The effect of air bubble position on ART outcome in frozen-thawed blastocyst transfer

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Research

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Abstract

Background

So far, only few literatures have studied the relationship between blastocyst transfer position and ART outcomes, and the conclusions are still controversial. Our study is to evaluate the effect of air bubble position on ART outcome and to find the optimal embryo transfer position in frozen-thawed blastocyst transfer.

Methods

This study included a retrospective cohort analysis of 399 frozen-thawed single blastocyst transfers ultrasound-guided performed between June 1, 2017 and November 30, 2020. All of the women scheduled for frozen-thawed single blastocyst transfers ultrasound-guided. The primary outcome is clinical pregnancy rate and the secondary outcome is live birth rate. Statistical analyses were conducted using One-way Anova, Kruscal Whallis H test, chi-square test and Smooth curve fitting.

Results

When BFD was less than 19 mm, there was no significant change in clinical pregnancy rate as BFD increased (OR = 0.95, 95% CI: 0.89 to 1.02, P = 0.1373); when BFD was more than 19 mm, the clinical pregnancy rate decreased by 16% for every 1 mm increase in BFD (OR = 0.84, 95% CI: 0.72 to 0.98, P = 0.0363). The effect of BFD on live birth rate were similar to that on clinical pregnancy rate, the inflection point was 19mm, when BFD was more than 19 mm, the live birth rate decreases by 58% for every 1 mm increase in BFD (OR = 0.42, 95% CI: 0.21 to 0.86, P = 0.0174)

Conclusions

The ideal pregnancy outcome can be achieved within 19mm from uterus fundus after single blastocyst transfer, The clinical pregnancy and live birth at a distance of more 19mm from the uterus fundus have a cliff-like downward trend.

Introduction

Embryo transfer (ET) is one of the most critical factors affecting assisted reproductive technology (ART). Many factors affecting embryo transfer are also related to the success of ART, such as catheter type, catheter loading technology, ultrasound technology [1–5]. During embryo transfer, we can clearly see the position of the catheter tip and the air bubbles by ultrasound. The position of the air bubble is an important indicator of the embryo position [6, 7].

There have been lots of studies exploring the relationship between embryo transfer position and ART outcome [8–11]. Most of literature have shown that the location of cleavage embryos cannot be too close to the uterus fundus, nor too far from the uterus fundus, and the middle of the uterine cavity is the best position to place the embryos for optimal ART outcome [12–14]. In addition, in the ASRM standard embryo transfer protocol template, a committee suggested that the tip of the catheter should be placed in the upper or center of the uterine cavity (more than 1 cm from the fundus of the uterus) and then released the embryos, which can optimize the pregnancy rate [15].

In recent years, studies have shown that selective single embryo transfer is the most effective method to reduce the risk of multiple pregnancy and the related risks to the mother and child after in vitro fertilization, while maintaining the overall pregnancy rate [16–18]. Hence, blastocyst transfer has become a common practice in IVF [19]. However, only a small part of the studies existed exclusively analyzing the influence of air bubble position on ART outcome in blastocyst transfer [20]. Thus, it is still unknown where is the best embryo position in blastocyst transfer and whether it is consistent with cleavage embryo transfer. Therefore, this study aims to explore the effect of air bubble position on ART outcome and to find the optimal embryo transfer position in frozen-thawed blastocyst transfer.

Materials And Methods

Participants

This study included a retrospective cohort analysis of 399 frozen-thawed embryo transfers (FET) ultrasound-guided embryo transfers performed in the reproductive center of SSL Central hospital of Dongguan City which is affiliated Southern medical university, China, between June 1, 2017 and November 30, 2020. Written informed consent was obtained from all patients.

The study included all women with infertility undergoing frozen-thawed cycle transfer of single blastocyst. The criteria used to exclude patients from the study were the following: (i) women of 40 years or above, (ii) difficulty of ET, (iii) presence of hydrosalpinx, (iv) the embryo flashes were not visualized, (v) cycle cancellation, (vi) endometrial thickness was <7mm.

