Uterine Factors Modify the Association Between Embryo Transfer Depth and Clinical Pregnancy: A Retrospective Study in 7849 Fresh Transfer In Vitro Fertilization Cycles

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Abstract

The embryo position is supposed to affect implantation following embryo transfer. However, embryo dislodging caused by uterine contraction may occurred after transfer. The retrospective study was to investigated whether the factors associated with uterine contractility, such as endometrial thickness and progesterone elevation, affect the association between embryo position and implantation. A total of 7849 fresh transfer cycles on conventional stimulation in a single IVF centre during the period 2013–2015 was reviewed. Patients were categorized according to quartiles of embryo-fundus distance (≤9, 9.1-11, 11.1-14, ≥1.4 mm, respectively). Adjusted for confounding factors, the odds ratio (OR) (95%CI) for clinical pregnancy was 0.90 (0.79-1.02), 0.86 (0.74-0.99) and 0.70 (0.60-0.82) respectively in quartiles 2 through 4, comparing with quartile 1. However, ORs were significantly increased when endometrial thickness was < 8 mm. The ORs comparing quartiles 2 through 4 with quartile 1 increased 1.96 (95%: 1.33-2.90), 1.20 (95%: 0.78-1.87) and 1.98 (95%: 1.20-3.26) fold respectively in cycles with an endometrial thickness < 8 mm than in cycles with a normal endometrial thickness (8-11 mm). Elevated progesterone on the day of hCG and blastocyst stage transfer reduced the ORs. Our data suggested an interaction between patient characteristics and embryo transfer techniques.

Introduction

Embryo transfer is the final and probably the most critical step toward achieving pregnancy in an IVF cycle. Even good quality embryos were created and satisfying endometrium were prepared, poor transfer technique may hinder the embryo implantation. Although there is no real consensus on the optimal practice of embryo transfer, several factors with respect to transfer technique have been associated with IVF outcomes [1,2].

Among the technical aspects of the transfer procedures have been studied, there is fair evidence regarding the effect of the site of embryo transfer on the implantation[2]. However, evidence available remained conflicting. In early practice of embryo transfer, the tip of the catheter has been empirically placed 5–10 mm from the uterine fundus[1,3–5], while other researchers argued for transferring embryo at lower in the uterine[6,7] or no influence of the depth of embryo transfer[8]. More recently, Coroleu et al. proposed an optimal positioning of the catheter at 15 to 20 mm from the fundus for ultrasound-guided transfer by showing that positioning catheter at 10 mm from the fundus decreased pregnancy rate significantly in their randomized trail containing 180 consecutive patients[9]. A meta-analysis published in 2007 stated that there is limited evidence supporting the superiority of lower cavity transfers compared with the traditional high cavity[10]. Since then, however, negative[11], positive[12] or no association[13,14] between the depth of catheter insertion and IVF outcome was still observed by different researchers. By showing that embryo transfer depth was similar between cycles led to a pregnancy and those did not, Kovacs et al. argued that transfer depth does not affect implantation and pregnancy rates when the transfer is in the middle or upper third of the uterus[13]. In a prospective study by Rovel et al., higher pregnancy and implantation rates were achieved when the tip was placed between 5 and 15 mm from fundus compared with >15 mm distance from fundus[15]. Finally, an embryo transfer guideline from American Society for Reproductive Medicine in 2017 concluded that “there is insufficient evidence for more specific recommendations regarding the positioning of the catheter at the time of embryo transfer”[2].

