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Original Article

Clinical outcome of IVF/ICSI cycles with an arrested embryo on day 3

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Abstract: Embryo development arrest commonly occurs during IVF/ICSI treatment, however, no research has reported whether the clinical outcomes were affected by the presence of an arrested embryo. In this study, we retrospectively compared the basic characteristics and clinical outcomes of 1590 cycles with at least one arrested embryo on day 3 (arrested group), and 2551 cycles with no arrested embryo (control group). There was no significant difference in female age, BMI and duration of infertility between the two groups ($P>0.05$), the antral follicle count was higher while the basic FSH level on day 3 of menses was lower in the arrested group ($P<0.001$). The arrested group got more ooctyes and 2PN embryos, but the high quality embryo number and rate, available embryo number and rate, clinical pregnancy rate, implantation rate and live birth rate in fresh embryo transfer cycles were significantly lower ($P<0.05$), the cumulative pregnancy rate and cumulative implantation rate were also significantly lower in the arrested group ($P<0.05$). However, there was no statistical difference in the pregnancy rate, implantation rate and live birth rate for patients transferred matched-cohort embryos of the same grade between the two groups. Multivariate regression analysis results showed that transferred high quality embryo rate is significantly associated with pregnancy outcome while the presence of an arrested embryo is not statistically associated with the pregnancy outcome. We deduce the presence of an arrested embryo in a cohort of embryos may associate with increased proportion of poor quality embryos, and affect the clinical outcome in IVF/ICSI cycles by transferring lower quality embryos, however, for embryos of the same grade, there was similar embryo development potential between the two groups.

Keywords: Embryo arrest, embryo quality, clinical outcome, IVF, ICSI

Introduction

When mammalian embryos are cultured in vitro, some of them stop cleavage and arrest at a certain development stage, called "early embryonic development arrest", in human the stage often at 2-4 cell stage, while mice at 2-cell stage and bovine at 4-8 cell stage [1, 2]. Developmental arrest of human embryos cultured in vitro is a common phenomenon during IVF or ICSI treatment, it has been shown that about 10% of all human embryos produced by IVF or ICSI arrested at the early cleavage stage, and about 40% of patients exhibit at least one arrested embryo per treatment cycle [2].

Generally, arrested embryos are discarded during IVF or ICSI treatment, which may decrease the total number of available embryos and affect the clinical outcome of the treatment cycle. Meanwhile, for a cohort of embryos retrieved from the same couple in the same IVF/ICSI treatment, whether the presence of an arrested embryo can reflect the quality of the sibling embryos is unknown. Oocyte quality predominantly determines the embryo quality [3-5], it has been shown that a giant oocyte in a cohort of retrieved oocytes is associated with abnormal cleavage in sibling embryos [6], and embryos exhibited lower cleavage rates and higher rates of fragmentation and asymmetry in patients with at least one non-cleaved embryo on day 3 compared with patients with 100% cleaved embryos on day 3 after fertilization [7]. Then, the presence of an arrested embryo in a cohort of embryos may reflect the poor oocyte quality and poor embryo quality produced by the same couple in the same IVF or ICSI treatment.

Jun et al. has reported that the rate of embryo arrest is associated with IVF cycle outcome, for cycles with embryo number ≥6, patients with embryo arrest rate >14.6% were 3 times more likely to result pregnancy failure compared with patients with embryo arrest rate ≤14.6% [8]. In their study, "arrest embryos" were defined as those with 4 or fewer cells on day 3 of in vitro culture, according to the Istanbul consensus wrote by alpha scientists in reproductive medicine and ESHRE special interest group of embryology [9], the definition is not appropriate and may include part of "slow embryos" that have 6 or fewer cells on day 3, but have cleaved during the preceding 24-h period. Meanwhile, the reasons caused the different clinical outcome was also not discussed in their study. According to the Istanbul consensus [9], "arrested embryos" are those that have not cleaved during a 24-h period, including those that arrest at the one-cell stage, the definition is consistent with previous reported by Lan et al. [10], and we follow this definition in this study.

To our knowledge, no data exist has shown whether the presence of an arrested embryo in a cohort of embryos may associate with the sibling embryo quality and the pregnancy outcome. In this study, a total of 4141 IVF/ICSI cycles were retrospectively analyzed to explore whether embryo quality and/or clinical pregnancy outcome in cycles with at least one arrested embryo differs from cycles with no embryo arrested during IVF or ICSI treatment.

