Does The Depth of Embryo Transfer Affect The Outcome of Intra Cytoplasmic Sperm Injection?

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Abstract

Background: ICSI (Intra cytoplasmic Sperm Injection) has been the treatment of choice in severe tubal infertility &for most other indications (male factors, ovarian dysfunction, tubal disease, endometriosis and uterine or cervical factors); ICSI is applied as the last resort therapy of infertility after the failure of other treatment modalities. Implantation is the final and most crucial step in ICSI , but also the least successful, as a few embryos replaced in the uterine cavity will result in a clinical pregnancy. The majority of women undergoing ICSI will reach embryo transfer (ET) stage, with good quality embryos available for transfer, but only a small proportion of them will ever achieve a clinical pregnancy or live birth. The most important factor for successful embryo transfer is not touching the endometrium of the fundus with replacement of the embryo in the lumen of the uterine cavity. Our study aimed to study the effect of the depth of embryo transfer into the uterine cavity on the success of ICSI cycle. Methods: This prospective cohort study was conducted at a private center starting from January 2020 to January 2022 . This study was conducted upon 120 women undergoing frozen ICSI cycle and they were divided into two groups: Group I : included 60 cases in whom the depth of embryo transfer was (>10 <15 mm) from the uterine fundus. Group II : included 60 cases in whom the depth of embryo transfer was (≤10 mm) from the uterine fundus. Results: In this study, Age in group I ranged 25-33 with mean value 28.1±3.65 and in group II ranged 26-35 with mean value 29.1±3.15. There was no statistical significant difference between the two studied groups regarding age (p value >0.05). BMI in group I was ranged 25-33.7 with mean value 28.3±2.0 and in group II ranged 24.69-31.6 with mean value 28.20±1.74. There was no statistical significant difference between the two studied groups regarding BMI (p value >0.05). Duration of infertility in group I was ranged 2-5 with mean value 3.10±0.98 and in group II was ranged 2-4.5 with mean value 3.45±0.87. There was no statistical significant difference between the two studied groups regarding duration of infertility (p value >0.05). Tubal factor in group I was 24 cases (40%) and in group II was 29 cases (48%), idiopathic factor was 19 cases(32%) in group I and 12 cases (20%) in group II, male factor in group I was 17cases (28%) and 19 cases (32%) in group II. Primary infertility was 48 cases (80%) in group I and 50 cases (84%) in group II, secondary infertility was 12 cases (20%) in group I and 10 cases (16%) in group II. In group I, number of embryo transferred ranged 2.1with mean value 1.25±0.33 and in group II ranged 2 with mean value 1.21±0.37. There was no statistical significant difference between the two studied groups regarding the number of embryo transferred. (p value >0.05). In group I, Endometrial thickness on day of ET mean ± sd was (11.2 ± 2.08) and in group II was (12.1 ± 2.2). There was statistical significant difference between the two studied groups regarding the Endometrial thickness on day of ET in group I (p value <0.05). As regarding items of fullness of bladder , ease of transfer , use of tenaclum and vaginal bleeding at time of embryo transfer there were no statistical significant difference between the two studied groups (p value >0.05). Moreover, Positive outcome was 25(41.7%) for 0.5-1.0cm and 37(61.5%) for >1cm.-5.1cm, while negative outcome was 35(58.3%) for 0.5-1.0 and 23(38.5%) for >1cm -1.5cm. There was statistical significant difference between group I (>10 <15 mm) from the uterine fundus with group II) ≤10 mm   (P< 0.05). Conclusion: Measuring the depth of embryo transfer into the uterine cavity has a significant role in improving success of ICSI cycle. The embryo transferring depth between (10mm and 15mm) significanty improve ICSI cycle success rate.

Key words: The Depth of Embryo Transfer, Outcome, Intra Cytoplasmic Sperm Injection.

1. Introduction

Infertility is defined as the inability of a couple to conceive after one year of regular unprotected intercourse under 35 years of age and after six months in women 35 or older. Infertility is a common condition about 8 -10 percent of the couples who are trying to conceive and not able to do so [1].

Medical treatment of infertility generally involves the use of fertility medication, medical device, surgery, or a combination of the above mentioned. If conservative medical treatments fail to achieve a full term pregnancy, the physician may suggest the patient undergo in vitro fertilization (IVF), and related techniques (ICSI, ZIFT, GIFT) which called assisted reproductive technology (ART) techniques [2].