Given the evidence from randomized trials remained limited[10], the cohort studies in large populations may contribute to verifying the conclusions draw from meta-analysis. However, one factor limited some of the studies examining the role of catheter depth is estimating the effect on pregnancy through simple bivariate analyses, without controlling for confounding effects by other important parameters[11,12], while several other studies controlled for different sets of confounding factors[16]. Many of the important parameters identified in recent studies that shown to be associated with the chance of pregnancy, such as endometrial thickness[17], quality of embryos transferred[16], number of oocyte retrieved[18] and progesterone elevation before transfer[19], were not included into the analyses in many previous studies, even the multivariate analysis was used. Moreover, some of these confounders, such as progesterone levels [20], endometrial thickness [21] and the stage of embryo transfer[22], may further affect the frequency and/or the direction of uterine contraction. The uterine contraction may result in embryo dislodging and dictate where the embryo will eventually implant following transfer. Given the significance of the embryo transfer depth is based on its influence on the optimal implantation site, we hypothesize that heterogeneity in important uterine factors may contribute to the lack of consensus in previous studies not only as sources of confounding but also as effect modifiers. The aim of the study is to explore whether the factors that correlated to uterine contraction modified the effect of embryo positon during embryo transfer.

Materials And Methods

Study subjects

The retrospective analysis was performed on patients who underwent IVF/ICSI treatment and fresh embryo transfer in the affiliated Chenggong Hospital of Xiamen University in the period between January 2013 and December 2015. Institutional Review Board approval for this retrospective study was obtained from the Ethical Committee of the Medical College Xiamen University. Informed consent was not necessary, because the research was based on non-identifiable records as approved by the ethics committee. All methods were performed in accordance with the relevant guidelines and regulations of the Institution.

Only patients undergoing conventional ovarian stimulation (agonist or antagonist) were reviewed. Patients on mild stimulation cycles, natural cycles and luteal phase stimulation cycles were excluded from the study (n=177). 48 cases of transfer lacked the record of catheter tip-fundus distance, and thus were excluded from the study. We also excluded patients identified as difficult-to-transfer (n=100) and patients had bacterial infection after transfer (n=3). No case was of the presence of blood in the catheter.

Stimulation protocols and laboratory procedures

In all stimulation cycles, patients received 2-3 ampoules (75-225 IU) gonadotropin per day during the gonadotropin stimulation. The initial and ongoing dosage was adjusted according to the patient's age, AFC, BMI and follicular growth response. Recombinant FSH (Gonal-F; Merck-Serono, Switzerland) or

domestic urinary HMG (HMG; Lizhu, China) was used for the gonadotropin stimulation. During the treatment, the ovarian response was monitored by trans-vaginal ultrasound measurements of follicular growth and serum E2 level every 1–3 days. Gonadotropin stimulation continued until ultrasonography revealed at least one follicle measuring ≥18 mm in mean diameter. 5000-10000IU human chorionic gonadotropin (hCG; Lizhu, China) was injected intramuscularly. Endometrial thickness and ultrasonic pattern of endometrium (Pattern A: a triple-line pattern consisting of a central hyperechoic line surrounded by two hypoechoic layers, pattern B: an intermediate isoechogenic pattern with the same reflectivity as the surrounding myometrium and a poorly defined central echogenic line, and pattern C: homogenous, hyperechogenic endometrium) were also evaluated on the day[23]. Oocyte retrieval was scheduled 34 to 36 hr after hCG administration. Oocyte retrieval was carried out under transvaginal ultrasound guidance.

Oocytes were inseminated using either conventional IVF or ICSI. The pronuclei were identified 17 to 18 hours later. On day 3, embryos were assigned quality grades and embryos that reached the 8-cell stage with even cleavage and less than 20% fragmentation were classified as good-quality embryos. For patients receiving blastocyst transfer, Gardner scale[24] was used to evaluate the embryo quality. Top quality embryos for transfer were defined as the following: the embryos with less than 10% fragment and on-time cell size on day3 and with good inner cell mass and good trophectoderm on day 5.

Embryo transfer

Fresh embryo transfers were performed on either day 3 or day 5. The number of embryos transferred ranged from 1-3 according to national regulations. Transferring three embryos was only considered in women with advanced age or repeated failure and no patients have more than 2 blastocyst transferred.

