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ASSISTED REPRODUCTION TECHNOLOGIES

Does IVF cleavage stage embryo quality affect pregnancy complications and neonatal outcomes in singleton gestations after double embryo transfers?

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Abstract
Purposes Embryo quality is associated with successful implantation and live births. Our retrospective study was carried out to determine whether or not cleavage stage embryo quality affects the miscarriage rate, pregnancy complications and neonatal outcomes of singletons conceived with assisted reproduction technology.
Method The current study included 11,721 In Vitro Fertilization-Embryo Transfer cycles (IVF-ET) between January 2009 (the date at which electronic medical records were implemented at our center) and March 2013. Only women< 40 years of age undergoing their first fresh embryo transfer cycle using non-donor oocytes were included.
Results Our study indicated that the transfer of poor-quality embryos resulted in higher miscarriage (19.77 % vs. 13.28 %, p=0.02) and lower ongoing pregnancy rates (15.33 % vs. 48.06 %, p<0.001). Logistic regression analysis performed on data derived from 744 cycles culminating in miscarriages versus 4,333 cycles culminating in live births, suggested that embryo quality (p=0.04) is significantly associated with miscarriage rate after adjusting for other confounding factors. Moreover, there were no differences in the mean birth weight, low birth weight (<2,500 g), very low birth weight (<1,500 g), gestational age, preterm delivery (<37 weeks), very preterm delivery (<32 weeks), congenital malformations, small-for-gestational-age singletons (SGA), and large-for-gestational-age singleton (LGA) rate (p>0.05). Similarly, pregnancy complications resulting from poor-quality embryos were not different from good-quality embryos (4.04 % vs. 2.57 %, p= 0.33). Finally, logistic regression suggested that embryo quality was not significantly associated with pregnancy complications after adjusting for other confounding factors (p=0.40).
Conclusions Our study suggests that transfer of poor-quality embryos did not increase the risk of adverse outcomes; however, the quality of cleavage stage embryos significantly affected the miscarriage rate and ongoing pregnancies.

Keywords Embryo quality · Pregnancy complications · Miscarriage · Singleton gestations · IVF/ICSI

Capsule The current study describes association between cleavage stage embryo quality and miscarriage based on 11721 IVF-ET cycles. However, embryo quality is not associated with obstetric and perinatal outcomes, transfer of poor-quality embryos is not responsible for a higher percentage of congenital malformations.

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Introduction

Embryo quality, as based on morphologic parameters (blastomere number and fragments) is the main predictor of successful implantation and live births in fresh cycles [1–3]. Top quality cleavage stage embryo at freezing, thawing, or transfer improves the likelihood of a live birth in frozen–thawed cycles [4]. It is well-known that adverse obstetric and perinatal outcomes of singletons and twins following IVF, such as low birth weight (LBW), preterm delivery (PTD) and congenital malformations, are more common than spontaneous conception [5–15]. Two studies have shown that singletons born from

frozen embryo transfer have better, or at least equivalent obstetric and perinatal outcomes compared with fresh embryo transfer [16, 17]. Less adverse outcomes in frozen cycles maybe associated with selective cryopreservation of high-quality embryos; however, a recent report from Pinborg et al. [18] has demonstrated an increased risk of large-for-gestational-age (LGA) newborns conceived after frozen transfer compared to fresh and natural conceptions. Knowledge regarding the relationship between embryo quality and neonatal outcome and congenital malformations is incomplete. Ebner et al. [19] reported that bad quality embryo is associated with a high percentage of congenital malformations based on 164 pregnancies. A recent study demonstrated that miscarriage and ongoing pregnancy rate, congenital malformations, pregnancy complications, and neonatal outcomes of singletons were comparable between the good and poor quality embryo groups [20].

With the conflicting views on this topic, the aim of the current study was to determine whether or not cleavage stage embryo quality is associated with miscarriage rate, SGA, LGA, congenital malformations of live singletons and pregnancy complications with a large, single-center dataset.

Materials and methods

Participant, stimulation protocol, and embryo culture

The current study included 11,721 IVF-ET fresh cycles performed at the Center for Reproductive Medicine between January 2009 (the start of electronic medical record use at our center) and March 2013. Only women<40 years of age undergoing their first fresh embryo transfer cycle using non-donor oocytes were included. Only cycles with cleavage stage and double embryo transfers were included. Patients who received a pre-implantation genetic diagnosis and cycles with donor sperm were excluded.

