



## Evaluation of the Effect of Embryo Transfer Methods on Pregnancy Outcomes: A Retrospective Study and Future Perspectives

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### Abstract

**Background:** Trans-abdominal ultrasound (TAUS) and transvaginal ultrasound (TVUS) are used for embryo transfer. However, few studies were conducted to compare the methods and assess their effect on pregnancy outcomes.

**Methods:** A retrospective cohort study was conducted at Mahdieh Hospital in Tehran, analyzing 506 ICSI cycles with fresh embryo transfer. The study period was from April 2019 to March 2022. Following the evaluation of patients' profile, they were divided into two groups of TAUS (n=250) and TVUS (n=256). The pregnancy outcomes included positive test of  $\beta$ -HCG, history of miscarriage, ectopic pregnancy (EP), clinical pregnancy, and the duration of the embryo transfer were compared between two groups. Mann-Whitney U test, Pearson Chi-Square test, Fisher's exact test, and logistic regression were used for data analysis.

**Results:** The rate of chemical and clinical pregnancy in the TAUS group was higher compared to the TVUS group, which was statistically significant ( $p < 0.05$ ). The rate of live term birth and live preterm birth was higher in the TAUS group compared to the TVUS group, though the difference was insignificant. Moreover, EP and abortion rates were higher in TVUS group compared to the TAUS group, but the differences were not statistically significant. The odds ratio of achieving pregnancy was higher with TAUS compared to TVUS, but this was only statistically significant for the age variable.

**Conclusion:** The use of TAUS method appears to be associated with improved pregnancy outcome, including higher rates of chemical and clinical pregnancy, compared to TVUS. Yet, further research is needed to confirm these findings and elucidate underlying mechanisms.

**Keywords:** Abdominal ultrasound, Embryo transfer, Infertility, Pregnancy outcomes, Vaginal ultrasound.

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### Introduction

Infertility is one of the problems that affects a large number of couples in the world. There are numerous causes and factors that contribute to the occurrence of infertility (1, 2). Approximately 15% of couples struggle with infert-

ity which may significantly affect their lifestyle and psychological well-being. Infertility in many cases can be related to both female and male partners, while in certain cases, it is associated with either the female or male individual (3). Assisted

reproductive technology (ART) is the most popular of method used to treat infertile couples (4). ART methods vary based on the clinical conditions of the patient, and each one is selected according to the specific needs and circumstance of the individual patient (3).

The success rate of ART depends on different factors. These factors include the female age, the quality of the oocytes, the structure and function of the uterus, and many other factors (5, 6). The quality of embryo transfer procedure is the most important factor that can affect the success of ART. Embryo transfer is influenced by several processes (7, 8). The first, is the selection of good quality embryos, the second is, appropriate catheters for transfer of the embryos into the uterus. Finally, the physician is responsible for accurately placing the embryo into the uterus. Each of these steps can independently affect the outcome of pregnancy (7).

In the past, embryo transfer was challenging due to the lack of advanced imaging techniques. This could cause the catheter to come into contact with endometrium, which affected the success rate of IVF (9, 10). Gradually, the use of imaging techniques for embryo transfer increased. Recently, both abdominal and vaginal ultrasound have been utilized for embryo transfer. Although each of these two methods has the same function and efficiency, but they have their own distinct advantages and disadvantages (11-13).

While recent studies have been conducted regarding the effect of TAUS and TVUS on pregnancy outcome of ART cycles, a majority of these studies have focused on investigating each method independently. Therefore, the purpose of the current study was to compare the effect of the two methods on pregnancy outcome of ICSI cycles (14).

### Methods

In this retrospective cohort study, the patients were selected from those who sought medical care at Mahdiah Hospital in Tehran between April 2019 and March 2022. In total, 506 patients who underwent intracytoplasmic sperm injection (ICSI) with fresh embryo transfer were selected for this analysis. For all patients in both groups, good quality sperm were selected for the ICSI procedure. Additionally, all embryos used for transfer were top grade of quality.

