
Uterine length measurement before embryo transfer in ICSI cycles: is there a role?

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Short title

Uterine length and Embryo Transfer

Abstract

Objective: To evaluate the embryo transfer (ET) technique by measuring the length of uterus prior to embryo transfer (ULMbET) in comparison to the standard transabdominal ultrasound-guided embryo transfer (TA-UGET).

Methods: This randomized controlled trial was carried out on 116 IVF-fresh ET cycles which were randomly assigned to either ULMbET (n = 58) or TA-UGET (n = 58). The transfer of one to three high-quality fresh embryos was performed either under the guidance of transabdominal ultrasound (TA-UGET) or after transvaginal ultrasound measurement of the uterine cavity (ULMbET group). The rates of clinical pregnancy, ongoing pregnancy, and patient distress during ET were the primary outcome measures. Secondary outcomes encompassed the duration of the ET procedure, ET percentage requiring a repeat ET, and ET percentage involving a blood-stained catheter.

Results: The two groups exhibited a comparable clinical, baseline, and ICSI cycle characteristics. Rates of clinical pregnancy (51.7% vs 48.3%, $P=0.710$) and ongoing pregnancy (34.5% vs 31.0%, $P=1$) were comparable among the groups of ULMbET and TA-UGET. The mean score of discomfort intensity and the proportion of patients experiencing moderate-to-severe discomfort during ET both increased significantly with TA-UGET (2.1 vs 1.3 visual analog scale points and 20.7% vs 3.4%; $P<0.001$ and $P=0.004$, respectively).

Conclusion: The reproductive outcomes of IVF attained with ULMbET are similar to those obtained with TA-UGET; however, it is more patient-tolerated and technically simple to be employed by a single operator.

Keywords: Uterine length, Embryo transfer, TA-UGET, ULMbET.

INTRODUCTION

The probability of pregnancy in human in-vitro fertilization (IVF) is influenced by a variety of technical aspects of embryo transfer (ET). Several factors may affect IVF outcome, including the catheter type (1), operator expertise (2), embryo discharge site (3–6), contamination with blood or mucus (7), the difficulty level in negotiating the cervical canal, and contractions of uterus (8,9).

Over the past several years, embryo transfer (ET) was performed by passing the catheter through the cervical canal and releasing the embryos blindly, generally midway through the endometrial cavity. The term "clinical touch" ET was used to refer to this procedure. Ultrasound imaging during ET was progressively implemented to enhance the embryo's discharge site. The tip of catheter was visualized in real time with transabdominal ultrasound guidance. Furthermore, the echogenic spot observed immediately after the embryo being discharged allows accurate assessment of the embryo's position post-transfer. Most recently, TAUS-guided ET has been found to have a more significant live birth and clinical pregnancy rates than clinical touching in a number of systematic reviews of randomized trials (10–14).

However, the TV-UGET method has been suggested as an option that may be advantageous in certain patients (e.g., obese or with retroverted uterus), easier (single operator) and more comfortable (no need for a full bladder) compared to TA-UGET (15, 16). Two retrospective trials (17, 18) was carried out to assess TV-UGET, and two randomized controlled trials (RCTs) (19, 20) were conducted to compare it with TA-UGET. Despite the fact that these trials demonstrated comparable IVF outcomes, they were not sufficiently powered to draw definitive conclusions. Additionally, the technique of TV-UGET appears to be relatively difficult for the physician and unpleasant for the patient as a result of the need to insert the speculum,

vaginal probe, and transfer catheter into the vagina. To date, it has not been extensively adopted as a consequence.

The objective of the current RCT was to evaluate the ET technique in relation to the measurement of uterine length prior to embryo transfer (ULMbET) in which transvaginal ultrasound was employed to determine the optimal site of embryo discharge prior to ET, rather than during it. ULMbET maintains the benefits of TV-UGET over TA-UGET, while also being more patient tolerated and less technically demanding for the operator.

SUBJECTS AND METHODS

A randomized controlled trial included 116 women undergoing IVF- fresh ET between March 2022 and January 2024 at a specialized fertility and gynecology center in which the standard (control) technique was TA-UGET, while ULMbET was considered an experimental technique. The local ethical committee of Menoufia University endorsed the investigation. A comprehensive informed consent form was signed by all enrolled patients.

