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Effect of sequential versus single-step culture medium on IVF treatments, including embryo and clinical outcomes: a prospective randomized study

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Abstract

Purpose Sequential media G5 series (Vitrolife) and single-step medium Continuous Single Culture Complete (CSC-C) (Irvine Scientific) are two different culture media. We want to examine difference between culturing effects of the two media.

Methods To compare the fertilization and early embryo development, a prospective randomized controlled trial with sibling oocytes in infertile patients, aged ≤ 45 years with ≥ 8 oocytes (226 cycles) was conducted. Each half of the retrieved oocytes from the same patient were randomly allocated to two culture media separately. The remaining fresh cycles were randomly assigned to two culture media during the same period (179 cycles). We compared the clinical outcomes based on the total fresh ET cycles in this periods, in which the transferred embryos were only from one culture medium.

Results Embryo outcomes: 226 cycles, included 176 IVF and 50 ICSI cycles, were analyzed, which correspond to 3518 inseminated or micro-injected oocytes. Clinical outcomes: 71 (CSC-C) and 71 (G5 series) fresh ET cycles were compared. There were no significant differences in clinical outcomes and general fertilization rate. However, the fertilization rate was superior in the CSC-C when compared with G5 in ICSI cycles (76.51% vs. 67.25%, $P = 0.008$). In addition, the compacted embryo development rate was significantly higher in CSC-C on day 3. The cycles that had compacted embryos on day 3 demonstrated better outcomes both in embryos as well as clinically.

Conclusions CSC-C had higher fertilization rates than G5 series in ICSI cycles. In addition, the compaction rates of day 3 embryos were significantly higher in CSC-C.

Keywords Embryo culture · Culture media · Embryo quality · Single-step · Sequential · Embryo compaction

Introduction

Embryo culture is essential for in vitro fertilization–embryo transfer (IVF-ET) therapy, and is closely related to the outcome of IVF treatment [1]. Embryo culture medium has close association with embryo and long contact time during the first 6 days of embryonic development. Today, there are many commercial culture media to select, and each has a different composition but all are based on two-design concepts: sequential culture or single-step culture. The former concept is based on "back to nature", and is designed according to the needs of different developmental stages of the embryo for nutrients before implantation. From day 0 to day 3, cleavage medium is used and moved the embryos to blastocyst medium on day 3 and maintained until day 5/6 [2–4]. The latter medium followed the principle of "let the embryo choose", wherein the nutrition needed is included in a single-step medium. The embryos were maintained in the same culture environment from days 0 to 5/6 without any abrupt change [5, 6]. Although the design theories of the two media are different, both of them are available in clinics, and which one is better has always been the research focus. Several studies have shown that fertilization and blastocyst

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formation rates are similar in both the culture media [7, 8], but other researchers have suggested that single-step media has increased blastocyst formation and top-quality blastocyst rate when compared with sequential media, while there is no difference in the clinical pregnancy, early miscarriage and sustained implantation rates [9–11].

The clinical outcomes after assisted reproductive technology (ART) might be attributed to many patient-related factors: subfertility, ovulation stimulation, hormone levels, maternal body mass index (BMI), smoking and alcohol consumption, or reproductive technologies itself [12, 13]. Different components in different culture medium can induce different gene expressions and DNA methylation patterns in various animal models [14, 15] as well as in human embryos [16, 17]. Therefore, there is no wonder that the culture system, which is the key part of ART, affects the clinical outcomes. Some studies have compared the effects of different commercial media for culturing [10], but there are many commercialized brands of culture medium, and CSC-C and G5 series are the two widely used media in single-step and sequential media. Hence, in this study, we aimed to know if there were any differences in the embryo and clinical outcomes between single-step medium CSC-C and sequential medium G5 series.