The inclusion criteria for this study comprised of

women aged 20 to 42 years who were candidates for ICSI. These women had previously undergone ICSI with an antagonist protocol as a result of primary or secondary infertility. Additionally, patients with an endometrial thickness ranging from 6 to 7 mm were also included. Patients with uterine anatomical malformation, BMI >38, and those with underlying diseases such as diabetes, hypertension, and rheumatic diseases were excluded. After selecting the eligible patients, they were assigned to either TAUS or TVUS group based on the embryo transfer technique utilized.

**Embryo transfer procedure:** In both groups, a speculum was inserted into the vagina under sterile conditions after being washed with physiological saline solution. In TVUS group, the patients were asked to empty the bladder prior to the procedure. Then, a catheter (Cook Medical, USA) was guided into the vagina and the speculum was subsequently removed. Embryo transfer was performed under the guidance of vaginal ultrasound. In the TAUS group, patients were asked to have a full bladder. During the transfer of the embryo using a catheter (Cook Medical, USA), the probe was placed on the abdomen and the transfer was carried out under ultrasound guidance (Honda Electronics Co., Japan). The vaginal probe was H-S2100, while the abdominal probe used was H-S2000. For all patients, luteal phase support was provided with a 400 mg progesterone suppository (cyclogest; Actoverco Pharmaceutical Company, Iran).

**Evaluation of pregnancy outcome:** The pregnancy outcomes assessed following embryo transfer included chemical and clinical pregnancy, ectopic pregnancy (EP), live birth, abortion, and intrauterine fetal death (IUFD). All the procedures performed in this research were in accordance with the ethical standards of the local Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.RETECH.REC. 1401-604), as well as the 1964 Helsinki declaration.

**Statistical analysis:** All analyses were performed using SPSS version 26 software (IBM, USA). The data were presented as interquartile range (IQR) and n (%). Due to the non-normal distribution of the data, nonparametric tests including Mann-Whitney U test, Pearson Chi-Square test, Fisher's exact test, and logistic regression were employed for data analysis. The level of significance was set at  $p < 0.05$ .

Results

**Demographic information of patients:** Table 1 shows the demographic data of the patients. These data include age, BMI, history of abortion, EP, history of endometriosis, infertility, and use of ART. There were no statistically significant differences in these baseline characteristics between the two groups.

**Evaluation of  $\beta$ -HCG and embryo age in the two groups:** The percentage of patients with positive  $\beta$ -HCG results was lower compared to those with negative results in both groups ( $p=0.038$ ). Also, the number of  $\beta$ -HCG positive test in cleavage embryos that were transferred in two groups was higher compared to  $\beta$ -HCG negative cases ( $p=0.016$ ). In contrast, there were fewer blastocyst embryos with positive  $\beta$ -HCG transferred compared to those with negative  $\beta$ -HCG ( $p=0.034$ ) (Table 2).

**Characteristics of embryo transfer in the two groups:** The maximum number of embryos transferred was 3 in both groups. The minimum number was 1, and this showed a statistically significant difference between the two groups ( $p=0.002$ ). The number of cleavage embryos transferred was the highest in both groups, though blastocyst embryos were also transferred in the two groups ( $p=0.028$ ) (Table 3).

Table 2. Measured  $\beta$ -HCG levels in both groups

Variables	Type of ultrasound		p-value*
	Vaginal (n=256)	Abdominal (n=250)	
$\beta$ -HCG	57 (22.3%)	76 (30.4%)	0.038
Cleavage stage	203 (79.3%)	175 (70.0%)	0.016
Blastocyst stage	52 (20.3%)	71 (28.4%)	0.034

\* The result of Chi-Square test

**Evaluation of the pregnancy outcome in the two groups:** The percentage of chemical ( $p=0.038$ ) and clinical ( $p=0.028$ ) pregnancy was significantly higher in the TAUS group compared to the TVUS group. Also, the percentage of live term and live preterm births was higher in the TAUS group compared to the TVUS group, though the difference was not statistically significant. On the other hand, the rates of EP and abortion were higher in the TVUS group compared to the TAUS group, but this difference was not statistically significant (Table 4).