Materials and methods

Institutional review board approval for this study was obtained from Ethical Committee of Medical College Xiamen University. All the subjects enrolled in this study were given written formal consent before participation.

Study participants

Patients underwent IVF or ICSI treatment in our center from January 2008 to May 2013 were retrospectively analyzed, fresh embryo transfer was routinely performed on day 2 or day 3 after insemination. A total of 4141 fresh embryo transfer cycles on day 3 after insemination were included, for cycles that available embryo number was 2 or less, embryo transfer was performed on day 2 after insemination, those cycles were excluded as there was no embryo observation and assessment on day 3. According to the Istanbul consensus [9] and previous report [10], embryos that have not cleaved during a 24-h period, including those that arrest at the one-cell stage were defined as "arrested embryos" in this study. Among the 4141 day 3 embryo transfer cycles, 1590 cycles with at least one arrested embryo were defined as the arrested group, and 2551 cycles with no arrested embryo were defined as the control group.

Ovarian stimulation protocol

Ovarian stimulation protocol was determined based on patient's age, ovarian reserve, cause of infertility, and other medical conditions. For most patients with normal ovarian reserve, GnRH agonist protocol was used as previously described [11], GnRH antagonist protocol, natural cycle and luteal-phase ovarian stimulation protocol were generally used for aged patients or those with poor ovarian reserve. Domestic urinary human menopausal gonadotropin (hMG; Lizhu Parma) or recombinant FSH (Gonal F; Merck Serono) was used for ovarian stimulation, and 5,000-10,000 IU human chorionic gonadotropin (hCG; Lizhu Pharma) was injected when at least one follicle ≥18 mm in mean diameter. Oocyte retrieval was performed using transvaginal ultrasound device 34-36 hours after hCG injection.

Embryo culture and assessment

Oocytes were routinely inseminated or injected within 4-6 h after retrieval, pronuclei were identified 17-18 hours after insemination. Embryos were cultured at 37°C in a humified atmosphere of 5% CO_2 in air and observed every day. Embryo grading system was based on numbers of embryo blastomere, fragmentation and symmetry [12-14]. Day 3 embryos were assessed and divided into four different grades: grade I embryos were those with 8 blastomeres, of even symmetry and no fragmentation; grade II embryos were those with 7-9 blastomeres or at compaction stage, of even symmetry and <25% fragmention, grade III embryos were those with ≥10 or 5-6 blastomeres, with even symmetry and <50% fragmentation, or those with 7-9 blastomeres of uneven symmetry or 25-50% fragmentation; grade IV embryos were those with ≤4 blastomeres, or those with ≥5 blastomeres, with uneven symmetry and >50% fragmentation. Embryos that had the same number of blasto-

Table 1. Basic characteristics of the two groups

	Arrested group (n=1590)	Control group (n=2551)	*P* value
Female age (y)	31.04 ± 3.96	31.23 ± 3.99	NS
Duration of infertility (y)	4 (4)	4 (4)	NS
Body mass index	21.04 ± 2.68	21.16 ± 2.60	NS
Antral follicle count on day 1 of stimulation	8.37 ± 3.49	7.84 ± 3.44	<0.001
FSH on day 3 of menses (mIU/ml)	5.97 (2.35)	6.32 (2.55)	<0.001
LH on day 3 of menses (mIU/ml)	4.49 ± 2.47	4.41 ± 2.57	NS
E_2 on day 3 of menses (pg/ml)	38.20 ± 23.27	38.06 ± 23.88	NS
Stimulation protocol			
GnRH agonist protocol (%)	84.59 (1345/1590)	78.87 (2012/2551)	<0.001
GnRH antagonist protocol (%)	14.34 (228/1590)	19.68 (502/2551)	<0.001
Infertility causes			
Tubal infertility (%)	976/1590 (61.38)	1681/2551 (65.90)	0.003
Endometriosis (%)	115/1590 (7.23)	147/2551 (5.76)	NS
PCOs (%)	91/1590 (5.72)	171/2551 (6.70)	NS
Male factor (%)	370/1590 (23.27)	523/2551 (20.50)	NS
Unknown factor (%)	38/1590 (2.39)	29/2551 (1.14)	0.002
ICSI (%)	460/1590 (28.93)	680/2551 (26.66)	NS
Patients with a previous IVF/ICSI failure (%)	242/1590 (15.22)	433/2551 (16.97)	NS
Patients with previous pregnancy history (%)	827/1590 (52.01)	1370/2551 (53.70)	NS

