不良胚移植後妊娠の周産期・新生児予後

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Comparative study of obstetric and neonatal outcomes of live births between poor- and good-quality embryo transfers

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Abstract

Purpose: To evaluate the effect of embryo quality on pregnancy outcomes.

Methods: This retrospective analysis included 80 live singleton births, resulting from morphologically good-quality embryo transfers, and 25 live singleton births that resulted from morphologically poor-quality embryo transfers between January, 2008 and December, 2014. Cleavage embryos that were graded as ≥2, according to the Veeck classification system, and blastocysts that were graded as ≥3BB, according to the Gardner classification system, were defined as good quality. The obstetric and neonatal outcomes were compared between the poor- and good-quality embryo transfer groups.

Results: The mean maternal age between the groups was similar. The blastocyst transfer rate was higher in the good-quality, than in the poor-quality, embryo transfer group. Other characteristics, including parity, infertility duration, the intracytoplasmic sperm injection rate, frozen-thawed embryo transfer rate, endometrial thickness, and hormone values before the embryo transfer, were similar between the groups. The obstetric and neonatal outcomes of live births between the two groups were not different in terms of preterm delivery, birthweight, small or large size for gestational age, malformation, umbilical artery cord pH of <7.20, hypertensive disorders of pregnancy, gestational diabetes mellitus, chorioamnionitis, placenta previa, and placental abruption.

Conclusion: The obstetric and neonatal outcomes of live births between the poor- and good-quality embryo transfers were equivalent.

KEYWORDS

assisted reproductive treatment, embryo quality, maternal complication, neonatal outcome, poor-quality embryo

1 | INTRODUCTION

Although the outcomes of assisted reproductive technology have continued to improve, the pregnancy rates in older women and those with diminished ovarian function remain low.[1,2] As a result of poor responses to ovarian stimulation, these women have few or sometimes no oocytes that are suitable for retrieval. In cases when all the obtained embryos are morphologically poor, most

women experience anxiety regarding obstetric and neonatal outcomes of embryo transfer. Thus, at times, the physician could be hesitant to transfer poor-quality embryos. The embryo quality is a major predictor of success of in vitro fertilization (IVF) and studies have shown that clinical pregnancy and live birth rates are lower in cases with poor-quality embryo transfer, than with good-quality embryo transfer.[3,4] However, the association between the embryo quality and the pregnancy outcome remains unclear. Recently, one study reported that the obstetric and neonatal outcomes of live births after poor-quality embryo transfers were equivalent to those after good-quality embryo transfers.[5] In contrast, another study demonstrated that poor-quality embryo transfers resulted in higher miscarriage rates and lower ongoing pregnancy rates after implantation, although the obstetric and neonatal outcomes of live births after poor-quality embryo transfers were equivalent to those after good-quality embryo transfers.[6] However, there have been few studies that have evaluated the association between embryo quality and pregnancy outcome till date. Therefore, the aim of the present study was to evaluate the effect of the embryo quality on the obstetric and neonatal outcomes of live births.

2 | MATERIALS AND METHODS

2.1 | Women, stimulation protocol, oocyte retrieval, and embryo culture

In total, 802 embryo transfers were performed between January, 2008 and February, 2014 at the University of the Ryukyus Hospital (Okinawa, Japan). Of these embryo transfers, 338 were good- and 365 were poor-quality embryo transfers; 99 cases of good- and poor-quality embryo transfers were excluded. Among these embryo transfers, 108 resulted in live singleton births. Among all the live singleton births, complete data were obtained from 80 good- and 25 poor-quality embryo transfers (Figure 1). Twins and singletons without complete data were excluded. The obstetric and neonatal outcomes between the two groups were analyzed.