The study enrolled participants who met the following inclusion criteria: (i) transfer of one to three high-quality fresh embryos, (ii) age of less than 43 years, and (iii) without uterine cause of infertility.

Patients who exhibited the following characteristics were excluded: (i) patients aged 43 years or older, (ii) patients who have uterine anatomical abnormalities, such as myomas, malformations or endometrial lesions, and (iii) patients with forbidden cervix necessitating catheter replacement during ET due to the potential stiffer catheter impact on the outcome of IVF.

Randomization:

There were 116 patients who underwent IVF-fresh ET. Each subject was selected at random to receive either TA-UGET (n = 58) or ULMbET (n = 58), utilizing a comput-

er-generated algorithm for randomization at the day of ET in a 1:1 ratio. A third individual, the laboratory secretary, implemented the random allocation sequence by reviewing the randomized list (while the clinicians were uninformed) and transmitting the randomized evaluation via telephone (allocation concealment).

In-vitro fertilization protocol

The controlled ovarian stimulation (COS) was achieved by administering a starting dose of 100-300 IU/day of either human menopausal gonadotropin or recombinant follicle-stimulating hormone according to a long GnRH agonist or antagonist protocol. The anti-Mullerian hormone, basal (day 3) FSH, antral follicle count, body mass index, and age were used to determine the initial dose. The gonadotropin dose was adjusted in accordance with the response of ovary on days 6th –7th of ovarian stimulation.

The GnRH agonist was administered subcutaneously at a dose of 0.1mg/day, beginning on the 21st day of the previous cycle in the classical "long" protocol. The confirmation of pituitary suppression occurred approximately two weeks prior to the initiation of COS, as evidenced by the presence of menstrual flow, serum estradiol levels below 50 pg/mL, and endometrial thickness below 5 mm. The 'antagonist' protocol involved the initiation of the GnRH antagonist at a dose of 0.25 mg/day on a flexible schedule if at least one follicle with a diameter of 14 mm or greater was found.

Serial transvaginal ultrasound and serum measurements of estradiol were implemented to monitor the COS from stimulation days 6th –7th. The COS was maintained until at least three follicles had attained a diameter of 18 mm and had appropriate estradiol levels. In order to trigger ovulation, 10,000 IU of hCG was injected intramuscularly at this point. Around 36–37 hours later, oocyte pick-up was performed using transvaginal ultrasonography and intracytoplasmic sperm

injection was implemented. Embryos were assessed on 3rd to 6th day of in-vitro culture, and 1 to 3 high-quality, fresh embryos were transferred to the uterus. Extra embryos of top quality were cryopreserved. Commencing on the day of oocyte retrieval, progesterone vaginal pessaries (400mg every 12 hours) and intramuscular progesterone (100 mg/day) were administered to support the luteal phase for a period of 15 days.

The serum hCG assay was performed 15 days after ET to assess the pregnancy. The clinical pregnancy was confirmed when at least one sac of gestation was visible during transvaginal ultrasound after an additional 2 weeks. Another ultrasound scan was scheduled at ten weeks to verify that the pregnancy was still ongoing.

Embryo transfer technique

ET was carried out to all cases included in the present investigation on the third day (stage of cleavage) or fifth day (stage of blastocyst) with one to three high-quality fresh embryos. All ultrasound assessments were conducted using the Voluson E10 (GE Healthcare) ultrasound, which was equipped with a trans-abdominal probe and a 6.5-MHz transvaginal probe.

After the patient was positioned in the lithotomy position, the cervix was exposed using a speculum. Saline solution was used to meticulously cleanse the cervix, and the cervical mucus was aspirated or extracted using moist gauze.