Numbers are mean ± standard deviation or medium (interquartile range), FSH=Follicle stimulating hormone; LH=Luteinizing hormone; E_2=Estradiol; NS=No significant difference.

mere from day 2 to day 3 after insemination and those arrested at one-cell stage were considered as arrested embryos. Grade I and grade II embryos were considered as high quality embryos, and the rest of embryos were considered as poor quality embryos. Grade I, grade II and grad III embryos were considered as available embryos, while grade IV embryos, arrested embryos and embryos with all blastomere degenerated or lysed were usually deserted.

Embryo transfer and pregnancy determinant

Fresh embryo transfer was carried on day 3 after insemination, frozen-thawed embryo transfer was carried 1-2 h after embryos were thawed. High quality embryos were preferentially considered for transfer, for cycles with no high quality embryo, grade III embryos may be transferred according to patient's personal will, for women aged ≤35 years, 1-2 embryos were usually transferred, and for women aged >35 years or had a previous IVF or ICSI treatment failure, 3 embryos may be transferred according to their personal will. Chemical pregnancy test (serum β-hCG determination) was performed 14 days after embryo transfer, and clinical pregnancy was defined by the presence of gestational sac and visualization of fetal heart beat by ultrasound 4 weeks after embryo transfer. Luteal phase support was sustained from the day of oocyte retrieval until 12 weeks for patients who became clinically pregnant.

Statistical analysis

Data analysis was performed using SPSS Version 19 statistical software. Continuous variables were analyzed using independent sample t-test or the Mann-Whitney test, dichotomous variables were compared using Chi-square test or Fisher's exact test, Multivariable logistic regression analysis was used to examine factors predicting pregnancy. *P*<0.05 was considered to be statistically significant.

Results

Incidence of arrested embryos

Of the 25468 embryos assessed from the 4141 cycles included in this study, 2401 embryos in 1590 cycles arrested, the arrested embryo rate is 9.43% and arrested cycle rate is 38.40%, similar with previously reported [2]. Of the 1590 arrested cycles, 1068 cycles with only one embryo arrested, 353 cycles with two

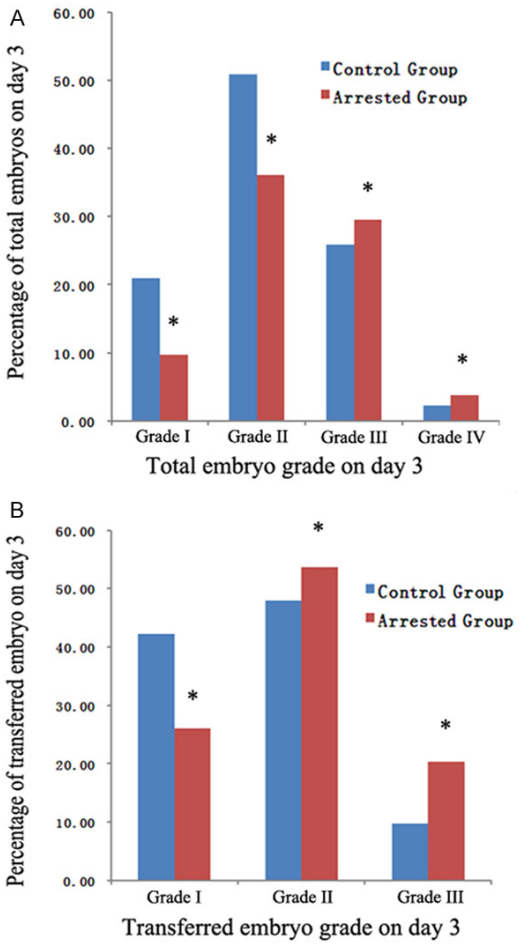

Figure 1. Distributions of total embryo grade and transferred embryo grade on day 3. A. Total embryo grade distribution. Grade I and grade II embryo rates were significantly decreased while grade III and grade IV embryo rates were significantly increased in the arrested group (N=1590 in arrested group, N=2551 in control group, **P*<0.01). B. Transferred embryo grade distribution. Transferred grade I embryo rate was significantly decreased while transferred grade II and grade III embryo rates were significantly increased in the arrested group (N=1590 in arrested group, N=2551 in control group, **P*<0.01).

arrested embryos, and 169 cycles with ≥3 arrested embryos.

