defined as detecting of viable intrauterine gestational sac(s) beyond 12 weeks of gestation.
- Secondary outcomes included:
 - Biochemical pregnancy rate. Biochemical pregnancy is defined as positive serum pregnancy test at least 12 days following ET.
 - Clinical pregnancy rate. Clinical pregnancy is defined as sonographic detection of intrauterine gestational sac(s), 10-14 days after a positive serum assay.
 - Ectopic pregnancy rate.
 - First trimester miscarriage rate.

Ethical Aspects

- The study protocol is in agreement to the Helsinki's Principles of Ethical Medical Research [last updated in Brazil 2013].
- All women have to sign informed written consent before participating in the study after thorough explanation of the purpose and procedure of the study.
- Every recruited woman has the right to withdraw from the study at any phase without being adversely affected regarding the medical service she should receive.

Sample Size Justification

Sample size was calculated using EpiInfo version 7.0, setting the power at 80% and the two-sided confidence level at 95%. Data from a previous similar study ⁽⁴⁾ showed that the clinical pregnancy rates in women who had difficult ET and those who had easy ET were 21.4% and 36.9%, respectively. The ratio of difficult-to-easy ETs in the same study was nearly 4:1.

Calculation according to these values produces a minimal sample size of 80 cases of difficult ET to comprise 20% of the total no. of recruited women. Therefore, eligible women fulfilling the inclusion/exclusion criteria were sequentially recruited until at least 80 cases of difficult ET were included, provided that they represent at maximum 20% of the total no. of cases.

Statistical Methods

Statistical analysis was performed using SPSS for Windows version 20.0. Data are presented in terms of range, mean and standard deviation (for numeric parametric variables); range, median and interquartile range (for numeric non-parametric variables); or number and percentage (for categorical variables). Difference between two groups was analyzed using independent student's t-test as well as the mean difference and its 95% confidence interval (for numeric parametric variables); Mann-Whitney's U-test (for numeric non-parametric variables); or chi-squared test as well as the odds ratios and their 95% confidence intervals (for categorical variables). Significance level is set at 0.05.

RESULTS

The current study was conducted at Dar Al-Teb IVF/ICSI Center during the period between January 2017 and January 2018. A total of 417 eligible women were recruited in the current study.

Table (1): Initial Characteristics of Included Women

Age (years)	Range	20 – 35
	Mean ± SD	27.24 ± 4.47
BMI (kg/m ²)	Range	19.38 – 34.19
	Mean ± SD	26.24 ± 3.29
Indication for IVF/ICSI	Tubal Factor Infertility	123 (29.5%)
	Male Factor Infertility	129 (30.9%)
	Unexplained Infertility	165 (39.6%)

SD standard deviation, BMI body mass index, IVF in vitro fertilization, ICSI intracytoplasmic sperm injection
Data presented as range, mean ± SD; or number (percentage). All included women underwent controlled ovarian hyperstimulation (COH) using the standard GnRHa long protocol, in preparation for their primary IVF/ICSI cycle.

Table (2): Characteristics of IVF/ICSI Cycles in Included Women

Duration of Ovarian Stimulation (days)	Range	10 – 20
	Median (IQR)	14 (12 – 16)
Total Dose of Gonadotropins (IU)	Range	1650 – 7150
	Mean ± SD	3834.05 ± 1343.89
No. of Oocytes Retrieved	Range	6 – 23
	Median (IQR)	14 (10 – 19)
No. of Fertilized Oocytes	Range	3 – 23
	Median (IQR)	11 (7 – 14)
Fertilization Rate	Range	0.5 – 1.0
	Mean ± SD	0.77 ± 0.15
No. of Embryos Transferred	2	189 (45.3%)
	3	228 (54.7%)

IQR interquartile range, SD standard deviation

IVF in vitro fertilization, ICSI intracytoplasmic sperm injection

Data presented as range, mean ± SD; range, median (IQR); or number (percentage)

Included women were divided into two groups:

- Group I (n=89), including women who had difficult ET procedure.
- Group II (n=335), including women who had easy ET procedure.

The rate of difficult ET was, therefore, 89/417 (21.3%).

Table (3): Signs of Difficult ET Procedure in Included Women of Group I

Use of Stylet	43 (52.4%)
Excessive Blood on Tip of ET Catheter	30 (36.6%)
Cervical Grasping and Traction	9 (11%)

ET embryo transfer

Data presented as number (percentage)

There were no significant differences between women of both groups regarding initial characteristics (table-4).