Women underwent controlled ovarian hyperstimulation with a GnRH agonist or antagonist protocol. Ovarian follicle development was monitored based on serum estradiol (E2) levels and transvaginal ultrasonographic measurements. When at least one follicle reached a mean diameter of 18 mm and the E2 concentration was>500 pg/ml, 10,000 units of urinary hCG (Serono, Aubonne, Switzerland) were administered before ultrasonography-guided oocyte retrieval. Luteal support was initiated on the day after oocyte retrieval using 60 mg of progesterone (Xianju Pharmacy, Zhejiang, China).

The main causes of infertility included male infertility, polycystic ovarian syndrome (PCOS), endometriosis, poor ovarian reserve, tubal factor infertility, unexplained infertility, or a combination of the aforementioned infertility factors.

IVF and ICSI were performed according to semen quality on the day of oocyte retrieval. The presence of two pronuclei and two polar bodies was observed 17–19 h after insemination or injection. Human embryos were cultured in one of the following four commercially available culture mediaand 20 % oxygen: G5™ (Vitrolife, Gothenburg, Sweden); Global (IVF Online, Toronto, Canada); Quinn's advantage medium (SAGE, Pasadena, CA, USA); and G5™ PLUS (Vitrolife). The corresponding protein sources used to supplement the media were as follows: HSA-solution™ (Vitrolife); HSAsolution (IVF Online); and Quinn's advantage SPS (SAGE). G5™ PLUS was ready-to-use from the supplier and included 5 mg/ml of HSA. In our center, one kind of medium was generally used for 3 consecutive days, then changed to another medium. Quinn's advantage medium was discontinued in July 2012; G5™ PLUS was used from January 2011 in our center. Embryo morphology was evaluated 68–72 h after insemination with respect to cell number and fragmentation.

Embryo quality

We defined the cleavage stage embryo as good quality if the embryo had seven or eight cells on day 3, contained<10 % anucleated fragments. The embryo was defined as poor or fair quality if the embryo had≤5 cells on day 3 and/or 30–50 % fragments. Cleavage grading was performed by three trained embryologists. In the good-quality embryo group all of transferred two embryos met the criteria for good quality. Similarly, all of transferred two embryos met the criteria for poor or fair quality in the poor-quality embryo group.

Clinical outcomes

Clinical outcomes were categorized as biochemical or clinical pregnancies, with the latter including ectopic pregnancies, miscarriages, ongoing pregnancies, stillbirths, and live births. Pregnancy complications, including pre-eclampsia, gestational diabetes, placenta previa, placenta abruption, premature rupture of fetal membranes, vaginal bleeding, and severe anemia, were assessed. Adverse neonatal outcomes included LBW (<2,500 g), very low birthweight (VLBW; <1,500 g), PTD (<37 weeks), very preterm delivery (VPTD; <32 weeks), SGA, LGA, and congenital malformations (neonatal brain injury, congenital heart disease, Down syndrome, hypertrophic pyloric stenosis, icterus hepatitis, or congenital cartilage disease). Calculating the proportion of SGA and LGA, we used the Chinese publication regarding birthweight reference at distinct gestational ages, including standard deviations.

Statistical analysis

All statistical analyses were performed with the Statistical Package for the Social Sciences software (SPSS, version 17.0; SPSS, Inc., Chicago, IL, USA). The basic characteristics of patients were compared using analysis of variance (continuous variables), and categorical variables were evaluated with

chi-squared tests. Logistic regression analyses were used to evaluate the possible relationship between embryo quality and miscarriage rate and pregnancy complications after adjusting for other potential confounding factors, including parental age, parental BMI, type of infertility, parity, cycles with ICSI, main cause of infertility, gestational age, method of delivery, birthweight, infant gender, culture media, and newborn complications. The results of logistic regression indicated independent effects of each factor on the miscarriage rate and pregnancy complications, which ruled out the possibility that other confounding factors influence the miscarriage rate and pregnancy complications.