For IVF, controlled ovarian stimulation, such as the gonadotropin-releasing hormone (GnRH) agonist long or short protocol or the GnRH antagonist protocol, was used for women with a normal ovarian reserve, whereas mild stimulation protocols with clomiphene citrate were used for those women with a poor ovarian

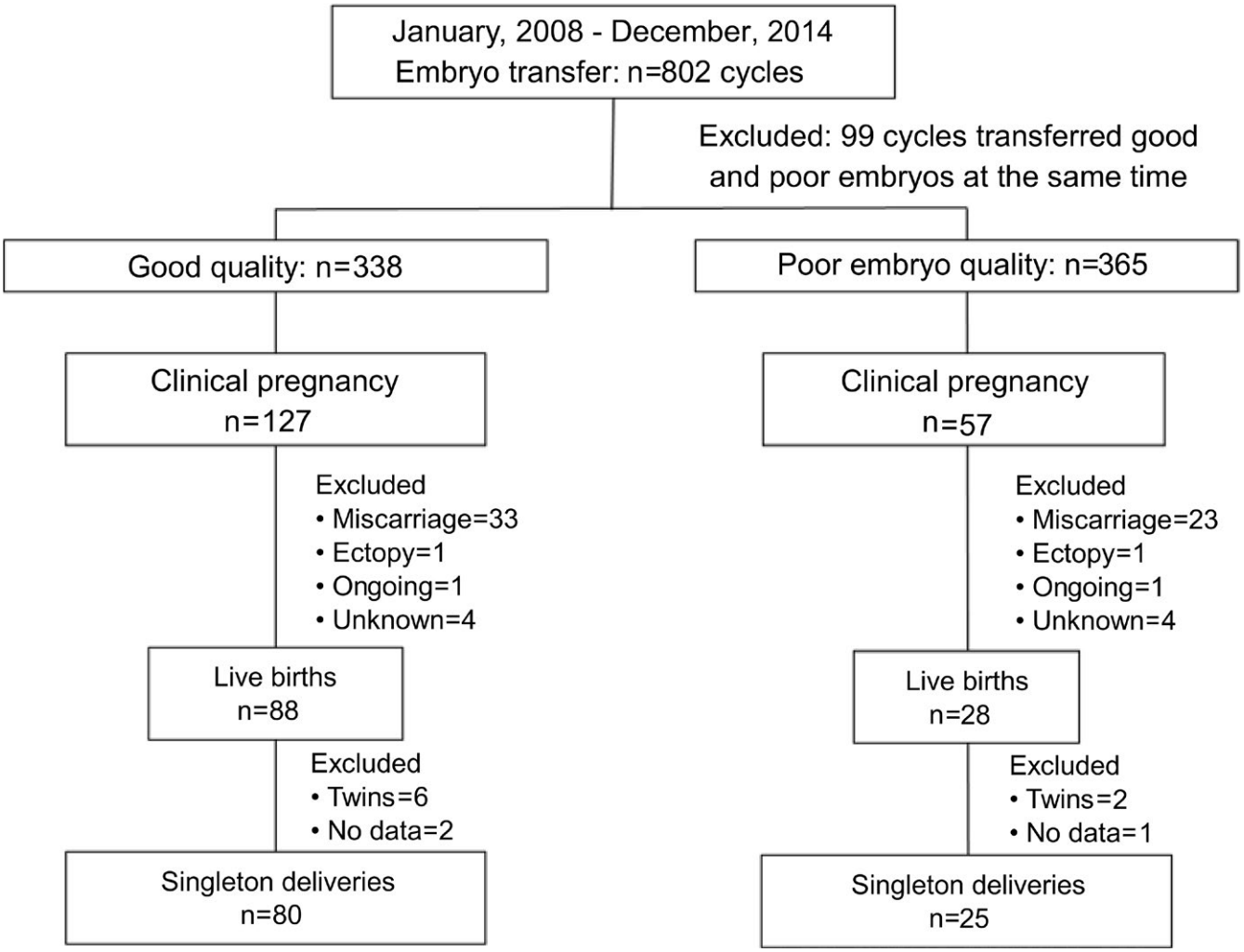

FIGURE 1 Outcomes of all the embryo transfers

reserve due to advanced age, endometriosis, or premature ovarian failure. When the dominant follicles reached ≥18 mm in diameter, 10 000 IU of human chorionic gonadotropin (hCG) was administered and an oocyte pick-up was performed 35 hours later under i.v. anesthesia or under local anesthesia if there were a few follicles. Fertilization was achieved by insemination or intracytoplasmic sperm injection (ICSI), depending on the state of the sperm. If there were >5 fertilized ova, all were cultured for 5 days and a blastocyst embryo transfer or cryopreservation was performed. If there were ≤4 fertilized ova, early-cleavage embryo transfer or cryopreservation was performed. Vitrification methods were used for embryo cryopreservation. The luteal phase was supported by the i.m. injection of 5000 IU of hCG per week for the cases of fresh embryo transfer. All the frozen-thawed embryo transfers were performed during an artificial cycle. A single embryo transfer was usually performed; however, a double embryo transfer was considered if the woman was aged >35 years or had undergone unsuccessful IVF treatment more than twice.

2.2 | Embryo quality

The embryo quality was assessed just before the embryo transfer. Cleavage embryos were defined as "good quality" if they were composed of ≥4 cells on day 2 or at least seven-to-eight cells on day 3 and contained <20% anucleate fragments, according to the Veeck classification system. The embryos that failed to meet these criteria were defined as "poor quality." The blastocysts were graded according to their size, density, inner cell mass (ICM), and trophectoderm