ART techniques generally start with stimulating the ovaries to increase egg production. After stimulation, the physician surgically extracts one or more eggs from the ovary, and unites them with sperm in a laboratory setting, with the intent of producing one or more embryos. Fertilization takes place outside the body, and the fertilized egg is reinserted into the woman's reproductive tract, in a procedure called embryo transfer [3].

Embryo transfer may be the most important, final step in IVF (in vitro fertilization) and intra-cytoplasmatic sperm injection (ICSI) treatment. The embryo transfer can be performed in three ways: [1] blind (clinical touch); [2] based on information on the length of the uterus (obtained by previous ultra-sonographic measurement or mock transfer) or; [3] guided by abdominal ultrasonography [4].

It is advantageous to place the embryos into the uterus as a traumatic manner as possible. Uterine

contractions, presence of blood on the catheter, bacterial contamination of the catheter, difficulty of transfer, and the type of catheter all influence the success rate of the IVF/ICSI treatment [5].

The embryos should be placed in an area within the uterus most likely to afford implantation. Some studies have suggested that ultrasound guidance of the embryo transfer procedure may be of benefit because the tip of the embryo transfer catheter can be visualized to ensure that the embryos are placed in the proper location. Some show that pregnancy and implantation rates can be dramatically affected by the physician performing the embryo transfer [6].

There is good and consistent evidence of benefit in ultrasound guidance, using trans-abdominal ultrasound to ensure correct placement, which is 1–2 cm from the endometrium of the uterine fundus. There is evidence of a significant increase in clinical pregnancy using ultrasound guidance compared with only "clinical touch". Anesthesia is generally not required. The optimal target for embryo placement, known as the maximal implantation point (MIP), is identified using 3D/4D ultrasound.

However, there is limited evidence that supports deposition of embryos in the midcavity of the uterus [7].

A high success rate has been reported with a technique of transabdominal ultrasound guidance in the presence of a full bladder [8].

Most IVF teams consider not touching the endometrium and the uterine fundus with replacement of the embryos in the lumen of the endometrial cavity the most important factors for successful embryo transfer [9].

It has been traditionally accepted that the embryo should be placed about 10 mm below the fundal endometrial surface, some authors have suggested that placing embryos rather lower in the uterine cavity may improve pregnancy rate [10].

The aim of this work was to study the effect of depth of embryo transfer site into the uterine cavity on clinical pregnancy rate during (ICSI) cycle.

2. Patients and Methods
Patients:
Study design:

This was a prospective clinical cohort study carried out in a private center starting from January 2020 to January 2022 . This study was conducted upon 120 women undergoing frozen ICSI cycle and they were divided into two groups:

- **Group I**: included 60 cases in whom the depth of embryo transfer was (>10 <15 mm) from the uterine fundus.
- **Group II**: included 60 cases in whom the depth of embryo transfer was (≤10 mm) from the uterine fundus.

The required sample for analysis was calculated according to the following equation : $N = (Z^2 \times P \times Q)/(E^2)$

- Z= constant=1.96
- P= Number of affected population
- Q=1-p=Number of unaffected population
- E = error =.05%
- So, N= $(1.96)^2$ x 0.08 x 0.92 / 0.0025 = 113 cases.
- All included women were informed about the aim of the study, the required procedures and all cases were subjected to informed written consent before inclusion.

Inclusion criteria
1. Age of patient between 25-35 years.
2. Main causes of infertility attributable to tubal, idiopathic or male factor.
3. Embryos quality should be ≥ A.
4. Less than four embryos were replaced into the uterine cavity.
5. Embryo transfer was carried out in frozen cycles.

Exclusion criteria
1. Any uterine or cervical pathology.
2. Any uterine or cervical anomalies.
3. Patients of repeated implantation failure.

The same transfer technique was maintained with all patients.

Methods:
Every patient was be subjected to:
1. Informed written consent.
2. Complete history taking to define the inclusion and exclusion criteria. 3. General examination of the patients.
3. Vaginal bimanual examination.
4. Vaginal ultrasound to exclude any pelvic diseases or uterine anomalies.