Results

Of 11,721 fresh cycles that were included in this study, there with 10,625 cycles with transfer of good-quality embryos and 1,096 cycles with transfer of poor- or fair-quality embryos, respectively, yielding 2,487 and 99 live birth singletons, respectively. Because all transfer cycles had two embryos, vanishing twins were excluded in the subsequent analysis of pregnancy complications and neonatal outcomes.

The patients and treatment characteristics are shown in Table 1. The mean maternal age was 1 year younger, the paternal age was 1 year younger, and a greater number of ICSI cycles were used in transfers for the good-quality embryo group ($p<0.001$). Moreover, the two groups differed in treatment characteristics, with a lower total dose of gonadotrophin (2,389.56 IU vs. 3,051.62 IU, $p<0.001$), and more oocytes retrieved in the good-quality embryo group (13.15 vs. 10.62, $p<0.001$). The fertilization rate in the good-quality embryo group was significantly higher than the poor-quality group (67.81 % vs. 42.14 %, $p<0.001$). As many parameters differed between the good-and poor-quality embryo groups, logistic regression was performed to determine factors influencing embryo quality; the results suggested that embryo quality is associated with maternal age ($p<0.001$), maternal BMI ($p<0.001$), dose of gonadotrophins ($p<0.001$), parity ($p<0.001$), number of oocytes retrieved ($p<0.001$), and cycles with ICSI ($p=0.002$; Supplementary Table 1).

Table 2 summarizes the clinical outcomes in the good- and poor-quality embryo groups. The number of clinical pregnancies per transfer in the good-quality embryo group was significantly higher than the poor-quality embryo group (50.26 % vs. 16.15 %, $p<0.001$); the same trend was observed with respect to the live birth rate per transfer between the two groups (39.60 % vs. 11.41 %, $p<0.001$). When pregnancy was achieved, the stillbirth and ectopic pregnancy rates were comparable between the two groups ($p>0.05$), however, the transfer of poor-quality embryos resulted in higher miscarriage (19.77 % vs. 13.28 %, $p=0.02$) and lower ongoing pregnancy rates (15.33 % vs. 48.06 %, $p<0.001$). Most miscarriages occurred in the first trimester; there was a significantly higher percentage of miscarriages in the poor-than good-quality embryo transfer group during the first trimester (15.82 % vs. 9.08 %, $p=0.002$), but the percentage of miscarriages was comparable in the second and third trimesters (3.95 % vs.4.19 %, $p=0.88$).

Logistic regression analysis as shown in Table 3, performed on data derived from 744 cycles with miscarriage versus 4,333 cycles with live birth, suggested that maternal age ($p=0.02$), paternal BMI ($p=0.04$) and embryo quality ($p=0.04$) are significantly associated with miscarriage rate after adjusting for other confounding factors including parental age, parental BMI, basal FSH, parity, causes of infertility, ICSI cycles, and culture media.

Table 4 demonstrates the pregnancy complications and neonatal outcomes of live birth singletons derived from good- or poor-quality embryo transfer. The characteristics of patients who had live birth singletons, including parental age,

Table 1 Patients and treatment characteristics

	Transfer of good quality embryo	Transfer of poor quality embryo	*P* value	OR(95 % C.I)
Cycles	10625	1096	——	
Maternal age	30.26±3.38	31.10±3.89	<0.001	
Paternal age	32.14±4.53	33.45±4.89	<0.001	
Maternal BMI	22.3±3.31	22.23±3.36	0.52	
Cycles with ICSI	4399(41.40 %)	260(23.72 %)	<0.001	
Dose of gonatrophin	2389.56±1089.26	3051.62±1496.57	<0.001	
Number of oocytes retrieved	13.15±5.69	10.62±6.35	<0.001	
Number of fertilized oocytes	8.92±4.34	4.47±3.34	<0.001	
Fertilization rate	94764/139741 (67.81 %)	4904/11637 (42.14 %)	<0.001	0.35(0.33–0.36)

Data are presented as the number (%) or mean±SD. Continuous variables were compared using analysis of variance, and categorical variables were evaluated with chi-squared tests