TABLE 1 Characteristics of live births after good- and poor-quality embryo transfers

Characteristic	Live birth after good-quality embryo transfer (n = 80)	Live birth after poor-quality embryo transfer (n = 25)	*P*-value
Maternal age (years ± SD[†])	35.50 ± 0.40	37.20 ± 0.90	.067
Body mass index (kg/m^2)	22.80 ± 0.38	21.90 ± 0.67	.260
Parity ≥1 (%, n)	33.80 (27)	40.00 (10)	.570
Primary infertility (%, n)	37.50 (30)	48.00 (12)	.350
Parity	.40 ± .08	.40 ± .1	.900
History of preterm delivery (%, n)	2.50 (2)	0 (0)	1.000
History of pregnancy hypertensive disorder (%, n)	2.50 (2)	.00 (0)	1.000
History of uterine surgery (%, n)[†]	40.00 (32)	36.00 (9)	.720
Duration of infertility (years)	4.30 ± .60	4.82 ± 1.00	.650
IVF/ICSI (%, n)			.480
IVF	60.00 (48)	52.00 (13)	
ICSI	40.00 (32)	48.00 (12)	
Day of transfer (%, n)			.023
Cleavage embryo transfer (days 2, 3)	15.00 (12)	36.00 (9)	
Blastocyst embryo transfer (days 5, 6)	85.00 (68)	64.00 (16)	
Fresh embryo transfer (%, n)	22.50 (18)	24.00 (6)	.880
Thawed embryo transfer (%, n)	77.50 (62)	76.00 (19)	
Endometrium before the embryo transfer (mm)	10.90 ± 0.30	11.70 ± 0.50	.150
Estrogen value before the embryo transfer (pg/mL)	1180.00 ± 184.00	704.00 ± 330.00	.210
Progesterone value before the embryo transfer (ng/mL)	0.54 ± 0.07	0.32 ± 0.13	.140
Cause of infertility			
Male factor (%, n)	42.90 (33)	40.90 (9)	
Tubal factor (%, n)	43.80 (35)	36.00 (9)	
Endometriosis (%, n)	3.80 (3)	4.00 (1)	
Polycystic ovarian syndrome (%, n)	13.80 (11)	0.00 (0)	
Uterine myoma (%, n)	12.00 (3)	10.00 (8)	
Unexplained (%, n)	10.10 (8)	20.00 (5)	

[†]Including myomectomy, cesarean section, and dilatation and curettage.
ICSI, intracytoplasmic sperm injection; IVF, in vitro fertilization; SD, standard deviation.