Assisted Reproduction Procedures

We performed endometrial preparation and FET in natural cycles, stimulation cycles, or hormone therapy cycles. In all women with regular menstrual cycles, FET was performed first in a natural cycle. From day 12 on, follicle growth was monitored by means of serum hormones and transabdominal ultrasound and women were regularly monitored every 2 days. When the dominant follicle diameter was >16 mm, endometrial thickness was ≥ 8 mm, $E_2 > 150$ pg/mL, $P < 1.5$ ng/mL, a bolus of urinary hCG (5,000 IU) was given to induce ovulation. Exogenous P supplementation (dydrogesterone 40 mg/d; Duphaston, Abbott Biologics, United States) was started after 5 days, and then blastocyst transfer was performed.

The preparation of artificial endometrium included sequential administration of E₂ valerate and injection of progesterone. In short, 2mg of E₂ valerate was taken at least twice daily for 14 to 16 days, and adjust the dose according to the endometrial thickness measured by vaginal ultrasound. If the endometrial thickness was ≥ 7 mm, E₂ >150 pg/mL, P <1.5 ng/mL, then progesterone injection would be started. Progesterone was injected daily at a dose of 20 mg, and FET was performed five days later. Each woman was transferred one blastocyst. After pregnancy, luteal phase support continued until 10 weeks of gestation.

The thawing process was performed by vitrification. After thawed, the embryos were transferred into G-2 Plus culture medium, and then cultured in a CO₂ incubator for 1 to 2 hours and transferred. The distance between the air bubble (hyperechogenic spot) and the fundal endometrial surface (embryo-fundus distance, EFD) was measured by transvaginal US when the transfer catheter had been retracted completely for a few seconds.

For the analysis, the women were divided into four groups based on air bubble-uterine fundus distance (BFD): Group 1 included 59 blastocyst transfers in which BFD is less than 10mm. Group 2 consisted of 225 blastocyst transfers in which BFD is 10 mm or more and less than 15 mm. Group 3 included 90 blastocyst transfers in which BFD is 15 mm or more and less than 20 mm. Group 4 consisted of 25 blastocyst transfers in which BFD is 20mm or more.

Embryo Transfer

After placing the woman with moderately full bladder in the lithotomy position, expose the cervix with a double valve speculum. Clean the mucus in the cervical canal with a sterile cotton swab immersed in the medium. Place the embryo in the atraumatic Cook Sydney embryo transfer catheter (Cook Medical, IN, USA), by using the "three-drop technique". Firstly, an air bubble was loaded into the catheter. Then, 20 μ L of medium was aspirated into the catheter and place the embryo in the smallest possible medium. The second air bubble was then loaded into the catheter. Finally, aspirate enough medium to bring the total volume to 30 μ L. The outer catheter was first inserted into the cervical canal. Once the guide was positioned before or after the internal os, the inner catheter was placed through the outer catheter. Place the tip of the inner catheter in place. Gently release the medium containing the blastocyst into the uterine cavity. The catheter was slowly withdrawn and examined by the same embryologist under a stereomicroscope to ensure that there were no blastocyst retained. After the operation, the patient remained supine for approximately 30 minutes.

Outcome Measures

Data collected included age, BMI, type of infertility, infertility duration, main cause of infertility, AMH, ART way, times of previous embryo transferred, protocol, blastocyst score, and multinucleation, the levels of Estrogen and progesterone on endometrial transformation day, endometrial thickness on day of ET, and cycle outcome.

The primary outcome of the study was the clinical pregnancy rate per cycle. Secondary outcome was the live birth rate. A clinical pregnancy was defined as the presence of a fetal sac visualized by transabdominal ultrasound examination at 6–8 weeks of amenorrhoea. Live birth was defined as the delivery of a living child.