All transfers were performed in the same room by seven experienced clinicians. Patients undergoing transfer received a mock transfer the day before embryo transfer was performed. During transfer procedure, all patients were placed in the lithotomy position and the cervix was exposed using a bivalve speculum. The external os was cleaned using a physiologic serum. The cervical mucus was removed cotton swab.

The outer catheter of the Cook catheter (K-JETS-7019-SIVF, Cook, IN, USA) was inserted under the guidance of abdominal ultrasonography. Embryos were loaded to inner catheter by the 'three-drop technique'[25]. The drop of medium containing the embryos was separated from a preceding and a following drop of medium by a bubble of air and the volume of both the air bubble and droplet did not exceed 10 μL.

The transferred matters were injected into uterine cavity with low speed under ultrasonic guidance. The position of injection was addressed to the thickest part of endometrium as possible [26]. The bubble generated following transfer was visualized under ultrasonography and distance from the position of the bubble to the fundal myometrium–endometrial interface was used a marker of the embryo position (transfer depth). The catheter was then gently removed and examined under a stereomicroscope to ensure that all embryos had been transferred. Following transfer, patients remained in bed for 30 minutes.

The luteal phase support was sustained with natural progesterone in oil (progesterone; XianJu, China), 60 mg i.m. daily from the oocyte retrieval day. A pregnancy test (serum β-hCG determination) was done 14 days after embryo transfer. Clinical pregnancy was defined as the presence of one or more gestational sacs detected on ultrasound scan performed 4 weeks after embryo transfer. If no evidence of intrauterine gestational sac was detected following β-hCG elevation, ectopic pregnancy was confirmed with surgical treatment.

Statistical analysis

For data analyses, transfer depth in all transfer cycles was categorized into quartiles. In order to test the effect of extreme values, 10% percentile and 90% of the distance were also used as categorization criteria in multivariate analyses.

The distribution of continuous variables across quartiles was described with the use of the mean and the standard deviation (SD). Categorical variables were presented as proportions and percentages of the total. One-way analysis of variance (ANOVA) with least significant difference (LSD) as a post hoc test was used for continuous variables. Dichotomous variables were analyzed by chi-square test or Fisher's exact test, as appropriate.

To perform multivariate analyses, we analyzed our data with generalized estimating equations (GEE) model. Multivariate analyses were performed to evaluate the association between catheter tip-fundus distance and the probability of clinical pregnancy, with adjustment for important confounding factors. The transfer depth was evaluated either as categorized value aforementioned or continuous value (per millimeter increased) in the multivariate analyses. Covariates were selected based on their clinical importance. Patient characteristics that known to be important for counseling IVF outcomes including age, BMI, antral follicle count (AFC), previous live birth or pregnancy, duration of infertility and etiologies of infertility, were included in the model [27]. Stimulation characteristics including stimulation dose, GnRH analogues used [28], number of oocytes[10], endometrial thickness and pattern[23] and progesterone elevation on the day of triggering[19] were also selected for they are known to influence the outcomes. Finally, the model was also controlled for other technical aspects of embryo transfer, including the development stage of transferred embryos, the presence of at least one good-quality embryo transferred and different clinicians that performed the embryo transfer.

To explore whether the covariates that correlated to uterine contraction modified the effect of embryo transfer depth, interaction terms were introduced into the model. The interactions between the embryo transfer depth and Blastocyst transfer[22], progesterone elevation[20], endometrial thickness[21] were studied based on previous knowledge. To facilitate the analysis, endometrial thickness on the day of hCG was categorized into thin (<8 mm), normal (8-11 mm) and thick (>11mm,) categories.

All calculations were performed with SPSS (version 19; IBM). In all analyses, $P < 0.05$ was considered significant, expect that Bonferroni-corrected P value ($P<0.0125$) was used in multiple comparison of chi-square test.