Patient characteristics

Basic characteristics of the arrested group and control group were shown in **Table 1**. No significant differences were found between the two groups in female age, duration of infertility, body mass index, LH level on day 3 of menses and E_2 level on day 3 of menses (*P*>0.05). The FSH level on day 3 of menses was significantly lower while the antral follicle count on day 1 of stimulation was significantly higher in the arrested group than in the control group (*P*<0.001). As for the simulation protocol, the arrested group adopted more GnRH agonist protocol and less antagonist protocol compared to the control group (*P*<0.001). More unknown infertility factor patients distributed in the arrested group, while more tubal infertility patients distributed in the control group (*P*<0.01), no significant difference was found in the distribution of patients with endometriosis, PCOs and male factors (*P*>0.05). There were similar percentages of patients with a previous IVF/ICSI failure or with previous pregnancy history in the two groups.

Distribution of embryo grades

Total day 3 embryo grades distribution and fresh transferred embryo grades distribution were shown in **Figure 1**. As to the total embryo grades distribution, grade 1 and grade II embryo rates were significantly decreased while grade III and grade IV embryo rates were significantly increased in the arrested group than in the control group (*P*<0.01). Meanwhile, as to fresh embryo transfer cycles, transferred grade I embryo rate was significantly decreased while grade II and grade III embryo rates were significantly increased in the arrested group than in the control group (*P*<0.01).

Stimulation and clinical variables of the two groups

Stimulation and clinical variables of the two groups were shown in **Table 2**. Gonadotropin usage dose and length of stimulation was similar between the two groups (*P*>0.05), the arrested group exhibited a more exuberant response to ovarian stimulation as reflected by more oocytes retrieved (*P*<0.001). Total mature oocyte number and normal fertilized embryo number were significantly higher in the arrested group (*P*<0.001), but the high quality embryo number and available embryo number were significantly lower in the arrested group (*P*<0.05). Mature oocyte rate and normal fertilized embryo rate were similar between the two groups, while high quality embryo rate (46.30% vs. 72.17%) and available embryo rate (69.36% vs. 89.87%) were significantly lower in the arrested group (*P*<0.001). Transferred fresh embryo number and endometrial thick-

Table 2. Cycle stimulation and clinical variables of the two groups

	Arrested group (n=1590)	Control group (n=2551)	*P* value
Total dose of GN (IU)	2035.20 ± 528.94	2019.20 ± 551.25	NS
Length of stimulation (d)	9.69 ± 2.07	9.60 ± 2.27	NS
E_2 on day of hCG (pg/ml)	2794 (2012)	2486 (1988)	<0.001
No. of oocytes retrieved	10.70 ± 4.23	9.01 ± 3.91	<0.001
No. of MII oocytes	9.67 ± 3.89	8.06 ± 3.56	<0.001
No. of 2PN embryos	7 (4)	5 (3)	<0.001
No. of high quality embryos	3 (4)	4 (2)	<0.001
No. of available embryos	4.98 ± 2.71	5.18 ± 2.45	0.016
No. of embryos transferred per fresh cycle	2.05 ± 0.35	2.06 ± 0.31	NS
Endometrial thickness on day of hCG (cm)	1.03 (0.30)	1.03 (0.29)	NS
% MII oocytes/total	91.15 ± 14.33	90.47 ± 12.48	NS
% 2PN zygotes/MII oocytes	75.18 ± 16.39	74.07 ± 18.27	NS
High quality embryo rate (%)	5288/11421 (46.30)	10620/14715 (72.17)	<0.001
Available embryo rate (%)	7922/11421 (69.36)	13224/14715 (89.87)	<0.001
Transferred high quality embryo rate (%)	2595/3262 (79.55)	4792/5312 (90.21)	<0.001
Clinical pregnancy per ET cyce (%)	831/1590 (52.26)	1438/2551 (56.37)	0.011
Implantation rate (%)	1135/3262 (34.79)	2030/5312 (38.22)	0.001
Live birth rate per ET cycle (%)	689/1590 (43.33)	1192/2551 (46.73)	0.033
Miscarriage rate (%)	107/831 (12.88)	195/1438 (13.56)	NS
Cumulative pregnancy rate (%)	1034/1590 (65.03)	1749/2551 (68.56)	0.019
Cumulative implantation rate (%)	1402/4375 (32.05)	2457/6893 (35.64)	<0.001