**The effect of ultrasound type on pregnancy outcome:** Table 5 shows the regression results, displaying the significance and lower and higher odds ratios for each of the variables. The odds ratio of IUFD in patients who had an abdominal

Table 1. Demographic information of patients

Variables		Type of ultrasound		p-value
		Vaginal (n=256)	Abdominal (n=250)	
Age (Mean $\pm$ SD)		33.00 $\pm$ 9.00	34.00 $\pm$ 8.00	0.105*
BMI (Mean $\pm$ SD)		26.47 $\pm$ 5.52	26.27 $\pm$ 5.38	0.754*
History of abortion	No	203 (79.3%)	202 (80.8%)	0.672**
	Yes	53 (20.7%)	48 (19.2%)	
Ectopic pregnancy	No	243 (94.9%)	238 (95.6%)	0.727
	Yes	13 (5.1%)	12 (4.4%)	
Endometriosis	No	235 (91.8%)	231 (92.8%)	0.682
	Yes	21 (8.2%)	19 (7.2%)	
Primary infertility	No	70 (27.3%)	61 (24.1%)	0.404
	Yes	186 (72.7%)	189 (75.9%)	
History of ART	No	72 (28.1%)	63 (25.0%)	0.427
	Yes	184 (71.9%)	187 (75.0%)	
Duration of infertility (years)	<5	153 (59.8%)	152 (60.9%)	0.170
	5-10	82 (32.0%)	66 (26.6%)	
	>10	21 (8.2%)	32 (12.5%)	

\* The result of Mann-Whitney U test

**Table 3.** Embryo transfer and associated age distribution in two groups

Variables		Type of ultrasound		p-value *
		Vaginal (n=256)	Abdominal (n=250)	
Number of transferred embryos	1	31 (12.1%)	53 (21.2%)	0.002
	2	138 (53.9%)	100 (40.0%)	
	3	87 (34.0%)	97 (38.8%)	
Embryo age	Cleavage stage	203 (79.3%)	175 (70.0%)	0.028 *
	Blastocyst stage	52 (20.3%)	71 (28.4%)	
	Others	1 (0.4%)	4 (1.6%)	

\* The result of Fisher's exact test

**Table 4.** Outcome of pregnancy

Variables		Type of ultrasound		p-value
		Vaginal (n=256)	Abdominal (n=250)	
Clinical pregnancy	Yes	45 (17.6%)	64 (25.6%)	0.028
	No	211 (82.4%)	186 (74.4%)	
Abortion	Yes	203 (79.3%)	196 (78.4%)	0.805
	No	53 (20.7%)	54 (21.6%)	
Ectopic pregnancy	Yes	4 (1.6%)	2 (0.8%)	0.686 *
	No	252 (98.4%)	248 (99.2%)	
Chemical pregnancy ( $\beta$ -HCG)	Yes	57 (22.3%)	76 (30.4%)	0.038
	No	199 (77.7%)	174 (69.6%)	
IUFD	Yes	1 (0.4%)	1 (0.4%)	1 *
	No	255 (99.6%)	249 (99.6%)	
Live term birth	Yes	31 (12.1%)	43 (17.2%)	0.105
	No	225 (87.9%)	207 (82.8%)	
Live preterm birth	Yes	5 (2.0%)	8 (3.2%)	0.375
	No	251 (98.0%)	242 (96.8%)	

\* The result of Fisher's exact test

ultrasound compared to those who had a vaginal ultrasound was 1.06 which is not significant. The odds ratio of live term birth and IUFD in patients who had an abdominal ultrasound compared to those who had a vaginal ultrasound was 0.60 which is not significant. The odds ratio of live preterm birth in patients who had an abdominal ultrasound compared to those who had a vaginal ultrasound was 0.60 which is not significant. The

odds ratio of IUFD in ectopic pregnancies among patients who underwent abdominal ultrasound compared to those who underwent vaginal ultrasound was 1.69. However, the difference is not statistically significant. The odds ratio for the age variable was 1.03. This indicates that for each one year increase in age, the significance of abdominal ultrasound is 1.03 times higher compared to vaginal ultrasound.