Results

7849 fresh transfer cycles from 6942 patients were included in the present study. The mean age of the patients was 31.44±4.38 years. The transfer depth ranged from 4-25 mm. The 10%, 25%, 50%, 75% and 90% percentile of the distance was 7, 9, 11, 14, 17 mm respectively. Using quartiles as cut-off values, the cycles were divided into four groups (quartile1-4). In 1735 cycles (22.1%), embryos were transferred at or within 9 mm of the fundus (quartile 1). In 2557 cycles (32.6%), embryos were transferred at a distance between 9.1 and 11 mm from the fundal endometrial surface (quartile 2). In 1933 cycles (24.6%), embryos were transferred at a distance >11 mm but ≤14 mm from the fundal endometrial surface (quartile 3). And finally, in 1624 cycles (20.7%), embryos were transferred at a distance>14 mm from the fundus (quartile 4).

The baseline characteristics of patients receiving transfer were summarized in Table 1. The overall baseline characteristics were similar between groups. However, the patients in quartile 2 were of longer duration of infertility, whereas the patients in quartile 4 had fewer previous attempts of transfer, lower proportion of PCOS, lower basal LH and more AFC than other three groups, as demonstrated by the post hoc test. In addition, significant heterogeneity in basal FSH levels was noted among groups, but the absolute differences were rather small.

Table 1
Baseline characteristics and ovarian stimulation parameters.

	Transfer depth quartiles				
Variables	Quartile 1 (≤9 mm, n=1735)	Quartile 2 (9.1-11 mm, n=2557)	Quartile 3 (11.1-13.9 mm, n=1933)	Quartile 4 (≥14 mm, n=1624)	P
Female`s age, year	31.29±4.51	31.45±4.35	31.51±4.41	31.50±4.23	0.400
Male`s age, year	33.28±5.11	33.45±5.04	33.60±5.09	33.40±4.85	0.279
Duration of infertility, year	4.612±3.33^{ab}	4.743±3.41^{b}	4.440±3.22^{a}	4.645±3.11^{ab}	0.024
Primary/Secondary (%)	804/931(46.3/53.7)	1236/1321(48.3/51.7)	917/1016(47.4/52.6)	813/811(50.1/49.9)	0.169
Previous attempt of ET	0.390±0.86^{a}	0.370±0.85^{a}	0.375±0.89^{a}	0.303±0.80^{b}	0.016
BMI, kg/cm^2	21.08±2.78	21.17±2.66	21.08±2.92	21.19±2.70	0.461
Additional etiologies					
PCOS (%)	115/1735(6.6)^{a}	159/2557(6.2)^{ab}	101/1933(5.2)^{ab}	75/1624(4.6)^{b}	0.039
Endometriosis (%)	203/1735(11.7)	285/2557(11.1)	237/1933(12.3)	216/1624(13.3)	0.200
Hydrosalpinix (%)	75/1735(4.3)	84/2557(3.3)	80/1933(4.1)	46/1624(2.8)	0.054
Male (%)	222/1735(12.8)	379/2557(14.8)	279/1933(14.4)	253/1624(15.6)	0.121
Basal FSH, IU/l	7.512±2.42^{ab}	7.656±2.60^{b}	7.633±2.45^{b}	7.420±2.88^{a}	0.017
Basal LH IU/l	4.92±3.06^{a}	4.72±2.85^{ab}	4.73±2.74^{ab}	4.59±2.57^{b}	0.009
Basal PRL, ng/ml	15.50±9.79	15.01±9.47	15.52±12.5	15.47±9.52	0.279
Basal E2, pg/ml	45.16±34.7	43.45±25.5	43.74±24.4	43.41±23.8	0.173
Basal T, ng/ml	0.44±1.20	0.53±2.41	0.54±2.48	0.54±2.99	0.556
Basal P, ng/ml	0.85±1.73	0.77±1.07	0.79±1.51	0.83±1.68	0.211
AFC	7.91±4.38^{a}	7.83±4.21^{a}	7.80±4.19^{a}	8.19±4.13^{b}	0.025
^{a,b,c} Groups do not share common letters differ significantly, P<0.0125 for chi square multiple comparison, P< 0.05 for other analyses					
BMI, body mass index; PCOS, polycystic ovarian syndrome; AFC, antral follicle count					
Numbers are means ± SD					