Numbers are mean ± standard deviation or medium (interquartile range). GN=Gonadotropin; hCG=Human chorionic gonadotropin; PN=Pronuclear; ET=Embryo transfer; NS=No significant difference.

ness on day of hCG was similar between the two groups, but the transferred high quality embryo rate (79.55% vs. 90.21%, *P*<0.001) in fresh embryo transfer cycles was significantly lower in the arrested group, and subsequently, the clinical pregnancy rate (52.26% vs. 56.37%, *P*=0.011), live birth rate (43.33% vs. 56.73%, *P*=0.033) and implantation rate (34.79% vs. 38.22%, *P*=0.001) in the arrested group were significantly lower than in the control group. Meanwhile, the cumulative pregnancy rate (63.05% vs. 68.56%, *P*=0.019) and cumulative implantation rate (32.05% vs. 35.64%, *P*<0.001) in the arrested group were also significantly lower compared to the control group, there was no significant difference in the miscarriage rate between the two groups in fresh embryo transfer cycles.

Basic characteristics and clinical variables of patients transferred matched-cohort embryos between the two groups

Results in **Table 2** showed the transferred high quality embryo rate in the arrested group was significantly lower than in the control group (79.55% vs. 90.21%, *P*<0.001) in fresh embryo transfer cycles, to test this influencing factor, patients underwent their first IVF/ICSI treatment and transferred 2 embryos of the same grade were selected and analyzed. Cycles were matched based on transferred embryo grade, 223 arrested cycles were matched with 681 control cycles in the grade I embryo transfer cycles, 469 arrested cycles were matched with 691 control cycles in the grade II embryo transfer cycles, and 102 arrested cycles were matched with 56 control cycles in the grade III embryo transfer cycles. As shown in **Table 3**, there was no significant difference between the two groups in basic characteristics like female age, BMI, duration of infertility and endometrial thickness on day of hCG (*P*>0.05), there was also no significant difference between the two groups of FSH level in cycles transferred grade I and grade III embryos (*P*>0.05), while FSH level in the arrested group was significant lower than in the control group in cycles transferred grade II embryos. The arrested group got more 2PN embryos (*P*<0.001), but there was no significant difference in the high quality embryo number be-

Table 3. Basic characteristics and clinical outcome variables in patients transferred matched-cohort embryos between the two groups

Grade I embryo transfer cycles	Arrested group (n=223)	Control group (n=681)	*P* value
Female age (y)	30.60 ± 3.64	30.45 ± 3.87	NS
Duration of infertility (y)	4 (4)	4 (4)	NS
Body mass index	20.93 ± 2.18	21.19 ± 2.63	NS
FSH on day 3 of menses (mIU/ml)	5.98 (2.38)	6.17 (2.39)	NS
No. of 2PN embryos	8.75 ± 3.36	7.19 ± 2.98	<0.001
No. of high quality embryos	6.21 ± 2.83	6.09 ± 2.66	NS
Endometrial thickness on day of hCG (cm)	1.04 (0.28)	1.04 (0.27)	NS
Clinical pregnancy rate (%)	133/223 (59.64)	436/681 (64.02)	NS
Implantation rate (%)	196/446 (43.95)	632/1362 (46.40)	NS
Live birth rate (%)	117/223 (52.47)	372/681 (54.63)	NS
Grade II embryo transfer cycles	Arrested group (n=469)	Control group (n=691)	*P* value
Female age (y)	30.54 ± 3.67	30.84 ± 3.77	NS
Duration of infertility (y)	4 (4)	4 (4)	NS
Body mass index	20.97 ± 2.89	21.05 ± 2.54	NS
FSH on day 3 of menses (mIU/ml)	5.93 (2.14)	6.41 (2.59)	<0.001
No. of 2PN embryos	7.47 ± 2.92	5.36 ± 2.19	<0.001
No. of high quality embryos	3.53 ± 1.74	3.62 ± 1.62	NS
Endometrial thickness on day of hCG (cm)	1.05 (0.31)	1.04 (0.32)	NS
Clinical pregnancy rate (%)	269/469 (57.36)	385/691 (55.72)	NS
Implantation rate (%)	363/938 (38.70)	539/1382 (39.00)	NS
Live birth rate (%)	222/469 (47.33)	317/691 (45.88)	NS
Grade III embryo transfer cycles	Arrested group (n=102)	Control group (n=56)	*P* value
Female age (y)	30.86 ± 3.72	31.04 ± 3.59	NS
Duration of infertility (y)	4 (5)	4 (3.5)	NS
Body mass index	20.73 ± 2.29	20.84 ± 2.57	NS
FSH on day 3 of menses (mIU/ml)	5.77 (2.22)	6.06 (2.26)	NS
No. of 2PN embryos	6.09 ± 2.76	4.18 ± 1.85	<0.001
No. of high quality embryos	0	0	
Endometrial thickness on day of hCG (cm)	1.08 (0.28)	0.97 (0.31)	NS
Clinical pregnancy rate (%)	36/102 (35.29)	17/56 (30.36)	NS
Implantation rate (%)	49/204 (24.02)	17/112 (15.18)	NS
Live birth rate (%)	22/102 (21.57)	12/56 (21.43)	NS