Materials and methods

Study design

This is a prospective cohort study conducted at the Reproductive Center of The First Affiliated Hospital of Xiamen University. The Ethics Committee of the First Affiliated Hospital of Xiamen University has approved this investigation. The patients who underwent only one fresh IVF or intracytoplasmic sperm injection (ICSI) cycle from March 2017 to November 2018 were enrolled. The study was designed into two parts, the outcomes of embryo quality were based on the first part, the patient's age ≤ 45 years with at least 8 follicles larger than 14 mm in diameter on the day of rhCG injection and retrieved oocytes number ≥ 8 were recruited in this part. The sibling oocytes of every recruited patient were randomly assigned to two different media (G5 series and CSC-C) ($n = 226$). The remaining cycles in the period of April 2017 to July 2017 were randomly assigned to the two culture medium (CSC-C: $n = 88$; G5 series: $n = 91$). The clinical outcomes were based on the total fresh ET cycles in this periods, in which the transferred embryos were only from G5 series medium or CSC-C medium (CSC-C: $n = 71$; G5 series: $n = 71$). Fresh ET cycles with ET embryos from both two culture media were excluded. The single-step medium culture used Continuous Single Culture Complete (CSC-C) (Irvine Scientific) during the whole culturing process with day 3 renewal, while the sequential media culture used G5 series (Vitrolife) and included G1™ plus and G2™ plus. G1™ plus was used till day 3 and ET was done using G2™ plus from day 3 until days 5 or 6. Except the culture media, the procedures, culture environment, and operators of the clinic are kept constant during the examined period.

About embryo outcomes, 226 cycles, with a total of 3518 sibling oocytes were recruited, and the data of fertilization, top-quality embryo, blastocyst formation and other indicators related to embryo quality of the two media were compared. About clinical outcomes, the clinical data after fresh ET with embryos from G5 series culture media ($n = 71$) were compared with paired data obtained from CSC-C culture media ($n = 71$).

Ovarian Stimulation for fresh IVF/ICSI cycles

Gonadotropin releasing hormone agonist (GnRH-a) (Diphereline, Ipsen, France) was injected on days 21–23 of previous menstrual cycle using long-acting protocol, and after reaching the downregulation standard, the recombinant human follicle-stimulating hormone (rFSH) (Gonal-f, Merck Serono, Germany) and/or human menopausal gonadotropin (hMG) (Lizhu group, Zhuhai, China) were given as appropriate to promote ovulation. The growth of the follicles was monitored by transvaginal ultrasound. When the diameter of at least 4 follicles was more than 17 mm or 2 follicles was more than 18 mm, 250 μg recombinant human chorionic gonadotropin (rhCG) (Merck Serono, Switzerland) was injected, and the oocyte retrieval was performed after 36–38 h. Progesterone (Xianju, Zhejiang, China) was used for luteal support.

Laboratory procedure

After been retrieved, the cumulus–oocyte complexes (COCs) were collected and washed in 1 mL G-MOPS plus medium (Vitrolife), and then the two culture medium systems were used. G5 series sub-group used G-IVF plus, G1™ plus and G2™ plus in different culture period, while CSC-C sub-group used only CSC-C the single-step media during the whole culture procedure. The COCs cultured in G5 series sub-group were transferred to a 1 mL G-IVF plus (Vitrolife), and those in CSC-C sub-group were transferred to 1 mL CSC-C (Irvine) randomly. COCs were cultured in an incubator for 2–3 h at 37 °C in an atmosphere of 6% CO_2 until insemination procedure. IVF or ICSI was decided according to the patient's condition. Density gradient centrifugation (Isolate, Irvine Scientific, USA) was used to prepare the sperms. The motile spermatozoa density for short co-incubation of gametes IVF was 300,000 per well (1 mL) and 200,000 per well (1 mL) due to long co-incubation of gametes IVF. ICSI was performed 3–6 h after oocyte

retrieval. After insemination, the embryos cultured in G-IVF plus (Vitrolife) were transferred into the culture dishes with 30 µL droplets of G1™ plus (Vitrolife) covered by mineral oil (Virolife) and those cultured in CSC-C (Irvine) were transferred into the culture dishes with 30 µl droplets of new CSC-C media covered by mineral oil (Irvine). The media renewal was performed on day 3 in both groups, in which G1™ plus was replaced by G2™ plus and CSC-C was replaced by new balanced CSC-C.