Table 2 presented the ovarian stimulation characteristics and IVF outcomes in the cycles studied. Besides the catheter tip-fundus distance, significant differences were also noted in GnRH analogues, E2 level on the day of hCG and endometrial thickness and endometrial type on the day of hCG among groups. But the starting and total dose, the oocytes yielded, and the number and quality of embryos transferred were comparable among groups. The clinical pregnancy rates and ectopic pregnancy rates were also similar among groups as evaluated with bivariate analyses.

Table 2
Outcome of ovarian stimulation, fertilization and embryo transfer.

	Transfer depth quartiles			
Variables	Quartile 1 (≤9 mm,n=1735)	Quartile 2 (9.1-11 mm, n=2557)	Quartile 3 (11.1-13.9 mm, n=1933)	Quartile 4 (≥14 mm, n=16
Antagonist/agonist (%)	349/1386(20.1/79.9)[a]	518/2039(20.3/79.7)[a]	350/1583(18.1/81.9)[ab]	232/1392(14.3/85.7)[b]
Starting dose of stimulation, IU	208.93±33.60	208.70±34.33	208.89±33.63	208.79±32.32
Total dose of gonadotropin, IU	2324.32±624.65	2320.07±621.09	2301.97±609.31	2351.75±604.94
E2 level on the day of hCG, pg/ml	2832.97±1568.52[ab]	2719.56±1576.04[a]	2756.70±1578.52[ab]	2864.02±1609.90[b]
Progesterone elevation, ng/ml	311/1735(17.9)	432/2557(16.9)	306/1933(15.8)	283/1624(17.4)
Endometrial thickness, mm	10.02±3.13[a]	10.49±3.18[b]	10.76±2.34[c]	11.63±2.69[d]
Endometrial pattern				
A/B/C (%)	354/1247/134(20.4/71.9/7.1)[a]	517/1787/253(20.2/69.9/9.9)[ab]	365/1341/227(18.9/69.4/11.7)[b]	271/1058/295(16.7/65.1/
Number of oocyte retrieved	10.63±5.44	10.48±5.60	10.63±5.72	10.96±5.43
ICSI/IVF (%)	455/1280(26.2/73.8)	704/1853(27.5/72.5)	536/1397(27.7/72.3)	455/1169(28/72)
Blastocyst/cleavage transferred (%)	149/1586(8.6/91.4)	215/2342(8.4/91.6)	158/1775(8.2/91.8)	149/1475(9.2/90.8)
Number of embryos transferred				
One/two/three (%)	361/1305/69(20.8/75.2/4)	512/1940/105(20/75.9/4.1)	400/1452/81(20.7/75.1/4.2)	318/1267/39(19.6/78/2.4
At least one top-quality embryo transferred (%)	407/1735(23.5)	612/2557(23.9)	441/1933(22.8)	428/1624(26.4)
Transfer depth, mm	6.754±1.27[a]	10.01±0.80[b]	12.83±0.79[c]	17.61±2.87[d]
Implantation rate, %	45.35±41.79	43.35±41.22	44.20±41.83	42.64±41.71
Ectopic pregnancy* (%)	10/1104(0.9)	24/1596(1.5)	15/1212(1.2)	10/997(1)
Clinical pregnancy (%)	1038/1735(59.8)	1498/2557(58.6)	1129/1933(58.4)	921/1624(56.7)
OR for clinical pregnancy	Ref.	0.896(0.785-1.024)	0.856(0.742-0.987)	0.704(0.604-0.821)

[a,b,c,d] Groups do not share common letters differ significantly, P<0.0125 for chi square multiple comparison, P< 0.05 for other analyses

*Ectopic pregnancy rate=ectopic pregnancies /(chemical pregnancies +clinical pregnancies +ectopic pregnancies)

**P for trend, ORs were adjusted for female`s age, duration of infertility, hydrosalpinx, the number of oocyte retrieved, starting dose of stimulation, type of GnR number of embryos transferred, endometrial thickness, endometrial pattern, progesterone elevation, the development stage of transferred embryos, the preser good-quality embryo transferred and providers of embryo transfer.