A single experienced clinician conducted all ETs using the same soft catheter (Wallace®, Smiths Medical International Limited, UK) loaded by an experienced embryologist to prevent any bias that may be associated with the operator's experience. Wallace catheters were utilized for all patients. If a stiffer catheter or a tenaculum was used in the event that ET was a challenge due to the presence of cervical stenosis, these cases were excluded from the subsequent analysis. The Wallace catheter outer sheath was inserted first,

followed by the soft inner cannula which contains the embryos (afterload technique). Then, the embryos were discharged and the catheter was gently withdrawn and meticulously examined under a microscope in order to retransfer any embryos that were retained in the catheter (repeat ET). Additionally, blood within the catheter was identified and recorded. The total time necessary to accomplish ET was measured and recorded, which is defined as the time from the delivery of the loaded catheter to the clinician to its return to the embryologist following embryo discharge. The scale of visual analog (VAS) questionnaire was administered to the patient a few minutes following the ET procedure to assess the level of distress experienced during the procedure (zero to ten (points, spanning from no to extreme discomfort. Before ET, a physician instructs the participants on how to complete the questionnaire and the patient's written response to the questionnaire was received a few minutes after ET procedure.

When TA-UGET was implemented, a nurse or an assistant will perform the transabdominal ultrasound imaging and the embryos were placed at a distance of approximately 1.5 cm from the fundal endometrium (6).

During the scheduled ULMbET, the clinician performed a transvaginal ultrasound scan and measured the cervical length and endometrial cavity length in a sagittal plane (internal cervical os and fundal endometrium distance) immediately before ET. After the probe was removed from the vagina, the speculum was inserted and the embryos were discharged at a specific position, which was determined by subtracting 1.5 cm from the total length of the uterine cavity.

Transvaginal ultrasound was conducted immediately following either ULMbET or TA-UGET to verify the echogenic spot(s) within the uterus, which are indicative of air bubbles that contain the embryos. The embryo's ultimate position was determined by measuring the echogenic spot(s) distance from the fundal endometrial surface after approximately 30 seconds.

Sample size estimation:

As per a review of the prior research (21) that demonstrated that patients in the TA-UGET group were more likely to report moderate-to-severe distress during ET (19.8% vs 1.2%; $P = 0.045$ and $P = 0.003$, respectively) than in the control group. In order to indicate a difference with a significance level of $\alpha = 0.05$ and 80% power, a sample size of 116 cases (58 per group) was determined to be necessary. The minimal sample size that can be determined using sample size pro and statistics was 104 and in order to avoid a 10% attrition rate, the sample size should be increased to at least 116 participants.

Statistical analysis

Statistical Package for the Social Sciences (IBM Corp., 2017) was employed to input data into a computer, revise, code, and tabulate it. "IBM SPSS Statistics for Windows, Version 25.0." IBM Corp. is located in Armonk, New York. We used the Kolmogorov-Smirnov test to determine if the data were normal. A non-parametric variable was compared among the two groups of participants using the Mann-Whitney U test to determine if the difference was statistically significant. We used the Chi-Square test to study two qualitative variables. A result that is statistically significant is defined as having a p-value that is less than 0.05 and a 95% confidence interval.

Outcome measures:

The primary outcomes of the study were the rates of clinical pregnancy, ongoing pregnancy, the patient distress intensity during ET (as measured by the point scale of VAS), and patients' percentage experiencing moderate-to-severe discomfort (defined as a VAS score of three or higher) during ET.

In order to ascertain clinical pregnancy, transabdominal ultrasound was employed to observe the cardiac activity of fetus among six and seven weeks of gestation. A viable pregnancy that persisted beyond twelve weeks of gestation was defined as an ongoing pregnancy.

The secondary outcomes involved the ET procedure duration, the echogenic spot(s) distance from the endometrial surface of fundus, cases percentage with repeat ET, cases percentage without visible echogenic spot(s), and ET percentage with a blood-stained catheter (blood within the inner cannula after ET).

RESULTS

The CONSORT flow diagram is demonstrated in Figure 1. One hundred thirty women had IVF- fresh ET between March 2022 and January 2024. Fourteen of them were excluded: five due to an inability to meet the inclusion criteria, three because they declined to participate in the trial, and six as the cycle of IVF was terminated prior to ET (no fertilized oocytes, insufficient response to COS or no available embryos). The study included 116 patients who were randomized into two groups on the day of ET in a 1:1 ratio: Fifty eight patients were arranged to undergo TA-UGET, while the remaining 58 patients were scheduled to receive ULMbET. Upon completion of the trial, all participants are prepared for analysis.

The clinical, baseline, and the ICSI cycle characteristics involved in the final analysis are demonstrated in Table 1. No significant differences were found among the two groups in any of the baseline or clinical variables that were analyzed. In addition, the two groups exhibited a comparable Mii oocytes yield, quality and number of the transferred embryos during the ICSI cycle.