Embryo assessment

After 16–20 h (day 1) of insemination, fertilization was observed under an inverted microscope, and the number of pronucleus (PN) was recorded, wherein 2PN indicates normal fertilization, and 1PN and ≥ 3PN indicates abnormal fertilization. The embryo morphology was scored based on cell number, fragmentation and symmetry [18]. Cleavage-stage assessment (day 3): (1) top embryo: D1 was 2PN, with cell number ≥ 6 (including compacted embryo), blastomere size was even or slightly uneven, morphology was regular or slightly irregular, no fragments or < 10%; (2) available embryo: day 1 was 2PN, with cell number ≥ 6 (including compacted embryo), and cell fragments < 40%. Blastocyst-stage assessment (day 5/6) is based on Gardner's scoring standard [19]. Good quality blastocysts include (1) expanding blastocysts (stage 3) with A or B score for inner cell mass and trophectoderm; (2) expanded blastocysts (stage 4), hatching blastocysts (stage 5) and fully hatched blastocysts (stage 6) with A or B score for trophectoderm.

Embryo transfer and Outcome measures

One or two best quality embryos were transferred in both groups on days 3 or 5 after oocyte retrieval. The number of ET embryos was determined based on age, endometrial thickness, progesterone levels, outcome of previous IVF cycles if the patient underwent and embryo quality.

Clinical pregnancy: 28–30 days after ET, B-ultrasound showed embryo bud and fetal heart activity. Implantation rate = number of fetal hearts/number of transferred embryos. Abortion rate = number of abortions women/number of pregnancies women. Live birth rate = number of deliveries with live births/number of ET cycles.

Statistical analysis

SPSS 20.0 software was used to analyze the data. Continuous variables were expressed as means ± SD. One-sample K–S test was used to test the normality, the Student's *t* test was used if the normal distribution was satisfied, and Wilcoxon rank sum test was used if it was not. Percentage (%) was used for categorical variables, and chi square test was used to compare the differences among groups. For the clinical outcomes of two culture media, including clinical pregnancy rate, abortion rate and live birth rate, logistic regression were used to adjust the baseline characteristics between the two groups. Statistical significance was set at $P < 0.05$.

Results

Laboratory outcomes

In the enrolled 226 cycles, a total of 3518 COCs were retrieved and randomly allocated to either sequential media: G5 series (Vitrolife) or single-step media: CSC-C (Irvine Scientific). Of these, 176 included IVF cycles and 50 included ICSI cycles. Table 1 presents the details of fundamental maternal characteristics of cycles for embryo outcomes.

A total of 1760 vs. 1758 COCs were assigned in G5 series and CSC-C culture media, respectively. The mature oocyte rate and fertilization rate were similar in both the culture media. The cleavage rate and good quality embryo rate on day 3 embryo quality outcomes showed no significant differences between the two culture media, but the embryo compaction rate of day 3 embryos was significantly higher in CSC-C than in G5 series (13.47% vs. 2.13%, $P < 0.001$) (Table 2). There were 76 cycles in CSC-C culture medium with at least one compacted embryo, which included 33.63% of the total cycles. Only 15 cycles (6.63% of the total cycles) in G5 series showed embryo compaction on day 3. The blastocyst development rate (63.66% vs. 60.18%, $P = 0.177$) and

Table 1 Maternal characteristics of cycles for embryo outcomes

Parameters	Value
Number of patients (*n.*)	226
Maternal age (years)	30.05 ± 3.94
Infertility years	3.52 ± 3.87
Body mass index (kg/m^2)	21.64 ± 3.38
Basal FSH (mIU/ml)	6.45 ± 2.18
Basal LH (mIU/ml)	5.61 ± 3.81
Basal E2 (pg/ml)	49.63 ± 22.30
Antral follicle count	17.00 ± 7.73
Main infertility factor (%)	
Tubal factor	43.81 (99/226)
Endometriosis	8.41 (19/226)
Polycystic ovarian syndrome	18.14 (41/226)
Male factor	19.47 (44/226)
Total dose of GN (IU)	2059.92 ± 528.13
Number of oocytes retrieved	15.81 ± 4.98
IVF cycles (*n.*)	176
ICSI cycles (*n.*)	50