Adjusted for aforementioned confounding factors, multivariate analyses revealed a decrease in clinical pregnancy rates in quartile 3 and quartile 4 with quartile 1 as reference. The odds ratios (OR) for clinical pregnancy comparing quartile 3 and quartile 4 with quartile 1 were 0.86(95%CI: 0.74-0.99) and 0.70(95%CI: 0.60-0.82) respectively. (Table 2)

To test the effect of extreme values of the transfer depth on clinical pregnancy and illustrate the trend of the change of pregnancy rates across the range of distance, we introduced 10% and 90% percentile of the transfer depth into analyses. In the six-group comparison using multivariate analysis, the ORs for clinical pregnancy of different distances (7.1-9 mm, 9.1-11 mm, 11.1-14 mm, 14.1-1.7 mm and >1.7mm) in comparison with the distance of ≤7mm was 0.91 (95%CI: 0.76-1.08), 0.89 (95%CI: 0.75-1.05), 0.84(95%CI: 0.72-0.99), 0.73 (95%CI: 0.60-0.88) and 0.64(95%CI: 0.51-0.80) respectively. A trend of decrease in clinical pregnancy with the increase of transfer depth was illustrated (Figure 1.) and the P value for trend was less than 0.001.

When the transfer depth was treated as continuous value, the OR for clinical pregnancy per millimeter increased was 0.97 (95%: 0.96-0.99) in multivariate analyses (supplemental Table 1). The ORs for clinical pregnancy of other covariates, which were included in multivariate analyses were also presented in

supplementary Table 1.

To explore whether the association between embryo transfer depth and pregnancy differs across stratum of potential effect modifiers, the interaction terms of endometrial thickness×embryo transfer depth, blastocyst transfer ×embryo transfer depth, progesterone elevation ×embryo transfer and transfer provider×embryo transfer depth was introduced into the model. When endometrial thickness on the day of hCG was categorized into thin (<8 mm, n=913), normal (8-11 mm, n=3760) and thick (>11mm, n=3176) categories, the ORs for pregnancy comparing quartile 2, quartile 3 and quartile 4 of tip-to-fundus distance with quartile 1 were 1.07(95%: 0.79-1.45) fold, 1.09(95%: 0.80-1.50) fold and 1.03(95%: 0.74-1.43) fold in thick group than in normal thickness group, respectively, suggesting insignificant change of the effect with increased endometrial thickness. To the contrast, the ORs increased 1.96(95%: 1.33-2.90) fold, 1.20(95%: 0.78-1.87) fold and 1.98(95%: 1.20-3.26) fold respectively in thin group comparing with normal group, suggesting an effect modification of thin endometrium. In cycles with thin endometrium, the adjusted ORs for pregnancy comparing quartile 3 and quartile 4 with quartile 1 was 1.72 (95%: 1.21-2.47), 1.03(95%: 0.68-1.55) and 1.44 (95%: 0.90-2.30), respectively.

On the other hand, both progesterone elevation and blastocyst transfer decreased ORs. The ORs comparing quartile 2 through 4 with quartile 1 decreased 0.52 (95%: 0.32-0.85), 0.60 (95%: 0.36-1.00) and 0.55 (95%: 0.33-0.93) fold comparing blastocyst transfer with cleavage stage transfer and decreased 0.86 (95%: 0.68-1.07), 0.79 (95%: 0.60-1.03) and 0.77 (95%: 0.58-1.01) fold comparing cycles with progesterone elevation with those without. Interaction (P for interaction term was 0.25) was not detected between embryo transfer providers and catheter tip-fundus distance (supplemental Table 2).