Table (4): Difference between Groups regarding Initial Characteristics

		Group I Difficult ET (n=89)	Group II Easy ET (n=335)	P
Age (years)	Range	20 – 35	20 – 35	0.608 ¹
	Mean ± SD	27.01 ± 4.49	27.29 ± 4.47	NS
BMI (kg/m²)	Range	19.38 – 34.19	19.72 – 33.33	0.341 ¹
	Mean ± SD	25.93 ± 3.1	26.32 ± 3.33	NS
Indication for IVF/ICSI	Infertility	22 (26.8%)	101 (30.1%)	0.778 ²
	Tubal Factor Infertility	25 (30.5%)	104 (31%)	
	Male Factor	35 (42.7%)	130 (38.8%)	
	Unexplained Infertility			NS

SD standard deviation

BMI body mass index

IVF in vitro fertilization - ICSI intracytoplasmic sperm injection

Data presented as range, mean ± SD; or number (percentage)

1 Analysis using independent student's t-test

2 Analysis using chi-squared test

NS non-significant

There were no significant differences between women of both groups regarding the characteristics of IVF/ICSI cycles (table-4).

Table (5): Difference between Groups regarding Characteristics of IVF/ICSI Cycles

		Group I Difficult ET (n=89)	Group II Easy ET (n=335)	P
Duration of Ovarian Stimulation (days)	Range	10 – 18	10 – 20	0.426 ¹
	Median (IQR)	14 (12 – 16)	14 (12 – 16)	NS
Total Dose of Gonadotropins (IU)	Range	1650 – 6750	1650 – 7150	0.438 ²
	Mean ± SD	3728.05 ± 1465.4	3860.0 ± 1313.48	NS
No. of Oocytes Retrieved	Range	6 – 23	6 – 23	0.375 ¹
	Median (IQR)	13 (9 – 19)	14 (10 – 19)	NS
No. of Fertilized Oocytes	Range	3 – 22	4 – 23	0.349 ¹
	Median (IQR)	10 (7 – 14)	11 (7 – 14)	NS
Fertilization Rate	Range	0.5 – 1.0	0.5 – 1.0	0.496 ²
	Mean ± SD	0.76 ± 0.14	0.78 ± 0.15	NS
No. of Embryos Transferred	2	36 (43.9%)	153 (45.7%)	0.773 ³
	3	46 (56.1%)	182 (54.3%)	NS

IQR interquartile range

SD standard deviation

IVF in vitro fertilization - ICSI intracytoplasmic sperm injection

Data presented as range, mean ± SD; range, median (IQR); or number (percentage)

1 Analysis using Mann-Whitney's U-test

2 Analysis using independent student's t-test

3 Analysis using chi-squared test

NS non-significant

The biochemical, clinical and ongoing clinical pregnancy rates were significantly lower in women who had difficult ET when compared to women who had easy ET [ORs 0.5, 95% CI (0.31 to 0.83); 0.48, 95% CI (0.29 to 0.79); 0.36, 95% CI (0.21 to 0.62); respectively] (table-5). The rates of ectopic pregnancy were comparable in both groups (table-5).

Table (6): Difference between Groups regarding Pregnancy Rates

	Group I Difficult ET (n=89)	Group II Easy ET (n=335)	OR (95% CI)	P¹
Biochemical Pregnancy	30 (36.6%)	179 (53.4%)	0.5 (0.31 to 0.83)	0.006 S
Clinical Pregnancy	27 (32.9%)	170 (50.7%)	0.48 (0.29 to 0.79)	0.004 S
Ongoing Clinical Pregnancy	20 (24.4%)	158 (47.2%)	0.36 (0.21 to 0.62)	<0.001 HS
Ectopic Pregnancy	1 (1.2%)	2 (0.6%)	2.06 (0.18 to 22.95)	0.896 NS

Data presented as number (percentage)

OR (95% CI) odds ratio and its 95% confidence interval

1 Analysis using chi-squared test

S significant – HS highly significant – NS non-significant

Among women with positive clinical pregnancy, difficult ET was significantly associated with almost 5-fold higher risk of first trimester miscarriage [OR 4.6, 95% CI (1.63 to 13.06), p=0.006] (table-6).

Table (7): Difference between Groups regarding First Trimester Miscarriage

Among women with Positive Clinical Pregnancy	Group I Difficult ET (n=27)	Group II Easy ET (n=170)	OR (95% CI)	P¹
First Trimester Miscarriage	7 (25.9%)	12 (7.1%)	4.6 (1.63 to 13.06)	0.006

Data presented as number (percentage)

OR (95% CI) odds ratio and its 95% confidence interval

1 Analysis using chi-squared test

S significant – HS highly significant – NS non-significant

Binary logistic regression analysis showed that no. of embryos transferred and difficulty of ET procedure were the only significant factors independently associated with positive ongoing clinical pregnancy (table-7).