Table 2 Embryo quality of sibling oocytes in two different culture media

Parameters	G5 series	CSCC	*P* value
No. of oocytes (*n*.)	1760	1758	
Mature oocytes rate (%)	85.23(1500/1760)	84.19(1480/1758)	0.399
Normal fertilization rate/oocytes (%)	64.43(1134/1760)	65.42(1150/1758)	0.697
Normal fertilization rate/mature oocytes (%)	75.60(1134/1500)	77.70(1150/1480)	0.548
Polyploid (> 2PN)/mature oocytes (%)	5.60(84/1500)	5.40(80/1480)	0.872
Cleavage stage			
Cleavage rate (%)	99.29(1126/1134)	98.78(1136/1150)	0.284
Good quality embryos rate (%)	53.20(599/1126)	50.62(575/1136)	0.223
Available embryos rate (%)	69.63(784/1126)	68.57(779/1136)	0.271
Day 3 compacted embryo development rate (%)	2.13(24/1126)	13.47(153/1136)	< 0.001
Blastocyst stage			
Blastocyst development rate (%)	63.66(424/666)	60.18(476/791)	0.177
Good blastocyst rate (%)	42.49(283/666)	38.56(305/791)	0.134
Day 5 Good blastocyst rate (%)	18.17(121/666)	17.83(141/791)	0.891
Day 6 Good blastocyst rate (%)	24.32(162/666)	20.73(164/791)	0.115

good blastocyst rate (41.89% vs. 38.56%, $P=0.134$) of G5 series and CSC-C showed no significant differences. The good blastocyst rates on day 5 and day 6, respectively, were 18.17% (G5), 17.83% (CSC-C) and 24.32% (G5) and 20.73% (CSC-C). This rather slow development may be due to day 3 transfer strategy of our center, meaning that the top quality embryos (one or two at least) were preferentially transferred or frozen on day 3. Only the remaining embryos were cultured to the blastocyst stage, hence the samples tend to be poorer embryos (Table 2).

The embryo quality of 176 IVF cycles and 50 ICSI cycles were analyzed separately. The results showed that the fertilization and normal fertilization of ICSI cycles ($n=50$) in CSC-C were higher than in G5 series (76.51% vs. 67.25%, $P=0.008$; 71.99% vs. 62.60%, $P=0.011$, respectively). The embryo compaction rate was significantly different in IVF and ICSI cycles of CSC-C medium and G5 series media (IVF 13.84% vs. 2.40%, $P<0.001$; ICSI: 12.02% vs. 0.93%, $P<0.001$, respectively), and the other embryo quality outcomes were similar in both the culture media (Table 3).

To find out the effects of embryo compaction, the cycles that developed compacted embryo on day 3 were compared with those that did not. The embryo outcomes were based on 226 cycles, and there were 76 and 15 compacted embryo cycles from CSC-C and G5 series media, respectively. The fertilization rate, good quality embryo rate, available embryo rate and blastocyst development rate were significantly higher in these cycles with compacted embryo when compared with non-compacted embryo cycles in both culture systems. Of the total 226 cycles in the sibling study, in 109 cycles (42 cycles with and 67 cycles without compacted embryos) a fresh ET was performed and these cycles were analysed for clinical outcome. Compacted embryo cycles had better outcomes at the clinical pregnancy rate and implantation rate and abortion rate though the differences were not significant. In addition, the live birth rate was

Table 3 Embryo quality of sibling oocytes in IVF and ICSI cycles of two different culture media