In terms of the primary outcomes, rates of clinical pregnancy (51.7% vs 48.3%, $P=0.710$) and ongoing pregnancy (34.5% vs 31.0%, $P=1$) of the ULMbET and TA-UGET groups did not differ statistically. The utilization of VAS to evaluate distress during ET led to substantially elevated levels of discomfort with TA-UGET (2.1 vs 1.3 points of VAS; P below 0.001). TA-UGET group also had a significantly higher patients proportion

experiencing moderate-to-severe discomfort (VAS > 3) (20.7% vs 3.4%; $P = 0.004$). The showed difference was most likely a result of the need to maintain a full bladder during TA-UGET (Table 2).

Concerning the secondary outcomes, the ULMbET group demonstrated a significantly decreased mean duration of the embryo transfer procedure (65.6 ± 14.9 s vs 72.5 ± 16.9 s, $P=0.027$). Alternatively, when comparing the groups based on the percentage of cases where an echogenic spot was not visible or regarding the distance between the spot(s) and the fundal endometrium, no significant differences were observed. At the same time, the rates of ET with blood in the catheter and rates of requiring a repeated ET procedure were similar (Table 2).

DISCUSSION

The outcomes of 'blind' clinical touch transfer technique have been compared to those of TA-UGET in numerous RCTs and systematic reviews (10–14). These reviews' results have generally indicated that TA-UGET offers some benefit. The TA-UGET procedure may facilitate ET by straightening the angle among the uterus and cervix, which may be responsible for the observed improvement in results when contrasted with clinical touch. Nevertheless, this approach has a few drawbacks: (i) The requirement for an ultrasound apparatus and an additional operator (a physician or nurse) who has received adequate training in transabdominal ultrasound. (ii) Ultrasound utilization to ascertain catheter tip's location is suboptimal in patients who are overweight or obese, or in retroversion of uterus. In order to more accurately determine the catheter's position, it may be necessary to move the catheter within the uterus; however, this may result in endometrial injury. (iii) The TA-UGET procedure requires more time than clinical touch, and (iv) For the duration of the procedure, the patient must maintain a full bladder, this could result in severe discomfort and distress. Subsequently, the pa-

tient's distress may result in uterine contractions.

TV-UGET use has also been suggested as a substitute for the standard TA-UGET procedure (15,16). This method offers several potential advantages, including the ability to ensure the uterocervical angle accurate detection in patients with uterine retroversion or obesity, as well as the catheter tip superior visualization compared to transabdominal ultrasound. Additionally, it does not necessitate a full bladder.

In comparison to clinical touch ET, two retrospective studies reported that the outcomes of IVF were significantly better with TV-UGET (17,18). In addition, two other recent RCTs demonstrated comparable clinical pregnancy and implantation rates when comparing TV-UGET and TA-UGET (19,20). The outer rigid portion of the transfer catheter, the vaginal ultrasound probe, and the speculum are all simultaneously held in the vagina for a period of several seconds in TV-UGET. Following the removal of the speculum, the probe is retained in the vagina during embryo release, and the interior, soft portion of the catheter which is loaded with the embryos, is inserted (19,20). If performed by a single operator, this technique is technically challenging and may cause distress for the patient, requiring manual dexterity.

The present investigation assessed a more tolerated ET technique that was performed using prior uterine length measurement (ULMbET). The most appropriate location for the embryo's discharge was determined by employing transvaginal ultrasound to measure the uterine length immediately prior to ET. In a retrospective study of 730 women, the same IVF outcomes as TA-UGET were achieved by utilizing uterine length measurement followed by clinical touch (22). In addition, a small prospective randomized study that included 26 patients also determined that the two methodologies were equivalent (23). Nevertheless, a more extensive recent randomized clinical trial that included 264

patients scheduled for frozen- thawed ET revealed a comparable pregnancy rates in the group that received a previous uterine length measurement and those with TA-UGET (23.4 versus 28%, $P=0.397$) (24).

In the current study, a prospective comparison of ULMbET and TA-UGET was carried out in patients who were undergoing IVF with fresh ET. The trial was planned as a randomized controlled trial with the aim of identifying any clinically relevant difference in the outcome of IVF among TA-UGET and ULMbET groups.