TABLE 2 Obstetric and perinatal outcomes

Characteristic	Live birth after good-quality embryo transfer (n = 80)	Live birth after poor-quality embryo transfer (n = 25)	*P*-value
Birthweight (g ± SD)	3005.0 ± 60.0	3007.0 ± 107.0	.98
Fresh embryo transfer	2918.0 ± 103.0	2780.0 ± 178.0	.51
Thawed embryo transfer	3030.0 ± 71.0	3079.0 ± 129.0	.74
Gestational age (weeks)	38.5 ± .2	39.1 ± .4	.22
Mode of delivery (%, n)			1.00
Vaginal delivery	60.00 (48)	60 (15)	
Cesarean section	40.00 (32)	40 (10)	
Small for gestational age (%, n)	6.25 (5)	16 (4)	.21
Large for gestational age (%, n)	7.50 (6)	16 (4)	.24
Low birthweight: <2500 g (%, n)	11.30 (9)	16 (4)	.50
Very low birthweight: <1500 g (%, n)	2.50 (2)	4 (1)	.56
Preterm delivery: <37 wk (%, n)	11.30 (9)	4 (1)	.45
Early preterm delivery: <32 wk (%, n)	1.30 (1)	0 (0)	1.00
Preterm-PROM[†](%, n)	5.00 (4)	0 (0)	.57
Hypertensive pregnancy disorders (%, n)	8.80 (7)	4 (1)	.68
Placental abruption (%, n)	.00 (0)	0 (0)	.00
Gestational diabetes mellitus (%, n)	12.50 (10)	0 (0)	.11
Chorioamnionitis (%, n)	2.50 (2)	0 (0)	1.00
Malformations (%, n)	.00 (0)	0 (0)	.00
Placenta previa (%, n)	.00 (0)	4 (1)	.24
Giant baby: >4000 g (%, n)	3.80 (3)	4 (1)	1.00
UmA[‡] pH: <7.2 (%, n)	3.80 (3)	8 (2)	.60

[†]Premature rupture of the membrane.
[‡]Umbilical artery cord.
SD, standard deviation.

development, according to the Gardner classification system. A grade of "3BB" or greater was defined as "good quality" and less than that was defined as "poor quality."

2.3 | Clinical outcomes

The maternal pregnancy complications and neonatal outcomes were evaluated. Maternal pregnancy complications included hypertensive disorders of pregnancy (HDP), placental abruption, gestational diabetes mellitus (GDM), chorioamnionitis (CAM), and placenta previa. The neonatal outcome analysis included the gestational age at delivery, birthweight, rate of low and very low birth weights, rate of preterm (PTD) and early preterm (early PTD) delivery, rate of caesarian section, small for gestational age (SGA) and large for gestational age (LGA) size, premature rupture of the membrane, malformation, giant baby, and an umbilical artery cord pH of <7.20. A low birthweight (LBW) was defined as <2500 g, very LBW as <1500 g, and giant baby as a birthweight of >4000 g. The PTD was defined as a birth occurring before gestational week 37 and early PTD was defined as a birth occurring before gestational week 32.

2.4 | Statistical analysis

For the statistical analyses, the categorical variables were assessed by using the chi-square test and Fisher's exact test for a small sample size. Differences in the continuous variables were evaluated by using Student's *t* test. A probability (*P*) value of <.05 was considered to be statistically significant.

3 | RESULTS

There were 802 embryo transfers that were performed at the authors' hospital between January, 2008 and February, 2014, which included 338 that were good quality, 365 that were poor quality,

and 99 of either good or poor quality that were excluded from this study.