Table (8): Association between Positive Ongoing Clinical Pregnancy and Measured Variables

Positive Ongoing Clinical Pregnancy	OR (95% CI)	P
Age	1.0 0.96 to 1.05	0.997 NS
BMI	1.04 0.98 to 1.11	0.221 NS
Total Dose of Gonadotropins	1.0 1.0 to 1.0	0.817 NS
No. of Oocytes Retrieved	0.88 0.71 to 1.09	0.231 NS
Fertilization Rate	0.25 0.01 to 11.59	0.481 NS
No. of Embryos Transferred	1.51 1.0 to 2.28	0.049 S
Difficulty of ET	0.33 0.19 to 0.58	<0.001 HS

BMI body mass index

ET embryo transfer

OR (95% CI) odds ratio and its 95% confidence interval

Analysis using binary logistic regression

NS non-significant – S significant – HS highly significant

Among women who had difficult ET, excessive blood on tip of ET catheter was associated with the least ongoing clinical pregnancy rate [5 (16.7%)] and the highest first trimester miscarriage rate [4 (44.4%)]; the differences, however, were not statistically significant (table-6).

Table (9): Difference between Difficulties in ET Procedure regarding Ongoing Clinical Pregnancy and First Trimester Miscarriage Rates

Among women with Difficult ET	Use of Stylet	Excessive Blood on tip of ET Catheter	Cervical Grasping and Traction	P ¹
Ongoing Clinical Pregnancy	11/43 (25.6%)	5/30 (16.7%)	4/9 (44.4%)	0.227 NS
First Trimester Miscarriage	1/12 (8.3%)	4/9 (44.4%)	2/6 (33.3%)	0.156 NS

Data presented as number (percentage)

ET embryo transfer

OR (95% CI) odds ratio and its 95% confidence interval

1 Analysis using chi-squared test

NS non-significant

DISCUSSION

Embryo transfer (ET) is one of the most crucial steps in IVF/ICSI treatment. Although it, apparently, seems simple, it is an integral part of IVF/ICSI and can affect the outcome of the whole treatment cycle⁽¹⁰⁾.

ET procedure is easy in most cases. In a quarter of cases, however, a difficult ET procedure is encountered⁽⁸⁾. Difficulties in ET procedure are often caused by a stenosed cervical os, tortuous cervical canal and extreme utero-cervical angle⁽⁸⁾.

The impact of difficult ET on pregnancy outcome is well known. Although some reports show no harmful effect^(6,11), most of studies show a detrimental effect on IVF outcome^(2,7,12). It has been estimated that poor ET technique accounts for as high as 30% of IVF/ICSI failures⁽⁴⁾.

In addition to the evident association between difficult ET and negative pregnancy outcome, an association between difficult ET and first trimester miscarriage, among women who had initially a positive clinical pregnancy outcome, has been practically and clinically observed. The aim of the current study was to assess the association between difficult ET and ongoing clinical pregnancy outcome.

The current prospective study was conducted at Dar Al-Teb Infertility and IVF Center during the period between January 2017 and January 2018. The study included women who underwent ET of fresh blastocyst-stage good-quality embryos after primary IVF/ICSI treatment cycle. Women who had poor response (< 5 oocytes retrieved), who had cleavage-stage ET and those had known endometrial or uterine defect were not included in the study. In addition, women who had retained embryos in the ET catheter

and underwent re-ET after reloading of retained embryos were excluded from the study.

Included women were categorized into one of two groups: group I, including women who had difficult ET; and group II, including women who had easy ET. In the current study, two commonly practised techniques were selected as a marker of difficult: use of a malleable easily curved stylet (which would negotiate a tortuous cervical canal), and grasping and traction of the anterior cervical canal (which would straighten an extremely anteфлекed uterus). In addition, presence of excessive blood on the tip of ET catheter was used in the current study as a sign of difficult ET as it indicates endometrial trauma, and is associated with poor outcome.

A total of 417 women were included in the study; 82 (21.3%) had difficult ET, while 335 (78.7%) had easy ET. Difficulty of ET was defined as use of stylet in 43 (52.4%) women, finding excessive blood on tip of ET catheter in 30 (36.6%) women, and cervical grasping and traction in 9 (11%) women.

The mean age of included women was 27.24 ± 4.47 years (range: 20 – 35 years). The mean BMI was 26.24 ± 3.29 kg/m² (range: 19.38 – 34.19 kg/m²). Indications of IVF/ICSI included tubal factor infertility in 123 (29.5%) women, male factor infertility in 129 (30.9%) women, and unexplained infertility in 165 (39.6%) women. There were no significant differences between women of both groups regarding age, BMI or indications for IVF/ICSI.