**Table 5.** Logistic regression model resulting from backward elimination (Wald) of variables which can differentiate ultrasound methods

		Variables in the equation						95% CI for EXP(B)	
		B	S.E.	Wald	df	Sig.	Exp (B)	Lower	Upper
Step 1 <sup>a</sup>	IUFD (1)	0.061	1.420	0.002	1	0.965	1.063	0.066	17.188
	Live term birth (1)	-0.507	0.259	3.826	1	0.050	0.602	0.362	1.001
	Live preterm birth (1)	-0.684	0.583	1.375	1	0.241	0.505	0.161	1.582
	Ectopic pregnancy (1)	0.526	0.878	0.358	1	0.549	1.691	0.303	9.452
	Age	0.036	0.015	5.382	1	0.020	1.037	1.006	1.068
	Constant	-0.717	1.860	0.149	1	0.700	0.488	-	-
Step 2 <sup>a</sup>	Live term birth (1)	-0.507	0.259	3.831	1	0.050	0.602	0.362	1.001
	Live preterm birth (1)	-0.684	0.583	1.376	1	0.241	0.505	0.161	1.582
	Ectopic pregnancy (1)	0.525	0.878	0.358	1	0.550	1.691	0.303	9.449
	Age	0.036	0.015	5.385	1	0.020	1.037	1.006	1.068
	Constant	-0.654	1.162	0.317	1	0.573	0.520	-	-
Step 3 <sup>a</sup>	Live term birth (1)	-0.515	0.259	3.962	1	0.047	0.597	0.359	0.992
	Live preterm birth (1)	-0.692	0.583	1.411	1	0.235	0.500	0.160	1.568
	Age	0.036	0.015	5.502	1	0.019	1.037	1.006	1.069
	Constant	-0.132	0.764	0.030	1	0.863	0.877	-	-
Step 4 <sup>a</sup>	Live term birth (1)	-0.491	0.258	3.619	1	0.057	0.612	0.369	1.015
	Age	0.035	0.015	5.082	1	0.024	1.035	1.005	1.067
	Constant	-0.771	0.541	2.034	1	0.154	0.463	-	-
Step 5 <sup>b</sup>	Age	0.031	0.015	4.114	1	0.043	1.031	1.001	1.062
	Constant	-1.056	0.516	4.182	1	0.041	0.348	-	-

a) Variable(s) entered on step 1: IUFD, live term birth, live preterm birth, ectopic pregnancy, age

b) Variable(s) entered on step 1: Age

### Discussion

Embryo transfer continues to be a challenge faced by infertility specialists. The use of imaging methods can be effective in embryo transfer and pregnancy success. However, TAUS and TVUS methods have their own distinct advantages and disadvantages. Therefore, the impact of each method on the success of pregnancy maybe different (15, 16).

The rate of chemical and clinical pregnancies was significantly higher in the TAUS group compared to the TVUS group ( $p < 0.05$ ). Additionally, the percentage of live term births and live preterm births was higher in the TAUS group compared to the TVUS group, though the difference was not statistically significant. In contrast, EP and abortion rates were higher in the TVUS group compared to the TAUS group, but the differences were not statistically significant. Furthermore, in the present study, the results showed that the use of TVUS compared to TAUS had a protective effect on live term birth.