Table 2 Cycle outcomes resulting from transfer of good or poor quality embryos

	Transfer of good quality embryo	Transfer of poor quality embryo	*P* value	OR(95 % C.I)
Cycles	10625	1096		
Implantation	7164/21250 (33.71)	200/2192 (9.12 %)	<0.001	0.20(0.17–0.23)
Clinical pregnancy	5340(50.26 %)	177(16.15 %)	<0.001	0.19(0.16–0.23)
Multiple pregnancy	1572(14.80 %)	22(2.01 %)	<0.001	0.12(0.08–0.18)
Live birth	4208(39.60 %)	125(11.41 %)	<0.001	0.20(0.16–0.24)
Clinical pregnancy outcome	5340	177	——	
Ongoing pregnancy	5106(48.06 %)	168(15.33 %)	<0.001	0.20(0.17–0.23)
Miscarriage	709(13.28 %)	35(19.77 %)	0.02	1.61(1.10–2.35)
-First trimester	485(9.08 %)	28(15.82 %)	0.002	1.88(1.24–2.85)
-Second or third trimester	224(4.19 %)	7(3.95 %)	0.88	
Stillbirth	9(0.17 %)	1(0.56 %)	0.28	
Ectopic pregnancy	237(4.44 %)	9(5.08 %)	0.68	
Unknown outcome	177(3.31)	7(3.95 %)		

Data are presented as the number (%) or mean±SD. Continuous variables were compared using analysis of variance, and categorical variables were evaluated with chi-squared tests

maternal BMI, type of infertility, and parity, were comparable between the good- and poor-quality embryo groups ($p>0.05$). These two groups differed with respect to ICSI cycles and the main cause of infertility ($p<0.05$). There were no differences in the mean birth weight, LBW, VLBW, gestational age, PTD, VPTD, SGA, LGA and congenital malformations ($p>0.05$).

Table 3 Logistic regression was performed on data derived from 5,077 IVF-ET cycles

	Miscarriage				
	B	S.E.	*P* value	OR	95.0 % CI for OR
Embryo quality	−0.42	0.21	0.04	0.66	0.44–0.99
Maternal age	0.04	0.02	0.02	1.04	1.01–1.07
Paternal age	0.00	0.01	0.81	1.00	0.98–1.03
Maternal BMI	0.00	0.00	0.33	1.00	1.00–1.01
Paternal BMI	−0.02	0.01	0.04	0.98	0.95–1.00
Basal FSH	−0.01	0.01	0.48	0.99	0.98–1.01
Parity	−0.05	0.09	0.55	0.95	0.80–1.13
Cause of infertility			0.10		
Cause of infertility(1)	0.79	0.53	0.14	2.21	0.78–6.30
Cause of infertility(2)	0.96	0.52	0.06	2.62	0.94–7.27
Cause of infertility(3)	1.04	0.53	0.05	2.83	1.00–8.02
Cycles with ICSI	−0.05	0.11	0.67	0.95	0.77–1.18
Culture media			0.82		
Culture media(1)	0.10	0.15	0.50	1.11	0.82–1.50
Culture media(2)	0.09	0.10	0.38	1.10	0.90–1.34
Culture media(3)	0.07	0.12	0.55	1.08	0.85–1.36
Constant	−3.01	0.74	<0.001	0.05	

Beta is the regression coefficient

S.E. is the standard error

Similarly, pregnancy complications resulting from poor-quality embryos were not different from good-quality embryos (4.04 % vs. 2.57 %, $p=0.33$).

Similarly, logistic regression was performed to determine the association between embryo quality and pregnancy complications, including other potential confounding factors (parental age, maternal BMI, type of infertility, parity, ICSI cycles, main cause of infertility, gestational age, method of delivery, birth weight, infant gender, and newborn complications. As shown in Table 5, embryo quality was not significantly associated with pregnancy complications after adjusting for other confounding factors ($p=0.40$).

Discussion

The purpose of the current study was to determine the risk of pregnancy complications and adverse outcomes of singleton gestations related to cleavage stage embryo quality. This retrospective study was a secondary analysis of pregnancy complications and neonatal outcomes in singleton gestations associated with embryo quality. We hypothesize that poor embryo quality is associated with a high risk of miscarriage and pregnancy complications. In agreement with recent findings [20], our study demonstrated no relationship between pregnancy complications and embryo quality. We also did not observe better neonatal outcomes in singleton gestations resulting from good-quality embryos compared with poor-quality embryos. Nevertheless, we have shown for the first time that cleavage stage embryo quality significantly affects miscarriage and on-going pregnancy rates.