The technique of ULMbET in the present investigation enabled the outcomes of IVF to be comparable to TA-UGET in terms of clinical and ongoing pregnancy rates, as we observed. Additionally, the procedure was far more well-tolerated by patients, who were able to circumvent the distress that is associated with protracted bladder distention. Furthermore, the time necessary to conduct ULMbET was significantly reduced. Aside from being easy to learn, the ULMbET technique is simple enough for a single operator to use. As a further benefit, ultrasound measures for ULMbET may be planned during the oocyte pick-up or even during cycle monitoring, which has further cut down the ET duration.

A number of limitations were present in the current research. Initially, the operator and the patient were unable to be unaware of the varying transfer techniques. Nevertheless, we did not evaluate the live birth rate.

In conclusion, the reproductive outcomes of IVF attained with ULMbET are similar to those obtained with TA-UGET; however, it is more patient-tolerated and technically simple to be employed by a single operator.

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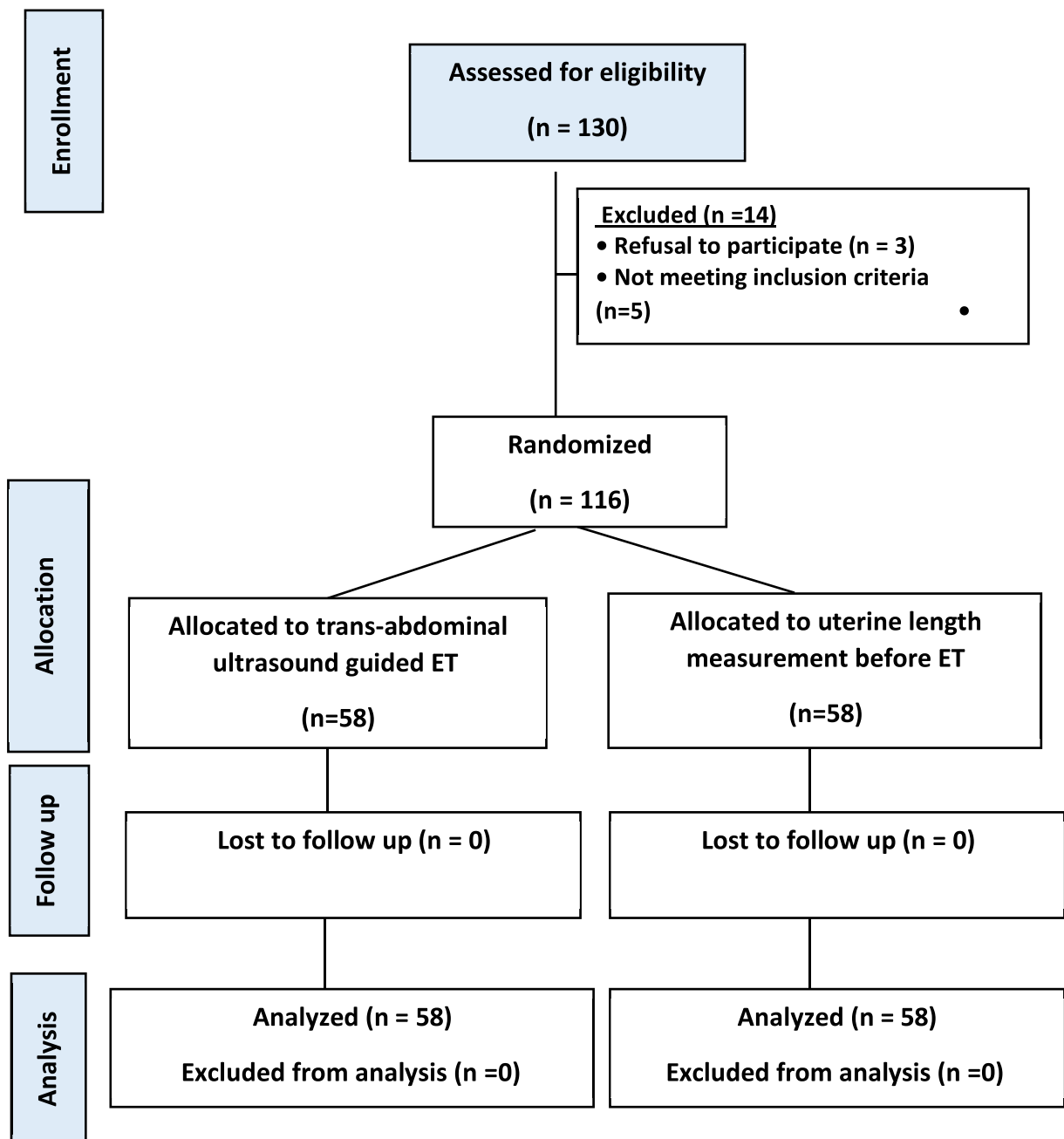