Discussion

In the present study, we demonstrated a negative association between the embryo position from fundus and pregnancy rate in a multivariate analysis containing 7849 fresh embryo transfer cycles. Moreover, our data suggested that the uterine factors that have been associated with changes in uterine contraction, such as thin endometrium, high progesterone and blastocyst stage transfer might modify the association between embryo transfer depth and pregnancy.

Efforts to find an idea embryo position during transfer procedure have been challenged by findings suggesting that embryos might undergo significant migration following replacement [29–32]. Saravelos et al. suggested that uterine factors such as uterine contractility may dictate where the embryo will eventually implant following transfer[32]. Several studies demonstrated that embryos might undergo significant migration following replacement [29–32]. However, the movement of embryos following transfer was not random and most embryo flashes undergone migration towards the fundus or remained static 60 mins following transfer[31]. The pregnancy rates among patients with embryo flashes located <15 mm from the fundus at 60 mins posttransfer were still significantly higher than those with embryo flashes located >15 mm from the fundus[31]. Therefore, it is suspected that the combination of uterine contraction and embryo transfer technique may determine the final location of implantation, and thus affect the chance of pregnancy.

Rombauts et al. showed that thinner endometrium is associated with increased ectopic pregnancy risk, whereas increased endometrial thickness is associated with higher placenta praevia risk[21,33], proposing that increased endometrial thickness is considered as a marker for increased fundus-to-cervix uterine peristalsis [21]. It hinted that directionality of embryo dislodging after transfer may differ in women with different endometrial thickness: patients with thin endometrium are more likely to undergo a tubal embryo migration, resulting increased ectopic pregnancy rate while patients with thicker endometrium might expel the embryos to cervix direction. Our study showed that the association between catheter depth and pregnancy rates may differ across endometrial thickness categories. The adjusted ORs were significantly increased in cycles with lower endometrial thickness and suggested a detrimental effect on pregnancy rate of deep fundus transfer in patients with thin endometrium. The observation may support the hypothesis that the endometrial thickness is associated with the directionality of uterine peristalsis and further affect the embryo migration following transfer.

Known as a relaxant of uterine contraction, progesterone level is another factor that may affect the embryo deposition [20,22]. Fanchin et al. suggested that uterine contraction frequency decreased in the patients with high progesterone levels, and was negatively correlated with progesterone concentrations on the day of embryo transfer[20]. Similarly, increased progesterone level during luteal phase may also response for the decreased uterine contractictility at the time of blastocyst transfer[22]. In such situations, the ORs comparing transfer at lower position with transfer at higher position were reduced. It is possible that with decreased uterine contraction frequency, the embryos are less likely to migrate away from their initial location and the embryo transfer depth is more likely to reflect the embryo position after transfer. Taken together, the data suggested the importance of considering the patients` uterine environment when evaluating the association between catheter depth and pregnancy rates.

Although conflicting with several previous studies[9,11,13,14,34], the positive association between transfer depth and implantation observed in our study consisted with the report of Pacchiarotti et al. [12], and echoed several observation in the early days [3–5]. The lower adjusted pregnancy rates observed in cycles with embryos transferred at a distance>14 mm from the fundus also partially confirmed the results of Rovei et al., which suggested that a transfer distance above 15 mm compromised the implantation and pregnancy rates[15]. The results were also logically in line with the studies suggesting an embryo position closer to the fundal myometrium–endometrial interface results in a better chance of pregnancy [14,35,36]. More recently, Bayram et al. further confirmed the negative association between the distance from fundus and implantation in a cohort transferring with euploid blastocyst[16].

A significant concern on transferring embryos close to fundus is that placing the catheter tip near the fundus might transfer the embryos into the tube, possibly leading to ectopic pregnancies[7,37]. In our study, no significant difference in the ectopic pregnancy rates among groups was observed. However, given the total events of ectopic pregnancies were relatively rare, it is immature to draw a firm conclusion in this regard from the present data.