Table 4 Live birth singletons delivered from transfer of good or poor quality embryos

	Transfer of good quality embryo	Transfer of poor quality embryo	P value	OR(95 % C.I)
Singletons	2487	99	——	
Maternal age	30.31±3.31	30.74±3.53	0.21	
Paternal age	32.22±4.47	32.22±4.19	0.99	
Maternal BMI	22.56±13.49	21.93±2.73	0.65	
Primary infertility	1421(57.14 %)	53(53.54 %)	0.48	
Primipara	2387(95.98 %)	92(92.93 %)	0.19	
Cycles with ICSI	1062(42.70 %)	30(30.30 %)	0.01	
Cause of infetility			0.00	
-Male	557(22.40 %)	10(10.10 %)		
-Female	1579(63.49 %)	69(69.70 %)		
-Mix	307(12.34 %)	20(20.20 %)		
-Unknown	44(1.77 %)	0		
Gestational age	38.60±1.71 %	38.64±2.18	0.83	
Method of delivery				
Natural birth	409(16.45)	15(15.15 %)	0.73	
Caesarean Section	2078(83.55 %)	84(84.85 %)		
Newborn complications	13(0.52 %)	1(1.01 %)	0.42	1.94(0.25–15.00)
Birthweight	3221.1±530.08	3374±665.42	0.34	
Male newborns	1294(52.03 %)	52(52.53 %)	0.92	
Small for GA	226(9.11 %)	13(13.13 %)	0.21	1.51(0.83–2.75)
Large for GA	364(14.68 %)	19(19.19 %)	0.25	1.39(0.83–2.31)
Low birthweight <2,500 g	88(3.54 %)	4(4.04 %)	0.78	
Very low birthweight <1,500 g	11(0.44 %)	1(1.01 %)	0.38	
Perterm birth <37 weeks	183(7.36 %)	9(9.09 %)	0.52	
Very preterm birth <32 weeks	16(0.64 %)	1(1.01 %)	0.49	
Pregnancy complications	64(2.57 %)	4(4.04 %)	0.33	1.59(0.57–4.47)

Data are presented as the number (%) or mean±SD. Continuous variables were compared using analysis of variance, and categorical variables were evaluated with chi-squared tests

Table 5 Logistic regression was performed on data derived from 2,586 live birth singletons

	Pregnancy complications				
	Beta	S.E.	*P* value	OR	95.0 % C.I.for OR
Embryo quality	0.48	0.56	0.40	1.61	0.54–4.85
Maternal age	0.08	0.05	0.14	1.08	0.98–1.19
Paternal age	0.01	0.04	0.82	1.01	0.94–1.08
Maternal BMI	0.00	0.00	0.59	1.00	0.10–1.00
Type of infertility	0.25	0.28	0.36	1.29	0.75–2.21
Parity	−17.65	3899.68	0.10	0.00	0.000
Cycles with ICSI	0.07	0.35	0.85	1.07	0.54–2.11
Cause of infertility		0.86			
Cause of infertility (1)	0.60	0.85	0.48	1.83	0.34–9.69
Cause of infertility (2)	0.25	0.46	0.59	1.29	0.52–3.18
Cause of infertility (3)	0.07	0.43	0.87	1.08	0.46–2.50
Gestational age	−0.20	0.09	0.03	0.82	0.68–0.98
Method of delivery	0.72	0.48	0.13	2.05	0.80–5.21
Neonatal complications	−0.19	1.22	0.87	0.82	0.08–9.04
Birthweight	0.00	0.00	0.27	1.00	0.10–1.00
Gender	0.26	0.26	0.32	1.30	0.77–2.17
Constant	−15.56	7537.08	0.10	0.00	