Figure (1): The CONSORT flow chart

Table (1): Baseline, clinical and ICSI cycle characteristics of the study population (N=116):

Studied variables	TA-UGET group N=58	ULMbET group N=58	Test of significance	P value
Age (years)				
Mean \pm SD	29 \pm 5.8	28.9 \pm 5.7	U=	0.899
Median(range)	29.5(19-42)	29(19-40)	1659.0	
BMI (kg/m²)				
Mean \pm SD	24.6 \pm 2.4	25.1 \pm 3.4	U=	0.397
Median(range)	24(20-30)	25(19-31)	1529.5	
Duration of subfertility (years)				
Mean \pm SD	4.4 \pm 1.5	4.4 \pm 1.6	U=	0.936
Median(range)	4(3-9)	4(3-9)	1668.0	
Main indication for IVF				
Unexplained	26(44.8%)	22(37.9%)	X ² = 10.2	0.109
Anovulation	2(3.4%)	11(19%)		
Diminished ovarian reserve	8(13.8%)	4(6.9%)		
Endometriosis	3(5.2%)	4(6.9%)		
Tubal	2(3.4%)	5(8.6%)		
Mixed	6(10.3%)	4(6.9%)		
Male factor	11(19%)	8(13.8%)		
Endometrial thickness (mm)				
Mean \pm SD	9.7 \pm 1.3	9.8 \pm 1.1	U=	0.534
Median (range)	9.5(7-12)	10(7-12)	1573.5	
Number of Mii oocytes				
Mean \pm SD	12.1 \pm 7.9	14.6 \pm 9.3	U=	0.116
Median (range)	10(2-34)	13(1-45)	1372.5	
Number of embryos transferred				
Mean \pm SD	2.3 \pm 0.5	2.3 \pm 0.5	U=	0.843
Median (range)	2(2-3)	2(2-3)	1653.0	
Quality of embryos transferred				
Grade A	53(91.4%)	54(93.1%)	X ² =	1.0
Grade B	5(8.6%)	4(6.9%)	0.120	

BMI: body mass Index; N: number; SD: standard deviation; U: Mann Whitney test; X²: chi square test.

Table (2): Outcomes of the ET procedures (N=116):

Studied variables	TA-UGET group N=58	ULMbET group N=58	Test of significance	P value
Clinical pregnancy	28(48.3%)	30(51.7%)	X ² = 0.138	0.710
Ongoing pregnancy	18(31.0%)	20(34.5%)	X ² = 0.036	1
Discomfort intensity (VAS points)				
Mean ±SD	2.1±0.9	1.3±0.5	U=	<0.001*
Median (range)	2(1-4)	1(1-3)	794.0	
Patients with moderate–severe discomfort (VAS > 3)	12(20.7%)	2(3.4%)	X ² = 8.123	0.004*
Duration of ET procedure				
Mean ±SD	72.5±16.9	65.6±14.9	U=	0.027*
Median (range)	72.5(42-101)	65.5(43-90)	1282.5	
Echogenic spot–fundus distance (mm)				
Mean ±SD	13.5±2.6	12.8±2.6	U=	0.295
Median (range)	13(10-22)	13(9-17)	1494.0	
ET with no visible echogenic spot	3(5.2%)	2(3.4%)	X ² = 0.209	1.0
Repeat ET	2(3.4%)	1(1.7%)	X ² = 0.342	1.0
Blood-stained catheter	7(12.1%)	5(8.6%)	X ² = 0.372	0.542

ET: embryo transfer; VAS: Visual analogue scale; SD: standard deviation; U: Mann Whitney test; X²: chi square test; * significant.