A total of 120 patients who met the above mentioned criteria were enrolled in this study. Randomization fulfilled by closed envelope manner into two study groups according to the distance between the tip of the catheter and the uterine fundus at the time of embryo deposition in the cavity ; group I (>10 <15 mm) and group II (≤10 mm) .

Our frozen ICSI cycle management consisted of : Hormone replacement treatment (HRT) as follows :

Treatment with oral E_2 (estradiol valerate) is started on the first, second or third day of the cycle to prime the endometrium and suppress spontaneous follicle growth. Estradiol is administered either at a fixed constant dose (8 mg daily) or in an incremental fashion; the most commonly used is 2 mg/day during days 1-7, 4 mg/day during days 8-12, 8 mg/day during days 13 to embryo transfer [11].

Usually, after 12 – 14 days of E2 administration, vaginal ultrasound examination is performed for endometrial thickness measurement and to confirm the absence of a leading follicle. When the endometrial thickness >7 mm, Progesterone supplementation is commenced, and timing of FET is scheduled accordingly [12].

Optimal exposure of P, in terms of timing and concentration, is crucial for the establishment and maintenance of an ongoing pregnancy. The available routes for P administration in an HRT cycle are vaginal,

intramuscular (im), subcutaneous (sc), oral and rectal. The vaginal administration, distinct form the other routes, has a first-pass uterine effect [13]. Vaginal P administration in our HRT cycle consisted of vaginal suppository 400mg twice daily (2x400 mg).

Since there is no corpus luteum in an HRT cycle, all the available P derives from exogenous administration The optimal length of luteal support in HRT cycles from a physiological point of view should be continued until the luteo-placental shift occurs which is still a matter of debate and generally the common practice is to continue until 10th-12th weeks of gestation [14].

Embryo transfer was performed on day 3 (in frozen cycles) as follows:

The embryo replacement catheter connected to an insulin syringe (Labotect, Trans-vaginal ET catheter set 4, Germany) was used for all embryo transfer (This is a soft silicon catheter that possesses a stiffer outer sheath that stabilizes the softer inner cannula which carries the embryos).

Ultrasound monitoring was performed using transabdominal imaging with a 3.5 MHz linear array probe.

The bladder was partially filled as two hundred milliliters of liquid were given to the patient 30 min before embryo transfer to both enhance imaging of the uterus and endometrial lining and to straighten any uterine flexion that may be present.

A speculum was then inserted into the vagina and the vaginal vault will be carefully swabbed with sterile saline solution.

The coaxial system loaded with the embryos in the transfer catheter will be then brought into the field.

Both transfer and guide catheter was advanced as a unit. The guide catheter should serve to stabilize the softer and more flexible transfer catheter.

The transfer catheter should be kept well within the rigid one to reduce any unintended movement of the transfer catheter tip. Care will be taken not to advance the guide catheter into the depth of endometrial cavity as it may disturb the endometrium lining. The guide catheter will be advanced just beyond the internal os. The transfer catheter may be then advanced to a definite distance from uterine fundus (>10 mm <15mm) and (≤10 mm), respectively in I and II groups.

This measurement may be easily verified using the callipers prior to injection of the embryos. After transfer, the catheter was held in place for 45 to 60 seconds to permit the embryos to settle away from catheter tip.

The transfer and guide catheters was then slowly withdrawn as a unit and inspected for any retained embryos and to detect bleeding. Transfer will performed by the same transfer technique in all patients included in this study.

Transvaginal ultrasound was done to measure the distance between embryo droplet and the endometrial lining of fundus of uterus after transfer [15].

Increasing serum concentration of beta hCG and the presence of intrauterine gestational sac demonstrated pregnancy and success of ICSI cycle.

3. Results

Table (1): shows comparison between the two studied groups regarding age. Age in group I ranged 25-33 with mean value 28.1±3.65 and in group II ranged 26-35 with mean value 29.1±3.15. There was no statistical significant difference between the two studied groups regarding age (P > 0.05).

Table (2): shows comparison between the two studied groups regarding BMI. BMI in group I was ranged 25-33.7 with mean value 28.3±2.0 and in group II ranged 24.69-31.6 with mean value 28.20±1.74. There was no statistical significant difference between the two studied groups regarding BMI (P > 0.05).