In a previous study by Wageh et al., there were no significant differences in rates of first and second trimester abortion, EP, and clinical and biochemical pregnancy between the two groups that underwent embryo transfer using TAUS and TVUS (17). Their results were not consistent with the findings of the present study. This lack of alignment between the two studies can be due to the number of included patients. In the study of Karavani et al., no significant relationship was found between the two groups in terms of implantation, abortion, and clinical and biochemical pregnancy. However, the aforementioned study utilized the ICSI method, whereas the current study employed the IVF method (18). In the study conducted by Mohamed Hassan et al., a higher incidence of biochemical and clinical pregnancy was observed in patients undergoing TVUS compared to TAVS (19). The participants in this study were exclusively obese women, which could be a reason for the differences in results between their study and present one. Also, Geran Malekkheili et

al. demonstrated that the rate of chemical pregnancy was higher in the TVUS group compared to the TAUS group, although the difference was not statistically significant (20).

In general, the current study found that the use of TAUS had a more favorable effect on pregnancy outcomes compared to TVUS, which differs from the findings of some previous similar studies. Several factors may have contributed to this difference. The current study was a retrospective analysis of patients' data, whereas the previous studies were prospective clinical trials. It is important to note that the expertise and technique of the clinicians performing embryo transfer can potentially influence the results.

In the present study, it was found that the duration of ET using TAUS was longer compared to TVUS, but it was not statistically significant ( $p=0.07$ ). Similar findings of longer transfer durations with TAUS compared to TVUS were reported in previous studies by Karavani et al. and Mohamed Hassan et al. (18, 19, 21). In contrast, Geran Malekkheili et al. found the duration of TVUS to be longer. This inconsistency across studies is likely related to differences in the skill and expertise of the clinicians performing the embryo transfer procedures (20).

Considering that the embryo transfer duration was longer with TAUS method and the patient's bladder needs to be full during this procedure, it can potentially cause more pain and discomfort for the patients. The number of transferred embryos was statistically equivalent between the two groups in the current study. However, only the number of Blastocyst stage transferred was significantly higher in the TAUS group compared to the TVUS group ( $p<0.05$ ).

In previous studies including the research of Samy et al. and Hasan, the number of transferred embryos was equal in both groups (19, 22). However, in Karavani et al.'s study, the number of transferred embryos was higher in the TVUS group compared to the present study (18). In the study of Malekkheili et al., the average number of transferred embryos was 2 (20). The number of transferred embryos, which varies in different studies, is influenced by the quality of the oocytes retrieved. In other words, the quality of oocytes plays a significant role in determining the number of cases that are eligible for transfer.

In the present study, the number of  $\beta$ -HCG positive cases (indicative of chemical pregnancy) was higher in the TAUS compared to the TVUS

group, although the difference was not statistically significant. Previous studies have found that Blastocyst stage exhibited higher levels of  $\beta$ -HCG in patients' serum. Additionally, embryos that were implanted at a distance of 10 mm from the endometrium demonstrated higher levels of  $\beta$ -HCG positivity, which correlated with an increased rate of clinical pregnancy (23-25).

In general, there was a significant relationship between the number of transferred embryos and the blastocyst embryos which were  $\beta$ -HCG positive. Furthermore, the proficiency in embryo transfer technique can also have a significant impact on the outcomes. In general, there is no significant difference in the pregnancy outcomes between the use of the two aforementioned methods. Considering that the duration of TAUS is longer and the requirement for the full bladder can cause pain and discomfort for the patient, it is advisable to have TAUS performed by skilled professionals.

Also, since TAUS requires an assistant to adjust the probe, the expertise of the operator can also have an effect on the pregnancy outcome. This aspect should be further investigated in future studies to gain a better understanding of its influence. It is also better to use one of the two cited methods based on the patient's condition, the available equipment, and the skill level of the specialists. Given that TVUS is generally associated with less discomfort for patients, prioritizing the use of this method can be preferable for enhancing patient comfort.

### Conclusion

The use of TAUS method appears to be associated with improved pregnancy outcome, including higher rates of chemical and clinical pregnancy, compared to TVUS. Meanwhile, the percentage of EP and abortion was higher with the TVUS method. In general, it can be concluded that the rate of successful pregnancy is higher when using the TAUS method compared to TVUS. Yet, further research is needed to confirm these findings and elucidate the underlying mechanisms.

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

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