Parameters	IVF ($n=176$)		*P* value	ICSI ($n=50$)		*P* value
	G5 series	CSC		G5 series	CSC	
No. of oocytes (*n*.)	1358	1363		402	395	
Fertilization rate (%)	74.89 (1017/1358)	74.76 (1019/1363)	0.965	67.25 (232/345)	76.51 (254/332)	0.008
Normal fertilization rate (%)	67.60 (918/1358)	66.84 (911/1363)	0.683	62.60 (216/345)	71.99 (239/332)	0.011
Polyploid (> 2PN) (%)	5.45(74/1358)	5.43(74/1363)	> 0.99	2.90 (10/345)	1.81 (6/332)	0.450
Cleavage rate (%)	99.24 (911/918)	99.12 (903/911)	0.802	99.54 (215/216)	97.49 (233/239)	0.125
Good quality embryos rate (%)	53.02 (483/911)	51.94 (469/903)	0.481	53.95 (116/215)	45.49 (106/233)	0.089
Available embryos rate (%)	69.26 (631/911)	69.77 (630/903)	0.838	71.16 (153/215)	63.95 (149/233)	0.108
Day 3 compacted embryo development rate (%)	2.40 (22/911)	13.84 (125/903)	< 0.001	0.93 (2/215)	12.02 (28/233)	< 0.001

significantly higher in compacted embryo cycles (57.14% vs. 35.82, $P=0.047$) (Table 4).

Clinical outcomes

In the 109 fresh ET cycles of the 226 cycles, 26 cycles that transferred embryos from both culture media were excluded, in the remaining 83 fresh ET cycles, 39 cycles were from CSC-C and 44 were from G5 series media. In the part cultured separately, there were 88 and 91 cycles from CSC-C and G5 series media, respectively; 32 and 27 cycles were fresh transferred from each culture medium. Taken together this results in 71 cycles' transferred embryos from G5 series media and 71 cycles' from CSC-C medium. The characteristics of fresh ET cycles and clinical outcomes are described in Table 5. The Endometriosis (2.82% vs. 14.08%, $P=0.031$) and PCOS (25.35% vs. 9.86%, $P=0.026$) rate in infertility factor had significant differences between G5 series and CSC-C culture media, respectively. There were 28 and 32 patients who kept the ongoing pregnancy in G5 series and CSC-C media separately. However, one pregnant patient died in week 8 due to unknown reasons and one patient had an ectopic pregnancy. One patient delivered a stillbirth at week 36 in CSC-C group. The clinical pregnancy rate, implantation rate, live birth rate and abortion rate showed no significant differences between G5 series and CSC-C media (Table 5).

Discussion

The present study demonstrated that the fertilization rate of ICSI cycles and the embryo compaction rate of day 3 were significantly higher in the single-step medium (CSC-C, Irvine Scientific) than in the sequential medium (G5 series, Vitrolife). The pregnancy and live birth rates showed no differences between these two culture media.

A series of genome-scale epigenetic transitions occur during embryo culture in vitro. DNA methylation is much more dynamic in this stage, and any epigenetic alterations may lead to irreversible consequences [20, 21]. The culture medium plays an important role in this process. Animal studies have found that embryo culture and transfer can cause aberrant expression of imprinted genes [22, 23], influencing the phenotype of offspring [24]. At present, commercial media are mainly divided into two categories: sequential and single step. There are several brands of media, but the composition varies. These differences might have some potential effects on embryo culture [25]. No difference was found in the total fertilization rate between CSC-C and G5 series,

Table 4 Embryo and clinical outcomes of cycles with or without compacted embryo

Parameters	G5 series		*P* value	CSCC		*P* value
	Compacted embryo cycles	Non-compacted embryo cycles		Compacted embryo cycles	Non-compacted embryo cycles	
Embryo outcomes						
No. of cycles (n.)	15	211		76	150	
Normal fertilization rate/oocytes (%)	70.90(95/134)	63.90(1039/1626)	0.111	73.71(443/601)	61.11(707/1157)	< 0.001
Normal fertilization rate/mature oocytes (%)	85.59(95/111)	74.73(1039/1389)	0.011	83.11(443/533)	74.66(707/947)	< 0.001
Polyploid (> 2PN)/mature oocytes (%)	2.7(3/111)	5.83(81/1389)	0.201	5.82(31/533)	5.17(49/947)	0.633
Cleavage rate (%)	100(95/95)	99.23(1031/1039)	> 0.99	99.32(440/443)	98.44(696/707)	0.270
Good quality embryos rate (%)	76.84(73/95)	51.01(526/1031)	< 0.001	68.64(302/440)	39.22(273/696)	< 0.001
Available embryos rate (%)	84.21(80/95)	68.28(704/1031)	0.001	79.77(351/440)	64.17(428/696)	< 0.001
Blastocyst development rate (%)	80(56/70)	61.74(368/596)	0.003	69.25(259/374)	52.04(217/417)	< 0.001
Available blastocyst rate (%)	52.86(37/70)	40.60(242/596)	0.049	45.99(172/374)	31.89(133/417)	< 0.001