Table (3): shows comparison between the two studied groups regarding duration of infertility. Duration of infertility in group I was ranged 2-5 with mean value 3.10±0.98 and in group II was ranged 2-4.5 with mean value 3.45±0.87. There was no statistical significant difference between the two studied groups regarding duration of infertility (P>0.05).

Table (1) Comparison between the two studied groups regarding age.

Age	Group I (60)	Group II (60)	Student t test	P
			1.61	0.11
Range	25 – 33	26 – 35		
Mean	28.1	29.1		
S.D.	3.65	3.15		

Table (2) Comparison between the two studied groups regarding **BMI.**

BMI	Group I (60)	Group II (60)	Student t test	P
Range	25-33.7	24.69-31.6	0.29	0.77
Mean	28.3	28.20		
S.D	2.0	1.74		

Table (4): shows comparison between the two studied groups regarding cause of infertility. Tubal in group I was 24 cases (40%) and in group II was 29 cases (48%), idiopathic was 19 cases(32%) in group I and 12 cases (20%) in group II, male factor in group I was 17cases (28%) and 19 cases (32%) in group II.

Table (5): shows comparison between the two studied groups regarding the type of infertility. Primary was 48 cases (80%) in group I and 50 cases (84%) in group II, secondary was 12 cases (20%) in group I and 10 cases (16%) in group II.

Table (6): shows comparison between the two studied groups regarding the Embryo transfer characteristics . There was no statistical significant difference between the two studied groups regarding the Embryo transfer characteristics (P > 0.05).

Table (7): shows comparison between the two groups regarding the clinical outcome pregnancy rate by different depth of embryo transfer. Positive outcome was 25(41.7%) for 0.5-1.0cm and 37(61.5%) for >1cm.-5.1cm, while negative outcome was 35(58.3%) for 0.5-1.0 and 23(38.5%) for >1cm -1.5cm. There was statistical significant difference between group I (>10 <15 mm) from the uterine fundus with group II) ≤10 mm (P< 0.05).

Table (3) Comparison between the two studied groups regarding duration of infertility **.**

	Group I (60)	**Group II (60)**	**Student t test**	**P value**
Duration of infertility (yrs)				
Range	2 – 5	2 – 4.5		
Mean	3.13	3.45		
S.D.	0.98	0.87	1.89	0.06

Table (4) Comparison between the two studied groups regarding cause of infertility.

	Group I (60)		**Group II (60)**		**X2**	**P value**
	No	**%**	**No**	**%**		
Tubal	24	40.0	29	48.3	2.16	0.34
Idiopathic	19	31.7	12	20.0		
Male factor	17	28.3	19	31.7		
Total	60		60			

Table (5) Comparison between the two studied groups regarding the type of infertility.

Type of infertility	**Group I**		**Group II**		**X2**	**P value**
	No	**%**	**N0**	**%**		
Primary	48	80.0	50	83.3	0.22	0.64
Secondary	12	20.0	10	16.7		
Total	60		60			

Table (6) Comparison between the two studied groups regarding the Embryo transfer characteristics.

	Group I **No= 60**	**Group II** **No= 60**	**Statistical test**	**P value**
Mean number of embryo transferred **mean ± sd**	2.1±1.2	2±1.6	Student t = 0.387	0.699
Endometrial thickness on day of ET **mean ± sd (mm)**	11.2 ± 2.08	12.1 ± 2.2	Student t = 0.233	0.681
Full bladder (n)%	(31) 51.7%	(29) 48.3%	0.133	0.72
Ease of transfer (n)%	(43) 71.7%	(41) 68.3%	0.159	0.69
Use of tenaclum (n)%	(4) 6.7%	(5) 8.3%	FET= 0.0	1.0
Bleeding (n)%	(3) 5.0%	(5) 8.3%	FET= 0.134	0.714

Table (7) Comparison between the two groups regarding the clinical outcome pregnancy rate by different depth of embryo transfer.