The median no. of retrieved oocytes in included women was 14 (range: 6 – 23; interquartile range: 10 – 19). The mean fertilization rate was 0.77

± 0.15 (range: 0.5 – 1.0). Of the included 417 women, 189 (45.4%) had 2 embryos transferred, while 228 (54.7%) had 3 embryos transferred. There were no significant differences between women of both groups regarding these variables.

The current study showed a significantly reduced IVF/ICSI cycle outcomes among women who had difficult ET. Difficult ET was significantly associated with almost 2-fold reduction in biochemical pregnancy rate [OR 0.5, 95% CI (0.31 to 0.83), $p=0.006$], almost 2-fold reduction in clinical pregnancy rate [OR 0.48, 95% CI (0.29 to 0.79), $p=0.004$], and almost 3-fold reduction in ongoing clinical pregnancy rate [20 (24.4%) vs. 158 (47.2%), respectively; OR 0.36, 95% CI (0.21 to 0.62), $p<0.001$]. A binary logistic regression analysis showed that difficult ET is independently associated with negative ongoing clinical pregnancy outcome [OR 0.33, 95% CI (0.19 to 0.58), $p<0.001$].

The results of published studies regarding the association between difficult ET and pregnancy outcome were conflicting, though most literature agrees with significant adverse impact.

In an early retrospective study conducted by Tur-Kapsa ⁽⁶⁾ 854 ET procedures were analyzed. In this study difficult ET was defined when cervical traction, cervical dilatation or multiple attempts due to retained embryos were required. In contrast to the results of the current study, Tur-Kapsa ⁽⁶⁾ found comparable clinical pregnancy rates in easy ET and those conditions (23.3%, 23.6%, 23.8% and 23.9%, respectively). This study, however, carried many flaws. First, the rate of difficult ET was quite low (< 15%). Second, no hint was given regarding the presence of blood on the outer sheath or tip of the catheter. Third, there was no control of the possible confounding variables: quality of embryos transferred, female partner good/poor response, and whether there was prior failed IVF/ICSI cycles were all not defined.

In another early study, Burke ⁽¹¹⁾ conducted a retrospective logistic regression analysis of 205 IVF/ICSI cycles to find the predictors of cycle outcomes. The authors found that the only variable that predicts outcome was no. of high-grade embryos transferred. They found that difficult ET is not a significant predictor of IVF/ICSI outcome. This analysis also disagreed with the results of the current study. Nevertheless, the sample size was rather small, and, again, no hint regarding the presence of blood on the tip of catheter or its outer sheath.

In a retrospective analysis of 342 ET procedures performed in a single Indian center between 2008 and 2010, Singh ⁽¹⁾ defined difficult ET procedure as that required additional instrument, firmer catheter or changing of the catheter.

The rate of difficult ET according to this definition in their study was 17%. In this study, the clinical pregnancy rates were significantly lower among women who had difficult ET (17.2% vs. 23.6%, $p=0.045$). Although Singh ⁽¹⁾ did not include presence of blood on the ET catheter in the definition of difficult ET, they found that such a finding was associated with significantly reduced clinical pregnancy rate when compared to bloodless transfers (13.3% vs. 24.1%, $p=0.032$). The results of this study go in agreement with the results of the current study; yet with much lower clinical pregnancy rates. This latter observation may be explained by the non-uniformity of the included cases. The authors included cases who had COH using both long agonist and antagonist protocols, cases with poor and good response, as well as cases who had cleavage-stage and blastocyst-stage ET.

In another retrospective analysis conducted by Ghamen ⁽⁸⁾ between June 2013 and May 2015, at two centers: Mansoura University Infertility Center and a private center, 744 ET procedures were analyzed. Difficult ET was defined, in this study, when cervical traction was needed, blood was encountered on outer sheath, blood was encountered on tip of the catheter, and when cervical sounding was needed. According to this definition, difficult ET rate was 27%. In this study, the clinical pregnancy rates were lower among women who had difficult ET [39.8% vs. 45.6%]; the difference was, however, statistically not significant. When difficulties in ET were analyzed individually, the authors found significant association with reduced clinical pregnancy rate only with blood on tip of the catheter and cervical sounding. Neither cervical traction nor blood on outer sheath was associated with reduced clinical pregnancy rate ⁽⁸⁾.