Beta is the regression coefficient

S.E. is the standard error

Previous studies have shown that adverse neonatal outcomes of singletons resulting from fresh embryo transfer could be improved by thawed embryo transfer [16, 17]. Better perinatal outcomes could be attributed to synchronization between embryo stage and endometrial receptivity in natural cycles. The other reasonable explanation is that selective cryopreservation of good-quality embryos may be responsible for less adverse neonatal outcomes. However, our results showed that there was no difference in neonatal outcomes of singleton gestations with the good- and poor-quality embryo groups. LBW was observed in 3–4 % of singletons in the current study and this cohort reported 7–9 % PTDs; our results were in agreement with previous findings, in which≤10 % adverse outcomes were reported [21–23]. Although, PTD, VPTD, LBW, VLBW, SGA, LGA and congenital malformations were higher in the poor-quality embryo group, the difference did not reach statistical significance. Likewise, logistic regression indicated that women undergoing poor-quality embryo transfer are not at increased risk for pregnancy complications after adjusting for potential confounding factors. A recent meta-analysis indicated a strong relationship between pregnancy complications and PCOS [24]; several studies support this finding [25, 26]. Therefore, adverse outcomes are related to assisted reproduction technology, but also patient-related factors [27, 28].

It is well-established that transfer of good-quality embryos increases the probability of implantation and live births, both with cleavage and blastocyst embryos [1–3, 29–32]. Knowledge regarding the association between cleavage stage embryo quality and risk of ongoing pregnancy or miscarriage is incomplete. A prior study suggested that trophectoderm grading of blastocysts is correlated with ongoing pregnancy and miscarriages [33]. In contrast, Oron et al. reported that when pregnancy is achieved, there is a similar probability of having a live birth with a poor-quality embryo transfer [20]. The Oron et al. study had a relatively small number of transfer cycles (1,193 cycles with good-quality embryos vs. 348 cycles with poor-quality embryos) and bias of embryo stage, both with cleavage and blastocyst embryos in their cohort. In light of the conflicting views, the current study had a large single-center dataset (10,625 cycles with good-quality embryos vs. 1,096 cycles with poor-quality embryos) and demonstrated that transfer of poor-quality cleavage embryos significantly influences the miscarriage and ongoing pregnancy rates; this effect was not attributed to confounding factors distributed over the good-quality and poor-quality embryo groups. Indeed logistic regression suggested that embryo quality (expressed as cell number and fragmentation) was correlated with risk of miscarriage after adjusting for potential confounding factors.

The main differences between the findings in the current study and the study of Oron et al. [20] were the number of transferred embryos and the quality criteria of cleavage stage embryos. Our study included only double embryo transfers; however, Oron et al. only used single embryo transfers. The quality criteria of embryo classification (percentage of embryo fragmentation) in the current study were slightly different from Oron et al. Moreover, the embryos in our study were cultured in four different culture media in contrast to one medium in the Oron et al. study. However, logistic regression suggested that culture medium, as an independent factor is not associated with the miscarriage rate after adjusting for other confounding factor.

The strength of our study was the large, single-center dataset, in which three embryologists graded cleavage stage embryos, thus reducing intra-observer variability from multiple centers. In contrast, the limitation of the current study was that the number of live birth singletons resulting from transfer of poor-quality embryos was still small. A small singleton cohort has a low power to detect a statistical difference in pregnancy complications and congenital malformations. We cannot intentionally transfer an increased number of poor-quality embryos in clinical practice. Cigarette smoking was not included in current study, as this confounding factor was not available from our database. Nevertheless, our study was used to assure patients that singletons resulting from poor-quality embryos are not at high-risk for adverse neonatal outcomes, and embryo quality was not correlated with pregnancy complications. However, patients should be informed that poor-quality embryos are associated with higher miscarriage and lower on-going pregnancy rates.

In conclusion, transfer of poor-quality embryos did not increase the risk of adverse outcomes, such as pregnancy complications, PTD, LBW, SGA and LGA; Likewise, transfer of poor-quality embryos is not responsible for a higher percentage of congenital malformations; however, the quality of cleavage stage embryos significantly affected miscarriage rate. Despite our findings, we are still far from fully understanding the association between embryo quality and pregnancy complications and congenital malformations. A concern for IVF efficiency, as well as safety for the newborn and pregnant women, should remain a priority in the field of ART.

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Conflicts of interest The authors declare that they have no conflicts of interest.

Ethical statement All human studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All persons gave their informed consent prior to their inclusion in the study.

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