Pregnancy **Rate**	**Group I** **(>10 <15 mm)**		**Group II** **(≤10 mm)**	
	Number	**%**	**number**	**%**
Positive	37	61.7	25	41.7
Negative	23	38.3	35	58.3
Total	60		60	
X2		4.81		
P value		0.028*		

4. Discussion

Implantation is the final and most crucial step in ICSI and IVF, but also the least successful, as a few embryos replaced in the uterine cavity will result in a clinical pregnancy.The majority of women undergoing IVF will reach embryo transfer (ET) stage, with good quality embryos available for transfer, but only a small proportion of them will ever achieve a clinical pregnancy or live birth.[16] Pregnancy rate after ET is dependent on multiple factors including embryo quality, endometrial receptivity and the technique of ET itself. [17] In the recent years, more stress has been placed in optimizing and standardizing the ET protocol. Factors such as ease of the procedure catheter choice, the depth of ET have proved to improve the clinical outcomes. The most important factor for successful embryo transfer is not touching the endometrium of the fundus with replacement of the embryo in the lumen of the uterine cavity[18]. In agreement with our study, *Arianna 2007*[15] study 104 infertile patients underwent ultrasound-guided embryo transfer were randomly assigned to two study groups according to the distance between the tip of the catheter and the uterine fundus at the time of embryo deposition in the cavity: group A: > 10 < 15 mm ; group B >5 <10 mm. In this study, There was no statistical significant difference between the two studied groups regarding age, BMI, type , duration and cause of infertility also number of embryos transferred nor endometrial thickness at the day of ET (p value >0.05). In our study positive outcome was 25(41.7%) for <10mm and 37(61.5%) for (>10 <15mm), while negative outcome was 35(58.3%) for <10mm and 23(38.5%) for (>10<15mm). There was statistical significant difference between group I (>10 <15 mm) from the uterine fundus with group II ≤10 mm (P< 0.05). Our data seems to show a significant increase in pregnancy rate and implantation rate in A group, where there was a distance > 10 < 15 mm between the embryo and uterine fundus. In agreement with our study, *Arianna 2007* found a statistically significantly difference in pregnancy rate (PR) that results 27.7% in A group vs. 14% in group B (P = 0,009) and in implantation rate (15.3% vs. 7.1%) respectively in group A and B) (P = 0.027). *Abdel Salam 2010* [19] studied a total of 90 patients who complaining of infertility and undergoing ICSI cycle. They were randomly assigned to three study groups according to the distance between the tip of the catheter and the uterine fundus at the time of embryodeposition in the cavity; group I (<0.75 cm); group II: (0.75 – <1.5 cm) ; group III (1.5–2 cm).Among the various aspects of embryo transfer, the site of embryo placement in the uterine cavity has been postulated to influence embryo implantation rates. The data in the study showed a significant increase in implantation rate and pregnancy rate in group II and group III, (p value= 0.003 and 0.001 respectively) where there was a distance 0.75–1.5 and 1.5–2 cm, respectively, between the tip of the catheter and the uterine fundus.

Ivanovski et al.,2012[20] conducted a prospective observational study upon 106 infertile patients, aged 24-42 years undergoing IVF/ICSI and embryo transfer. The patients were divided into two groups according to the distance between the tip of the catheter and the uterine fundus at the time of embryodeposition in the cavity: group A: 10 +/- 2.5 mm; group B: 15 +/- 2.5 mm .There were not significantly differences between the two groups in term of embryo transfer characteristics (number of embryo transferred, endometrial thickness at day of ET, full bladder, ease of transfer, use of tenaculum, bleeding). But their results demonstrated that pregnancy rate is significantly influenced by transfer distance from the fundus where the pregnancy rate decreases from 46.2% in group B to 28.8% in group A (p < 0.05) which is in agreement with our results. *Neeta et al.,2017* [21] conducted a retrospective analysis of 743 ultrasound-guided ET in in vitro fertilization (IVF) cycles from a single center over a period of 4 years. The distance between the fundal endometrial surface and the air bubble was measured, and accordingly, patients were divided into four groups (≤10 mm; >10 and ≤15 mm; >15 and 20 mm; >20 and <25 mm). Clinical pregnancy rate was significantly more in groups 2 and 3 compared to the other groups. Similarly, pregnancy outcome will be 3.1 (95% confidence limits: 1.5–6.4) times higher for distance group >15 and <20 mm compared to less than 10-mm distance group. The lower PRs found in the group transfers directed toward the uterine fundus (<10mm) may be a result of more traumatic transfers, resulting in more frequent uterine contractions, negatively affecting PRs. Retrospective analysis (from May 2005 to November 2008) of 5,055 ultrasound-guided embryo transfers belonging to 3,930 infertile couples. The distance between the fundal endometrial surface and the catheter tip was measured and accordingly, patients were divided into five groups. Pregnancy rates and ongoing PRs are higher if the embryos are replaced at a distance >10 mm from the fundal endometrial surface. In addition because significantly more embryos were replaced in cycles where the transfers occurred at a distance of >20 mm, Finally they concluded that a distance (>10 mm to <20 mm) seems to be the best site for embryo transfer to achieve higher PRs. [22]