Figure 1 represents the cycle outcomes from the good- and poor-quality embryo transfers. Of all the live singleton births, complete data were obtained from 80 good and 25 poor embryo transfers. Twins and singletons without complete data were excluded. Table 1 summarizes the patients' characteristics for the singleton live births after good and poor embryo transfers. There was no significant difference in age, infertility duration, parity, primary infertility population, ICSI rate, or fresh embryo transfer rate. With respect to the cause of infertility, the ratio of the male factor was relatively high in both groups. There were more cleavage embryo transfers in the poor-quality embryo transfer group than in the good-quality embryo transfer group. Table 2 summarizes the maternal complications and neonatal outcomes of the singleton live births in the good- and poor-quality embryo transfer groups. There was no significant difference in the maternal pregnancy complications of GDM, CAM, HDP, placental abruption, and placenta previa. According to the neonatal outcomes, there was also no significant difference in the mean birthweight, gestational age, delivery mode, SGA, LGA, PTD rate, LBW rate, and umbilical artery pH of <7.2. As there was no case with a malformation in this study, chromosomal examination was not performed. The obstetric and neonatal outcomes of the live births after a poor-quality embryo transfer were equivalent to those after a good-quality embryo transfer.

4 | DISCUSSION

The results of this study demonstrate that the obstetric and neonatal outcomes of the live births after the poor-quality embryo transfers were equivalent to those after the good-quality embryo transfers and that the embryo quality was not associated with increased risks of adverse obstetric and neonatal outcomes.

The embryo quality might have an effect on obstetric and neonatal outcomes; however, there was no difference between the poor- and the good-quality embryo transfers in this study. Few studies that evaluated the possible effects of the embryo quality on obstetric and neonatal outcomes arrived at the same results as those of the present study, although each of these studies assessed the obstetric and neonatal outcomes according to different parameters. One of the studies that was mentioned conducted a retrospective cohort study that included 1541 fresh single embryo transfers and reported that the embryo quality was not associated with the perinatal outcome in terms of malformation, SGA, PTD rate, LBW rate, pre-eclampsia, GDM, CAM, or placental abruption between the poor- and the good-quality embryo transfers.[5] One report reviewed 340 singleton births after single-cleavage embryo transfers and observed no significant difference in the parameters of the mean birthweight and infant height, umbilical blood analysis, placental weight, or umbilical cord length between the poor- and the good-quality embryo transfers.[7] One of the reports reviewed 11 721 cleavage-stage double embryo transfers and demonstrated that the live births after the poor-quality embryo transfers achieved the same pregnancy outcomes as those after the good-quality embryo transfers did in terms of mean gestational week, delivery mode, SGA, LGA, PTD rate, and LBW.[6] In agreement with these reports, this review's results demonstrate that the poor-quality embryos did not increase the prevalence of adverse obstetric and neonatal complications. These findings could be useful for women with anxiety regarding the obstetric and neonatal outcomes after poor-quality embryo transfer.

Many studies have demonstrated a strong association between morphologically poor-quality embryos, a low clinical pregnancy rate, and low live birth rate per transfer.[3-6] However, relatively few studies have reported associations between the embryo quality and miscarriage and live birth rates of clinical pregnancies due to the lack of evidence in evaluating these issues. This study demonstrated that the live birth rate per clinical pregnancy was significantly lower and that the miscarriage rate per clinical pregnancy was higher in the poor-quality embryo transfer group than in the good-quality embryo transfer group (69.1% vs 49.1% and 26% vs 40.4%, $P = .0088$ and .053, respectively). One previous study reported that once clinical pregnancy was achieved, the subsequent miscarriage and live birth rates of the clinical pregnancies were equivalent between the poor- and good-quality embryo transfer groups.[5] On the other contrary, another previous study demonstrated that a poor-quality embryo transfer resulted in higher miscarriage rates and lower ongoing pregnancy rates after achieving clinical pregnancy.[6] While the former study[5] combined both cleavage and blastocyst embryos, the latter study evaluated only cleavage embryos and speculated that the embryo quality at the cleavage stage might affect the subsequent pregnancy outcomes of clinical pregnancy.[6] As a possible reason for the lower ongoing pregnancy rate after a poor-quality cleavage-stage embryo transfer, a group of authors reported that cleavage blockage, which is associated often with poor-quality embryos, might indicate developmental disturbances that are related to chromosomal abnormalities.[8] Another study also reported that the highest rate of complex aneuploidy was detected in cleavage-stage embryos, whereas at the blastocyst stage, the aneuploidy rate was lower.[9] Considering the findings of these reports, morphologically poor cleavage-stage embryos could have more chromosomal abnormalities that might result in a higher miscarriage rate than poor blastocyst-stage embryos. However, the results indicated that once clinical pregnancy was achieved after the transfer of morphologically poor embryos, which would not usually be chosen for embryo transfer, approximately half of these clinical pregnancies resulted in live births, which might be beneficial for older women and those with a diminished ovarian reserve who often have only morphologically poor embryos.