Statistical Analysis

Continuous variables were presented as Mean $\pm$ SD (normal distribution), categorical variables were presented as N (%). One-way Anova (normal distribution), Kruskal Wallis H (skewed distribution) test, and chi-square test (categorical variable) were used to determine the statistical differences in the mean and proportion between groups. Smooth curve fitting was used to identify the non-linear relationships between embryo transfer position and clinical pregnancy rate. The method on how to use smooth curve fitting has been described in detail by Motulsky et al.[21]. If the non-linear correlation was observed, segmented regression model, LRT test (likelihood ratio test, to compare the difference between Model I and Model II) and Bootstrap resampling method were performed to calculate the threshold effect of the BFD on clinical pregnancy rate. When the ratio between BFD and clinical pregnancy rate appears obvious in smoothed curve, recursive method calculates automatically the inflection point, where the maximum model likelihood will be used [22]. The present study listed non-adjusted models and multivariate adjusted models. Based on the recommendation of STROBE statement, we showed unadjusted, minimally adjusted analysis results, and results from a fully adjusted analysis.

All of the analyses were performed with the statistical software packages R (<http://www.R-project.org>, The R Foundation) and EmpowerStats (<http://www.empowerstats.com>, X&Y Solutions, Inc., Boston, MA). P values less than 0.05 (two-sided) were considered statistically significant.

Results

Baseline characteristics were listed in Table 1. The number of the women in the four subgroups was 59, 225, 90, 25 respectively. The four subgroups were no significant difference in terms of age, BMI, duration of infertility, type of infertility, main cause of infertility, times of previous embryo transferred, AMH, E₂ and P levels, endometrial thickness on day of ET (in millimeters), ART way, protocol and live birth rate (P>0.05). There were significant differences in clinical pregnancy rate among the four subgroups (P<0.05).

Table 1
Patients' baseline characteristics and clinical parameters.

Variables	BFD(mm)				P value
	Group 1 <10	Group 2 10 to <15	Group 3 15 to <20	Group 4 ≥20	
No. of participants	59	225	90	25	
Age (y)	31.14 ± 4.32	30.99 ± 4.11	31.02 ± 4.31	31.44 ± 4.76	0.962
BMI (kg/m ²)	21.54 ± 3.27	21.66 ± 3.16	21.33 ± 3.28	22.34 ± 3.04	0.549
Duration of infertility (y)	3.94 ± 2.68	3.74 ± 2.76	3.62 ± 2.26	3.60 ± 2.25	0.896
Type of infertility					0.205
Primarily	22 (37.29%)	101 (44.89%)	40 (44.44%)	7 (28.00%)	
Secondary	37 (62.71%)	124 (55.11%)	50 (55.56%)	18 (72.00%)	
Main cause of infertility					0.485
Tubal factor	38 (64.41%)	154 (68.44%)	66 (73.33%)	20 (80.00%)	
Endometriosis	0 (0.00%)	2 (0.89%)	3 (3.33%)	0 (0.00%)	
Ovulation disorder	3 (5.08%)	9 (4.00%)	4 (4.44%)	0 (0.00%)	
Male factor	15 (25.42%)	56 (24.89%)	15 (16.67%)	5 (20.00%)	
Unexplained	3 (5.08%)	4 (1.78%)	2 (2.22%)	0 (0.00%)	
times of previous embryo transferred					0.690
1	39 (66.10%)	142 (63.11%)	58 (64.44%)	13 (52.00%)	
2	10 (16.95%)	50 (22.22%)	20 (22.22%)	9 (36.00%)	
≥ 3	10 (16.95%)	33 (14.67%)	12 (13.33%)	3 (12.00%)	
AMH (ng/mL)	5.14 ± 2.52	5.31 ± 3.04	5.33 ± 2.95	5.58 ± 2.04	0.948
E ₂ level (pg/mL)	387.53 ± 403.61	319.67 ± 383.02	413.09 ± 539.59	291.52 ± 234.73	0.267
P level (ng/ml)	0.28 ± 0.20	0.28 ± 0.21	0.25 ± 0.18	0.23 ± 0.19	0.587
Endometrial thickness (mm)	9.44 ± 1.99	10.04 ± 1.88	9.81 ± 1.83	10.40 ± 1.85	0.085
ART way					0.205
IVF	48 (81.36%)	175 (77.78%)	77 (85.56%)	17 (68.00%)	