Davar et al.,2020[23] studied 180 women who were candidate for IVF/ ICSI/ embryo transfer were equally assigned to two groups based on the distance between the fundal endometrial surface and catheter tip to investigate implantation, chemical and clinical pregnancy (group A: 15 ± 5 mm and group B: 25 ± 5 mm, respectively). The subjects in the group B showed significantly higher implantation rate, chemical and clinical pregnancy rate compared to the group A (p = 0.03, 0.01, 0.04, respectively). They concluded that the depth of embryo replacement inside the uterine cavity at a distance of 25 ± 5 mm beneath fundal endometrial surface have better effects on the pregnancy outcomes of IVF/ICSI cycles and can be considered as an important factor to improve the success of IVF cycles.

Since the earliest descriptions of ICSI, laboratory aspects of this procedure were considered the driving forces behind improved pregnancy rates and embryo transfer techniques were considered of secondary importance.

Techniques, equipment and materials for embryo transfer catheters remained relatively unchanged until recently. Earlier techniques used single lumen, relatively rigid catheters passed into the uterine cavity under clinical touch only. Although the production of quality embryos remains the goal, there is an increasing appreciation of the embryo transfer technique as a variable that can significantly impact pregnancy rates. Several factors have emerged that can impact success of any embryo transfer including the vaginal flora, experience of the clinician performing the transfer [24], and volume of transfer media. Evidence suggests that blind embryo transfer may result in placement of the embryos outside the uterine cavity in as much as 25–30% of cases. [25]

What we want to show in our study is that, although base line patient clinical characteristics ,treatment cycle characteristics, ovarian response and any variable of transfer (difficulty of transfer , bleeding and use of tenaculum) were all similar in the two studied groups, there is a statistically significant difference in PR and IR between the two groups . we avoided any impact of the physician factor on implantation rate because all transfers were carried out by the same provider.

The two most significant changes in embryo transfer techniques are improvements in catheter quality and guidance techniques enabling accurate and atraumatic embryo replacement. There is a trend in deviating from rigid, single lumen catheters—towards the use and continued development of coaxial soft tipped catheter systems; and a trend away from replacement of the embryos under tactile guidance only—toward ultrasound guidance for embryo transfers. These improvements have resulted in a more efficient transfer technique and improved pregnancy rates.

Corolue [8], demonstrated that embryo transfer under ultrasound guidance compared with the clinical touch method improved pregnancy rates and these findings were confirmed by others . In using the clinical touch method, differences in the skill of the physician to very gently approach the fundus, never miss the touch moment and thereby creating a minimally traumatic transfer may account for the differences in pregnancy rates. In contrast, the fixed distance technique makes it possible for any provider to replace the embryos a traumatically, as the fundus need not to be touched for assessing the correct position of the catheter tip in the cavity at the time of expelling the embryos.

Roseboom et al. [26], found that the change in embryo transfer technique was paralleled by a significant increase in the overall pregnancy rates. Obviously, the change in pregnancy rates after the switch may have been caused by many known or unknown factors. He analyzed factors that are well known to play a role in the outcome of pregnancy after embryo transfer and that were selected from his own data by multivariate logistic regression. He could not find clear differences in these factors before and after the switch in technique, apart from the factor embryo quality that may have contributed partly to the observed difference.

To ensure that the embryos are expelled at the right distance from the uterine fundus , the sounding length of the uterus should be measured before the transfer. Mock transfer procedure will provide essential information on the depth of replacement but may be considered as disproportionate burden for the patient as well as the doctor [27].

5. Conclusion

Measuring the depth of embryo transfer into the uterine cavity has a significant role in improving success of ICSI cycle. The embryo transferring depth between (10mm and 15mm) significanty improve ICSI cycle success rate.

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