According to the Veeck classification system that is used to evaluate the quality of cleavage embryos, "good quality" was defined as ≥4 cells on day 2 or at least seven-to-eight cells on day 3 and <20% anucleate fragments. "Poor-quality" embryos were defined as those that failed to meet the above-mentioned criteria. The Gardner classification system was used to grade the blastocysts according to their size, density, ICM, and trophectoderm development and grade 3BB or higher was defined as "good quality" and a

grade below this was defined as "poor quality." Until now, relatively few studies have included a subgroup analysis of only poor-quality embryo transfers in order to identify the morphological features that can result in better pregnancy outcomes, such as a comparison between fair- and poor-quality embryo transfers, according to the Veeck classification system, developmental speed, and percentage fragmentation at various cleavage stages.[7] Moreover, the few studies that have analyzed three morphological parameters (ie blastocoel expansion, ICM, and the trophectoderm grade, according to the Gardner classification system) have shown that blastocoel expansion and the trophectoderm grade were significant predictors of a live birth.[10-12] As the present study did not include a subgroup analysis to identify the morphological features that result in better pregnancy outcomes, another study with a larger sample size has been planned to evaluate these factors. In recent years, with the introduction of time-lapse imaging, many studies have reported that the addition of time-lapse morphokinetics to conventional morphological evaluation in the selection of embryos for transfer has improved IVF outcomes.[13-15] However, these studies did not assess the long-term outcomes, such as miscarriage rates, ongoing pregnancy rates, or perinatal outcomes. Therefore, further studies are warranted to confirm the value of time-lapse imaging.

A limitation of the present study was the small sample size. Furthermore, the patients' characteristics and factors associated with IVF treatment, such as the rate of blastocyst transfer, could not be adjusted for, which could have affected the pregnancy outcomes. Also, in addition to the embryo quality, the subsequent process after implantation until delivery could be influenced by other factors, such as uterine characteristics and events at the delivery. Thus, predicting the obstetric and neonatal outcomes, based on morphological embryo quality alone, might be difficult. Until now, there is only one facility that has reported the obstetric and neonatal outcomes of live births after poor-quality embryo transfers in Japan. The number is still not sufficient, as only 79 cases of pregnancy have resulted from poor-quality embryo transfers. The authors believe that this study is valuable because it conducts the same survey for each facility and forms a consensus in Japan.

In conclusion, the results of the present study demonstrated that the obstetric and neonatal outcomes of the live births after a poor-quality embryo transfer were equivalent to those of the live births after a good-quality embryo transfer and that a poor embryo quality was not associated with an increased risk of adverse obstetric and neonatal outcomes.

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DISCLOSURES

Conflict of interest: The author declares no conflict of interest. *Human Rights Statement and Informed Consent*: This retrospective study was approved by the Institutional Review Board of the University of the Ryukyus Hospital (August 16, 2016; No. 984). All the procedures were followed in accordance with the ethical standards of the responsible committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from all the patients to be included in the study. *Animal studies:* This article does not contain any study with animal participants that have been performed by any of the authors.

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