Variables	BFD(mm)				P value
	Group 1 <10	Group 2 10 to <15	Group 3 15 to <20	Group 4 ≥20	
ICSI	11 (18.64%)	50 (22.22%)	13 (14.44%)	8 (32.00%)	
Protocol					0.485
Natural cycle	3 (5.08%)	18 (8.00%)	6 (6.67%)	3 (12.00%)	
Hormone therapy cycle	46 (77.97%)	145 (64.44%)	53 (58.89%)	13 (52.00%)	
Stimulation cycle	10 (16.95%)	62 (27.56%)	31 (34.44%)	9 (36.00%)	
Clinical pregnancy	45 (76.27%)	153 (68.00%)	58 (64.44%)	10 (40.00%)	0.013
Abortion/Ectopic gestation					0.058
Ectopic gestation	2 (4.35%)	1 (0.65%)	0 (0.00%)	0 (0.00%)	
Abortion	7 (15.22%)	27 (17.53%)	8 (13.56%)	5 (45.45%)	
Live birth	37 (82.22%)	126 (82.35%)	51 (87.93%)	6 (60.00%)	0.192

The results of univariate analysis were shown in Table 2. Only age, ≥ 3 times of embryo transferred, were significantly correlated with lower clinical pregnancy rate ($P < 0.05$). There was no significant correlation between other variables and clinical pregnancy rate ($P > 0.05$). Only age, secondary infertility were significantly correlated with lower live birth rate ($P < 0.05$). There was no significant correlation between other variables and live birth rate ($P > 0.05$).

Table 2
Univariate analysis between the variables and ART outcomes.

	Statistics	Clinical pregnancy		Live birth	
		β /OR (95%CI)	P value	β /OR (95%CI)	P value
Age (y)	31.05 $\pm$ 4.22	0.91 (0.87, 0.96)	0.0005	0.90 (0.84, 0.98)	0.0132
BMI (kg/m ²)	21.61 $\pm$ 3.19	0.96 (0.90, 1.03)	0.2354	0.98 (0.89, 1.09)	0.7437
Duration of infertility (y)	3.73 $\pm$ 2.61	0.95 (0.88, 1.03)	0.1814	1.04 (0.91, 1.18)	0.5767
AMH (ng/mL)	5.30 $\pm$ 2.89	1.04 (0.96, 1.12)	0.3584	1.06 (0.95, 1.20)	0.3012
Type of infertility					
Primarily	170 (42.61%)	1		1	
Secondary	229 (57.39%)	1.02 (0.67, 1.55)	0.9429	0.36 (0.18, 0.75)	0.0064
Main cause of infertility					
Tubal factor	278 (69.67%)	1		1	
Endometriosis	5 (1.25%)	2.08 (0.23, 18.84)	0.5161	–	–
Ovulation disorder	16 (4.01%)	1.14 (0.39, 3.38)	0.8105	–	–
Male factor	91 (22.81%)	1.17 (0.70, 1.94)	0.5502	1.74 (0.76, 3.97)	0.1870
Unexplained	9(2.26%)	0.65 (0.17, 2.47)	0.5264	1.01 (0.11, 9.34)	0.9904
times of previous embryo transferred					
1	252 (63.16%)	1		1	
2	89 (22.31%)	0.79 (0.47, 1.32)	0.3749	0.94 (0.43, 2.07)	0.8790
≥ 3	58 (14.54%)	0.49 (0.27, 0.87)	0.0153	0.67 (0.26, 1.70)	0.4025
E ₂ level (pg/mL)	349.90 $\pm$ 422.65	1.00 (1.00, 1.00)	0.2680	1.01 (0.11, 9.34)	0.9904