The reason for those conflicting results, in addition to the flaws explained in the first two studies, actually originates from the variability of what a 'difficult ET' is. This is quite clear when the rates of difficult ET in various studies ranged between 7.7% ⁽¹³⁾ and 41% ⁽¹⁴⁾.

The different impact of various difficulty categories shown by the study conducted by **Ghanem** ⁽⁸⁾ emphasizes this fact: difficult ET is a very heterogeneous spectrum.

One of the findings of the current study also goes in agreement with that explanation, since subgroup analysis of the individual categories of difficulties of ET showed that presence of excessive blood on the tip of ET catheter was associated with the least ongoing clinical pregnancy rate and the highest first trimester miscarriage rate, when compared to use of stylet or cervical grasping and traction [16.7% vs. 25.6% vs. 44.4% and 44.4% vs. 8.3% vs. 33.3%; respectively and respectively];

though the results were underpowered to show a significant difference, if it ever exists.

The observation that presence of blood on the catheter is associated with poor outcome has been also showed by numerable previous studies ^(2,7,8,14,15,16).

More specifically, the presence of blood on the tip of the catheter, rather than on the outer sheath was shown to be associated with poor outcome ⁽⁸⁾. This is clinically logical as presence of blood on the tip of the catheter is a marker of endometrial trauma; which would affect implantation, while presence of blood on the outer sheath may be caused by bleeding from the ectocervix or even the cervical canal, which should not necessarily adversely affect implantation.

The impact of cervical grasping and traction in published literature was conflicting. In a review published by Mansour and Aboulghar, the authors assumed that cervical grasping induces oxytocin release and uterine contraction which can expel the deposited embryos ⁽³⁾. The authors built their assumption based on an early non-randomized study ⁽¹⁷⁾.

Subsequently such an assumption was refuted by a randomized controlled trial conducted by **Tremellen** ⁽¹⁸⁾ which showed that sexual intercourse (which induces prostaglandin release and uterine contractions) in the peri-transfer period did not affect IVF/ICSI cycle outcomes.

In addition, in the retrospective logistic regression analysis conducted by **Tomas** ⁽¹⁴⁾ on 4807 ET procedures (which were categorized into easy, moderate or difficult), cervical traction (which was categorized as moderate) was associated with similar clinical pregnancy rate to that for easy ET procedures ⁽¹⁴⁾.

In the retrospective analysis published by **Sallam** ⁽¹⁶⁾ on 784 cases, cervical traction was not shown to be harmful to pregnancy outcome.

On the contrary, several other studies showed an adverse impact of cervical grasping and traction on ET outcome ^(1,13,15).

A novel assumption and a unique outcome of the current study was the ongoing clinical pregnancy rate. It has been practically observed that even in women who had positive biochemical and clinical pregnancy outcome after difficult ET procedures, a significant proportion of them develop first trimester miscarriage (unpublished observational study).

In the current study, among women who had positive clinical pregnancy outcome, the rate of first trimester miscarriage was significantly higher among those who had had difficult ET. Difficult ET was significantly associated with almost 5-fold higher risk of first trimester miscarriage [OR 4.6, 95% CI (1.63 to 13.06), p=0.006].

Several other points of strength for the current study are also highlighted; the most significant of which was the prospective nature of the study. Most, if not all, of published studies are retrospective analyses.

Moreover, possible confounding variables were controlled as far as possible, including quality of the embryos, experience of the operator, the used catheter and the technique.

Only transfer procedures of fresh good-quality blastocyst-stage embryos in primary IVF/ICSI cycles were analyzed. The impact of the quality of embryos on pregnancy outcome was, therefore, controlled.

ET procedure is an operator dependent one ^(1,19). All ET procedures were performed by a single expert operator with an 8-year experience of IVF/ICSI hand skills. The impact of the operator as a confounding variable was, therefore, controlled.

All procedures were performed under ultrasound guidance and using the same catheter. Therefore, technical confounders were also controlled.

Yet, a point of weakness of this study was the relatively small sizes of different categories of difficulty in ET procedures and the too small no. of ectopic pregnancy, making this study underpowered to reliably govern such outcomes.

In conclusion, difficult ET is associated with significantly reduced biochemical, clinical and ongoing clinical pregnancy rates. Even in women who had positive clinical pregnancy outcome, a significant association was observed between difficult ET and first trimester miscarriage.

Conclusion

In conclusion, difficult ET is associated with significantly reduced biochemical, clinical and ongoing clinical pregnancy rates.

RECOMMENDATIONS

ET should be smooth with easy passage of the transfer catheter. Since any uterine manipulation during ET adversely affects IVF results, therefore precaution should be taken to identify possibly difficult ET cases in advance.

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