Due to the limitation of retrospective nature, there were numbers of residual or unmeasured confounding might still be present in the present study. There were many other parameters during the embryo transfer procedure (1), such as fundal level of the uterine cavity, length of uterine cavity, and transfer speed, were

not recorded in the study, and thus the interactions between the parameters were unknown. The embryo transfer procedure is also affected by individual anatomy of patients and the preference of providers. Despite that we excluded the difficult-to-transfer patients from the study and controlled for high number of variables including different clinicians providing the transfer, the potential biases introduced by unknown/unmeasured factors should still be highlighted.

In summary, we reexamine the effect of embryo transfer depth on pregnancy rates in an IVF population containing 7849 cycles, and suggested that factors that associated with uterine contraction, including thin endometrium and progesterone elevation significantly affect the association between embryo transfer depth and clinical pregnancy. The potential modification effects of uterine-related factors may partially explain the heterogeneity among studies and warrant future studies on individualized embryo transfer.

Declarations

Authors` contribution

X.S, J.C. and L.L. are responsible for the concept design and performed the statistical analysis. X.S and H.C. scrutinized patients' files. X.S, J.C. and L.L. wrote the manuscript. H.C, X.J and J.R. contributed to the interpretation of the results and editing of the manuscript

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Conflict of interest

None declared.

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Figures

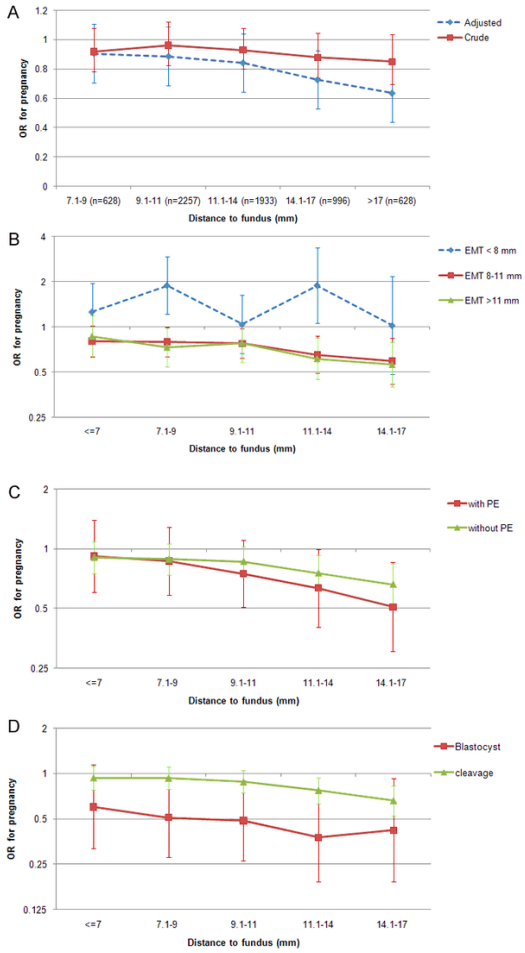

Figure 1

ORs (95% CI) for clinical pregnancy adjusted for female`s age, duration of infertility, hydrosalpinx, the number of oocyte retrieved, starting dose of stimulation, type of GnRH analogues, the number of embryos transferred, endometrial thickness, endometrial pattern, progesterone elevation, the development stage of transferred embryos, the presence of at least one good-quality embryo transferred and providers of embryo transfer through different transfer depth levels, using fundus distance ≤7mm (n=1107) as reference. (A) Adjusted and unadjusted ORs for pregnancy. (B) Adjusted ORs for pregnancy across endometrial thickness (EMT) categories. (C) Adjusted ORs for pregnancy in cycles with and without progesterone elevation (PE) (D) Adjusted ORs for pregnancy in cleavage transfer cycles and blastocyst transfer cycles.

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