	Statistics	Clinical pregnancy		Live birth	
		β /OR (95%CI)	P value	β /OR (95%CI)	P value
P level (ng/ml)	0.27 $\pm$ 0.20	0.61 (0.22, 1.73)	0.3551	2.78 (0.40, 19.21)	0.3006
Endometrial thickness (mm)	9.92 $\pm$ 1.89	1.06 (0.95, 1.19) 0.2950	0.2950	0.93 (0.78, 1.10)	0.4041
ART way					
IVF	323 (79.75%)	1		1	
ICSI	82 (20.25%)	0.97 (0.58, 1.61)	0.9066	1.05 (0.64, 1.73)	0.8380
Protocol					
Natural cycle	29 (7.16%)	1		1	
Hormone therapy cycle	257 (63.46%)	1.29 (0.59, 2.81)	0.5263	1.21 (0.55, 2.66)	0.6433
Stimulation cycle	119 (29.38%)	1.50 (0.65, 3.46)	0.3367	0.80 (0.34, 1.85)	0.5989

To evaluate the relationship between BFD and ART outcome (clinical pregnancy and live birth), 3 models including crude model, minimally adjusted model and fully adjusted model were established in Table 3. In crude model, BFD showed negative correlation with clinical pregnancy (OR = 0.93, 95% CI: 0.87 to 0.98, P = 0.011). The result did not have obvious changes in 2 adjusted models. For the purpose of sensitivity analysis, BFD was also analyzed as categorical variable, and found that the same trend was observed as well.

Table 3
Relationship between BFD and ART outcome in different models

Exposure	Crude Model		Minimally adjusted model		Fully adjusted model	
	β /OR (95%CI)	P value	β /OR (95%CI)	P value	β /OR (95%CI)	P value
Clinical pregnancy						
BFD(mm)	0.92 (0.87, 0.97)	0.0021	0.93 (0.87, 0.99)	0.0147	0.93 (0.87, 0.98)	0.0110
BFD(mm)						
<10	1		1		1	
10 to <15	0.66 (0.34, 1.28)	0.2205	0.58 (0.28, 1.20)	0.1435	0.57 (0.27, 1.17)	0.1270
15 to <20	0.56 (0.27, 1.18)	0.1286	0.55 (0.24, 1.25)	0.1520	0.54 (0.24, 1.22)	0.1393
≥ 20	0.21 (0.08, 0.56)	0.0020	0.21 (0.07, 0.66)	0.0072	0.20 (0.06, 0.62)	0.0055
Live birth						
BFD(mm)	0.99 (0.91, 1.08)	0.8649	1.01 (0.92, 1.10)	0.9084	1.00 (0.91, 1.10)	0.9442
BFD(mm)						
<10	1		1		1	
10 to <15	1.01 (0.42, 2.41)	0.9839	1.04 (0.41, 2.69)	0.9282	1.05 (0.40, 2.72)	0.9274
15 to <20	1.58 (0.52, 4.73)	0.4177	1.76 (0.54, 5.81)	0.3502	1.71 (0.52, 5.64)	0.3805
≥ 20	0.32 (0.07, 1.42)	0.1354	0.33 (0.06, 1.72)	0.1888	0.31 (0.06, 1.68)	0.1750

We also used smooth fitting curves to explore the relationship between BFD and clinical pregnancy rate/live birth rate (Fig. 1/Fig. 2). We found that with the increase of BFD, both clinical pregnancy rate and live birth rate decreased. Table 4 and Table 5 presented the threshold effect analysis for relationship between BFD and clinical pregnancy rate/live birth rate. A inflection point was found and it's 19 mm. After adjusting the variables including age, main cause of infertility, ART ways, times of previous embryo transferred, type of infertility, duration of infertility, BMI, AMH, protocol, endometrial thickness, embryo score and embryo multinucleation, we found that when BFD was less than 19 mm, there is no significant change in clinical pregnancy rate and live birth rate as BFD increased (OR = 0.95, 95% CI: 0.89 to 1.02, P=

0.1373; and OR = 1.06, 95% CI: 0.96 to 1.17, P= 0.2665; respectively); when BFD was more than 19 mm, the clinical pregnancy rate decreases by 16% for each increase in BFD (OR = 0.84, 95% CI: 0.72 to 0.98, P = 0.0363) and the live birth rate decreases by 58% for each increase in BFD (OR = 0.42, 95% CI: 0.21 to 0.86, P = 0.0174).