Numbers are mean ± standard deviation or medium (interquartile range); NS=No significant difference.

tween the two groups ($P>0.05$), there was also no significant difference in the clinical pregnancy rate, implantation rate and live birth rate between the two groups among all the three different embryo grade transfer cycles ($P>0.05$).

Multivariate logistic regression analysis results

In multivariate logistic regression analysis of pregnancy, the effect of embryo arrest (with arrested embryo versus without arrested embryo) was adjusted for clinical factors experientially associated with IVF/ICSI pregnancy outcome, and variables different between the two patient cohorts, including antral follicle count, FSH level on day 3 of menses, stimulation protocols (antagonist versus agonist), infertility indicators (tubal, endometriosis, PCOs, male factor and unknown factor), E_2 levels on the day of triggering, number of oocytes retrieved and transferred high quality embryo rate were selected as potential confounding factors. Other factors like female age, duration of infertility, type of infertility (primary versus secondary), BMI, basal LH and E_2 levels, insemination protocols (ICSI versus IVF), total gonadotropin dose used, endometrial thickness on the day of hCG and transferred embryo num-

Table 4. Multivariate logistic regression analysis of pregnancy per transfer cycle

Predictors	Categories	Sig.	OR (95% CI)
Female age	Per year increased	<0.001	0.954 (0.936-0.973)
Duration of infertility	Per year increased	0.23	0.986 (0.964-1.009)
Primary infertility	Primary versus secondary	0.495	1.052 (0.91-1.216)
BMI	Per unit increased	0.205	0.984 (0.959-1.009)
Basal FSH	Per unit increased	0.07	1.031 (0.998-1.065)
Basal LH	Per unit increased	0.003	1.044 (1.015-1.074)
Basal E_2	Per unit increased	0.021	0.997 (0.994-1)
Antral follicle conut	Per AFC increased	0.028	1.022 (1.002-1.042)
Stimulation protocol	Antagonist versus agonist	0.001	0.727 (0.603-0.876)
Insemination protocol	ICSI versus IVF	0.492	1.101 (0.837-1.448)
Tubal factor	Without versus with	0.545	1.077 (0.847-1.368)
Endometriosis	Without versus with	0.541	1.085 (0.834-1.412)
PCOs	Without versus with	0.142	1.227 (0.934-1.613)
Male factor	Without versus with	0.821	0.96 (0.677-1.363)
Unknown	Without versus with	0.795	1.386 (0.118-16.269)
Total gondadotropin dose	Per log unit increased	0.003	2.486 (1.373-4.5)
Endometrial thickness	Per unit increased	<0.001	2.013 (1.493-2.715)
E_2 on the day of triggering	Per log unit increased	<0.001	0.511 (0.371-0.704)
Oocytes retrieved	Per oocyte increased	0.211	1.013 (0.993-1.033)
Number of embryos transferred			
ET number (1)	Two versus one	<0.001	3.197 (1.772-5.766)
ET number (2)	Three versus one	<0.001	3.614 (1.946-6.71)
Transferred high quality embryo rate	Per percentage increased	<0.001	1.008 (1.004-1.011)
Arrested embryo	With versus without	0.983	1.002 (0.862-1.164)