Clinical outcomes	Compacted embryo cycles	Non-compacted embryo cycles	*P* value
No. of ET cycles (n.)	42	67	
Clinical pregnancy rate (%)	61.90(26/42)	44.78(30/67)	0.115
Implantation rate (%)	50(30/60)	35.85(38/106)	0.100
Abortion rate (%)	7.69(2/26)	16.67(5/30)	0.431
Live birth rate (%)	57.14(24/42)	35.82(24/67)	0.047

Table 5 Cycle characteristics and clinical outcomes after fresh ET of two culture media

Parameters	G5 series	CSCC	*P* value
No. of ET cycles (*n*.)	71	71	
Maternal age (years)	29.42 ± 3.72	30.32 ± 4.41	0.191
Infertility years	3.20 ± 2.03	4.02 ± 3.47	0.085
Body mass index (kg/m^2)	21.54 ± 2.93	22.08 ± 2.94	0.273
Basal FSH (mIU/ml)	6.74 ± 2.58	6.64 ± 1.99	0.803
Basal LH (mIU/ml)	5.26 ± 3.32	5.08 ± 2.65	0.718
Basal E2 (pg/ml)	48.04 ± 22.96	51.07 ± 27.05	0.473
AFC	15.08 ± 6.98	14.42 ± 8.02	0.601
Total dose of GN (IU)	2090.67 ± 592.69	2134.46 ± 643.49	0.674
No. of oocytes retrieved	12.30 ± 5.36	11.69 ± 6.22	0.535
No. of transferred embryos	1.52 ± 0.50	1.42 ± 0.49	0.242
Type of infertility			0.393
Primary infertility	63.38 (45/71)	54.93 (39/71)	
Secondary infertility	36.62 (26/71)	45.07 (32/71)	
Fertilization			0.841
IVF	76.05 (54/71)	78.87 (56/71)	
ICSI	23.95 (17/71)	21.13 (15/71)	
Tubal factor	64.79 (46/71)	76.06 (54/71)	0.198
Endometriosis	2.82 (2/71)	14.08 (10/71)	0.031
PCOS	25.35 (18/71)	9.86 (7/71)	0.026
Stage of transferred embryo			0.298
Cleavage	67.61 (48/71)	57.75 (41/71)	
Blastocyst	32.39 (23/71)	42.25 (30/71)	
At least one good quality embryo	90.14 (64/71)	92.96 (66/71)	0.764
Outcomes			
Clinical pregnancy rate	45.07 (32/71)	53.52 (38/71)	0.401
Abortion rate	12.50 (4/32)	15.79 (6/38)	0.745
Live birth	36.62 (26/71)	43.66 (31/71)	0.494
		Adjusted OR (95%CI)	Adjusted *P* value
Clinical pregnancy rate	Ref	1.82 [0.80–4.14]	0.155
Abortion rate	Ref	1.77 [0.32–9.98]	0.516
Live birth	Ref	1.99 [0.85–4.66]	0.111