Table 4
Threshold Effect Analysis of BFD and clinical pregnancy rate using Piece-wise Linear Regression.

Inflection point of BFD (mm)	OR	95%CI	P value
<19	0.95	0.89 to 1.02	0.1373
≥19	0.84	0.72 to 0.98	0.0363

Table 5
Threshold Effect Analysis of BFD and live birth rate using Piece-wise Linear Regression.

Inflection point of BFD (mm)	OR	95%CI	P value
<19	1.06	0.96 to 1.17	0.2665
≥19	0.42	0.21 to 0.86	0.0174

Discussion

Single blastocyst transfer occurs near the planting window, the endometrial receptivity is stronger, and can reduce the risk of multiple pregnancy[20, 23, 24], thus it has become the main trend of IVF-ET. However, most of the previous studies using catheter tip placement or bubble positioning to study the optimal embryo position mainly analyzed the IVF cycle of embryos with cleavage stage, and did not pay significant attention to blastocyst transfer.

This study explored the effect of air bubble position on ART outcome in frozen-thawed single blastocyst transfer. We found that the clinical pregnancy was significantly higher when the flash is less than 20 mm from the fundus of the uterus after blastocyst transfer, compared with more than 20 mm (P <0.05). Our results are consistent with the findings of a retrospective cohort study suggesting[20]. However, our study is contradictory to many studies that consider that the best results would be obtained when placing the embryo in the middle of the uterine cavity rather than near the uterus fundus[12–14]. It is important to note that we and the retrospective cohort study explored embryo transfer at the blastocyst stage[20], not at the cleavage stage[12–14].

In addition, the smooth curve fitting and threshold effect model showed that the closer the air bubble is from uterus fundus, the higher the clinical pregnancy rate. On the contrary, the air bubble position away from the uterus fundus resulted in a decrease in the clinical pregnancy rate and live birth rate. Especially when the distance between the air bubble and the uterus fundus > 19mm, the clinical pregnancy rate

decreased significantly. Each time the distance between the air bubble and the uterus fundus increased by 1 mm, the clinical pregnancy rate decreased by 16% (OR = 0.84, 95% CI: 0.72 to 0.98, P = 0.0363). When placing the blastocyst more than 19 mm away from the uterus fundus, live birth rate significantly lower than the uterus fundus < 19mm.

In recent years, studies have found that it is possible for the embryos to migrate in the uterine cavity after embryo transfer. Whether in the cleavage embryo stage or the blastocyst stage, most of the embryos moved to the fundus, and the clinical pregnancy rate of the group that moved to the fundus was higher than that of the stationary group and the group that moved to the cervix, which also proved the position near the uterine cavity central and fundus is indeed suitable for embryo transfer[23, 24]. Meanwhile, the location of the gestational sacs are mostly in the upper part of the uterine cavity in spontaneous conception(89.1%) [25]. The frozen-thawed single blastocyst transfers are closer to the natural conception, without super-physiological hormones in the ovulation stimulation cycle and this may be another reason for better ART outcome was obtained when air bubble were observed near the uterus fundus after embryo transfer in this study.

Moreover, the fundal endometrium is more conducive to embryo implantation because of a lower endometrial wavelike activity and an increased endometrial tissue blood flow[26–28]. Some researchers oppose placing embryos too close to the fundal endometrium because they fear that uterine contractions caused by high replacement has a negative impact on ART outcome [29–31]. A retrospective study found that air bubbles moving towards the uterine fundus with ejection were associated with higher pregnancy rates [32]. Thus, this may prove a point, it does not touch the mucosa membrane during migration, it is still beneficial to place the embryo near uterine cavity central and the fundus[8]. In contrast, the farther the transfer position is from the fundus of the uterus, the lower the pregnancy rate, which may be associated with an increased risk of embryo expulsion from the uterine cavity [33].