BMI=Body mass index, PCOs=Poly Cystic Ovary Syndrome, ET=Embryo transfer.

ber (two versus one, three versus two) were also selected based on clinical experience. Although the numbers of retrieved oocytes, MII oocytes, 2PN embryos and high quality embryos were all significantly different between the two groups, only the number of ooctyes retrieved was involved in the model to avoid colinearity. Hosmer-Lemshow goodness-of-fit χ^2 test statistics was 9.229 (P=0.323), which suggested that the multivariable model was a good fit (*P*>0.05). Adjusted for the potential confounding factors mentioned above, the odd ratio of arrest group versus control group on pregnancy rate was 1.002 (0.862-1.164), whereas several other factors like transferred high quality embryo rate and female age were significantly associated with pregnancy outcome (as shown in **Table 4**).

Discussion

It is a common phenomenon for early embryo cleavage arrest cultured in vitro during IVF or ICSI treatment, however, rare studies have reported the outcome of cycles with arrested embryos, and no research has shown whether the presence of an arrested embryo on day 3 is associated with the sibling embryo quality and affect the clinical pregnancy outcome.

In this study, there was no significant difference between the two groups in basic characteristics like female age, duration of infertility and body mass index, the basic FSH level on day 3 of menses was lower while antral follicle number on day 1 of stimulation was higher in the arrested group (*P*<0.001, **Table 1**). The two groups consumed similar dose of gonadotropin, but the arrested group exhibited a more exuberant response to ovarian stimulation as reflected by more mature oocytes retrieved and 2PN embryos after insemination (*P*<0.001, **Table 2**), according to previously reported predictive factors like total oocytes number and embryo quantity [15-17], the arrested group might got a better clinical pregnancy

outcome compared to the control group, however, results showed the high quality embryo rate, available embryo rate, clinical pregnancy rate, implantation rate and live birth rate in fresh embryo transfer cycles of the arrested group were significantly lower than the control group ($P<0.05$, **Table 2**). Meanwhile, the cumulative pregnancy rate and cumulative implantation rate in the arrested group were also significantly lower than in the control group ($P<0.05$).

We found the total embryo quality was greatly decreased as reflected by a significant decrease of high quality embryo (grade I and grade II) number and rate, as well as an increase in the low quality embryo (grade III and grade IV) rate ($P<0.01$, **Figure 1**), besides, fresh transferred grade I embryo rate was significantly decreased while fresh transferred grade II and grade III embryo rates were significantly increased as shown in **Figure 1** ($P<0.01$), and total fresh transferred high quality embryo rate in the arrested group was significantly lower than in the control group ($P<0.01$, **Table 2**). Quality of transferred embryo was significantly associated with clinical pregnancy outcomes in IVF or ICSI cycles [18]. High quality embryos were preferentially considered for transfer during our routine clinical work, the lower transferred high quality embryo rate in fresh transfer cycles reflect the lower total high quality embryo number and rate in the arrested group. Meanwhile, the decreased cumulative pregnancy rate and cumulative implantation rate in the arrested group may caused by the decrease of total available embryo number and rate. However, there was no significant difference in clinical pregnancy rate, implantation rate and live birth rate between the two groups of matched-cohort embryo transfer cycles in patients underwent their first IVF/ICSI treatment and transferred 2 embryos of the same grade, this result confirmed that transferred embryo quality is greatly associated with clinical outcomes in IVF/ICSI cycles, and there was similar development potential of embryos in the same grade between the two group. Multivariate regression analysis results also showed transferred high quality embryo rate is significantly associated with IVF/ICSI pregnancy outcome, while the presence of an arrested embryo is not statistically associated with the pregnancy outcome. So we deduce the presence of an arrested embryo in a cohort of embryos may associate with increased proportion of poor quality embryos, and affect the clinical outcome in IVF/ICSI cycles by transferring lower quality embryos.