confirming the results of previous studies [26, 27]. However, when comparing only ICSI cycles, the results showed that CSC-C had a higher fertilization rate compared with G5 series. In addition, previous studies did not investigate this part of the data. In the present study, the embryos in the two medium groups were from the same patient, and the laboratory procedure was exactly the same. After completing the ICSI operation, the embryos were randomly assigned to two media for cultivation, and the culture environment also remained the same to exclude other influencing factors. However, the fertilization rate was different only in ICSI, probably because oocytes were mechanically stimulated during the injection process of ICSI procedure. The penetration of the needle and the aspiration of the cytoplasm by negative pressure had an impact on oocytes. The sibling zygotes after ICSI showed different rates of development in four different media [28], implying that the culture media had effects on the recovery of stimulated oocytes. Fertilization and recovery processes need energy, and the difference in energy-containing substances of the two media might be responsible for this result. The single-step medium contains pyruvate, glucose and lactate during the whole culture process but the sequential medium contains a very low glucose concentration during the cleavage stage. According to the previous reports, the fluid in the fallopian tube and uterine had different contents of lactate and glucose [29, 30]. This also formed the basis for the design of the sequential medium. However, previous data were based on the analysis of samples collected from non-pregnant women, and the actual situation of the luteal phase still remains unknown.

The embryo compaction rate was significantly higher in the CSC-C medium than in the G5 series on day 3. The embryo qualities (including the fertilization rate, day 3 good embryo rate and blastocyst development rate) were

better in the compacted embryo cycles than in the non-compacted embryo cycles. In addition, the live birth rate significantly increased when the cycle contained compacted embryos. Some previous studies showed that compaction was strongly associated with implantation potential and ET-compacted embryos increased the implantation rates of IVF treatments [31, 32]. Embryos cultured in the single-step medium group had more cells on days 2 and 3 than those cultured in the sequential medium [26]. According to a study on murine embryo, the single-step medium showed an increased growth rate, and had more hatching embryos compared with the sequential medium on day 3 [33]. ET-compacted embryos increased the success rate of IVF treatments. In another study that used only the single-step medium (global medium) for culturing, the embryo compaction rate was 17.8% (91/509) on day 3, which was even higher than the rate in the present study (13.47%; cultured in CSC-C, Irvine), proving that early embryo compaction happened more frequently in the single-step medium. Compacted embryos on day 3 indicated not only better developmental potential but also a better clinical outcome. The study presented accurate data in this aspect on two kinds of culture media, which was not demonstrated earlier.

Compaction is the first identifiable morphogenetic process during mammalian embryogenesis; it is considered critical for the divergence of cell lineages and subsequent development, such as blastocyst formation [22, 34]. In the sequential medium, pyruvate and non-essential amino acids are the energy-containing substances in the cleavage embryo stage, which has almost no glucose or very little glucose; glucose and essential amino acids are added in the blastocyst stage. However, the single-step medium contains all nutrients needed for embryonic development from the cleavage to the blastocyst stage. Compaction represents the first transportation of the epithelium of the conceptus [35]; near the process of compaction, both glucose uptake and utilization by embryos increase [2]. Sequential medium artificially divides the culture of embryos into two periods, but in the natural state, the development of embryos may not be so accurate. For faster-developing embryos, the single-step medium provides nutrients for the whole in vitro developmental stage, and hence compaction can appear earlier. The supply of nutrients may be responsible for the differences in embryonic development in both culture media. In addition, this might be the reason as to why the blastocyst formation rate in the single-step medium was higher in some studies [36]. This impact seemed to be limited, and on the whole, no significant difference in embryo quality was found between the two media on days 3 and 5. Delayed specific nutrition supply for a specific period seems to have little effect on the developmental potential of the embryos. This was confirmed by subsequent clinical outcomes. The clinical pregnancy rate and implantation rate were not different between the two media, and this result was consistent with previous findings [8, 9]. However, it might also be because of the small sample size, which led to smaller differences that could not be detected. This was one of the limitations of present study.

In conclusion, this prospective study revealed that the culture medium could affect the fertilization rate of zygotes after ICSI, and both culture media were equally effective for in vitro culturing of embryos. The findings were consistent with pre-existing data; early embryonic compaction appeared more frequently in the single-step medium. Compaction was associated with better developmental potential and live birth rate. This phenomenon might provide more clues regarding when the transfer of embryos was chosen. Further larger sample size randomized experiments are warranted to confirm this conclusion.

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Declarations

Conflict of interest The authors declare that they have no competing interests.

Ethics approval and consent to participate This study was approved by the Ethics Committee of First Affiliated Hospital of Xiamen University.

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