There are some advantages to this research. First of all, in most other reports, the distance between air bubble and the uterus fundus was classified in 5 mm increments [12–14], which may result in errors caused by artificial grouping. We make up for this deficiency by using the smooth fitting curve and threshold effect model. Second, we have made adjustments to reduce interference from other factors. Third, this study explored the frozen-thawed blastocysts transfer, which was rarely discussed by previous researchers, and the result was different from that in fresh cleavage embryo transfer.

This study also has some limitations. Firstly, this is a retrospective study which makes many constraints unavoidable. Since there was no AMH testing project before 2016, the AMH data of some patients was missing. Besides, in this study, the position of the embryo is replaced by the position of the bubble, not the position of the embryo itself, which may also not necessarily be true. Lastly, morphometric characteristics of the cavity, such as geometric shape, size and rotation, were not observed in this study.

In conclusion, The clinical pregnancy and live birth at a distance of 19mm from the uterus fundus have a cliff-like downward trend. Therefore, the ideal pregnancy outcome can be achieved within 19mm from uterus fundus after single blastocyst transfer.

Limitations And Extrapolation Potential To The Human Situation

Since there was no AMH testing project before 2016, the AMH data of some patients was missing. Besides, morphometric characteristics of the cavity, such as geometric shape, size and rotation, were not observed in this study. This study explored the effect of air bubble position on ART outcome in frozen-thawed blastocyst transfer and the results were different from those in cleavage embryo transfer.

Conclusions

The ideal pregnancy outcome can be achieved within 19mm from uterus fundus after single blastocyst transfer, The clinical pregnancy and live birth at a distance of more 19mm from the uterus fundus have a cliff-like downward trend.

Abbreviations

ART: Assisted Reproductive Technology; ET: Embryo transfer; FET: Frozen-thawed Embryo transfer; IVF: In vitro fertilization; *hCG*: Human chorionic gonadotropin;

BFD: bubble-uterine fundus distance; EFD: embryo-fundus distance;

AMH: Anti-Müllerian hormone

Declarations

Ethics approval

All procedures performed in studies involving human participants were based on the ethical standards of the institutional and national research committee, as well as the Helsinki Declaration of 1964 and its subsequent amendments or similar ethical standards. So this retrospective analysis doesn't need the approval from the institutional review board.

Consent for publication

All writers agreed to public the study

Availability of data and material

The data was collected from the reproductive center of SSL Central hospital of Dongguan City which is affiliated Southern medical university, China. All data are available from the corresponding author by request.

Competing interests

No conflict of interest need to be declared.

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Authors' contributions

TT.Y participated in the study design, the statistical analysis and wrote the main parts of the manuscript. XY.S and XM.C participated in clinical diagnosis and treatment. QY.L and Z.L participated in embryo operation and performing scoring of the embryos. QM.L and RH.P participated in follow-up visit and collecting the data. B.C participated the study design, supervising the development of the manuscript and revised the manuscript for final approval.

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Figures

Figure 1

Association between BFD and clinical pregnancy rate. A threshold, nonlinear association between BFD and clinical pregnancy was found in a generalized additive model (GAM) . Solid red line represents the smooth curve fit between variables. Blue bands represent the 95% of confidence interval from the fit. All adjusted for age, main cause of infertility, ART ways, times of previous embryo transferred, type of infertility, duration of infertility, BMI, AMH, protocol, endometrial thickness, embryo score and embryo multinucleation.

Figure 2

Association between BFD and live birth rate. A threshold, nonlinear association between BFD and live birth rate was found in a generalized additive model (GAM) . Solid red line represents the smooth curve fit between variables. Blue bands represent the 95% of confidence interval from the fit. All adjusted for age, main cause of infertility, ART ways, times of previous embryo transferred, type of infertility, duration of infertility, BMI, AMH, protocol, endometrial thickness, embryo score and embryo multinucleation.