Early embryo development is mainly dependent on the RNA and protein synthesis during oogenesis [19, 20], poor oocyte quality may cause infertiliy of women and failure of in vitro fertilization treatment [21-23]. It has been reported by many scientists that embryo arrest is caused by the failure of embryonic genome activation from maternal control [24-26]. As the oocyte-maternal transition is controlled by the maternal factors, oocyte quality is especially important for early embryo development before oocyte-maternal transition [27], development incompetence of oocyte may cause subsequent embryo development arrest [28, 29]. Estradiol level was important for oocyte maturation, only the optimal concentration of estradiol can produce competent oocytes and embryos [30]. In this study, the estradiol level on day of hCG was significantly higher in the arrested group, the high level of estradiol may directly effect oocyte maturation and the subsequent embryo development. Proportion of dominant follicles is greatly associated with oocyte development potential [31]. The antral follicle count and total retrieved oocytes were significantly higher in the arrested group, this may arise from recruitment of smaller, less competent follicles and result oocytes of less development potential and subsequent embryos of inadequate quality.

Chromosome abnormality is another important reason cause embryo development arrest, studies have shown incidence of chromosome abnormalities in arrested embryos was significantly higher than in normally developed embryos [32-34]. Chromosome aneuploid is the main type of chromosome abnormality [35-37], but researches has shown aneuploid did not lead to embryo cleavage arrest probably because the embryonic genome is not fully active [9, 38]. Post-meiosis abnormalities like mosaicism, monospermic polyploidy and haploidy are significantly associated with embryo cleavage arrest, there is a significant relationship between embryo development potential and post-meiosis abnormality [39, 40]. Besides, post-meiosis abnormalities may also cause zygotic dysmorphism, as reflected by poor embryo grade assessed morphological-

ly [41], so we deduce embryos in the arrested group in this study may have a higher rate of post-meiosis abnormality than the control group, and thus the poor quality embryo proportion was significantly increased in the arrested group. Post-meiotic abnormalities are not affected by maternal age [9], that may explain why the arrested group got a poorer high quality embryo rate although there was no significant different in the female age between the two groups.

Paternal factors [42, 43], ovarian hyperstimulation protocol [44] and the inadequate in vitro culture system [45] may also cause embryo development arrest during IVF or ICSI treatment. Besides, patient inherent factors like different infertility causes may also associate with ooctye quality and subsequent embryo quality [46, 47]. It has been shown that mild ovarian stimulation protocol significantly reduced the abnormal and mosaic embryo rate compared to the conventional GnRH agonist protocol [44]. In this study, GnRH agonist protocol rate was significantly higher in the arrested group, that may related with a higher embryo abnormal and mosaic rate in the arrested group, patient rate with tubal infertility factor was lower while unknown factor rate was higher in the arrested group may reflect patients inherent oocye and embryo quality difference.

This study has some limitations. All the fresh transfer cycles analyzed in this study were performed embryo transfer on day 3 after insemination, for these cycles with 2 or less available embryos, embryo transfer was performed on day 2, these cycles were excluded as there was no embryo observation on day 3, this may get rid of some poor condition patients and increase the total clinical pregnancy rate and live birth rate in both the study group and the control group. Besides, according to our routine protocol, day 5 embryo transfers were rarely carried, and those cycles were also excluded in this study, this may exclude those embryos that stop cleavage from day 3 to day 4 or from day 4 to day 5 after insemination.

In conclusion, this study firstly reported that the presence of an arrested embryo on day 3 is associated with the decrease of numbers and proportions of high quality embryo and available embryo, and this may caused by embryo post-meiosis abnormality. A lower pregnancy rate and implantation rate in the arrested group may caused by poorer transferred embryo quality, for cycles transferred matched-cohort embryos, there was no significant difference in pregnancy rate and implantation rate between the arrested group and control group. It has been reported that embryo development arrest rate is independent of any of known variables like patient age [8], the causes of embryo development arrest are complex and remain unclear, with the development of molecular biology, many candidate genes related to the embryo arrest has been studied [26], and several genes related to the embryo genome activation have been identified, like CENPF (Centromere protein F) [48], Zar 1 (Zygote arrest *1*) [49], and Mater (Maternal effect, Nalp5) [50]. However, in the other hand, active embryo development arrest can drive incompetent embryos out of further development and is positive for species conservation [29, 51].

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Disclosure of conflict of interest

None.

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