



## Original Article

## Influence of cleavage-stage embryo quality on the in-vitro fertilization outcome after single embryo transfer in fresh cycles

Yu Sun <sup>a</sup>, Enshu Li <sup>b</sup>, Guofang Feng <sup>a</sup>, Miao Li <sup>a</sup>, Yanling Fu <sup>a</sup>, Jiali You <sup>a</sup>, Xiaozhen Liu <sup>a</sup>, Yimin Zhu <sup>a,\*</sup>

<sup>a</sup> Department of Reproductive Endocrinology, Women's Hospital, School of Medicine, Zhejiang University, Hangzhou, 310006, China

<sup>b</sup> Reproductive Endocrinology Laboratory, The Key Laboratory of Assisted Reproduction, Ministry of Education, Hangzhou, 310006, China

## ARTICLE INFO

Article history:  
Accepted 9 July 2020

Keywords:  
Embryo quality  
Pregnancy outcome  
Single-embryo transfer  
In vitro fertilization

## ABSTRACT

**Objective:** Embryo quality is crucial for determining the outcome of embryo implantation. This study aimed to assess the impact of embryo quality on the outcome of *in vitro* fertilization/single-embryo transfer (IVF-SET).

**Materials and methods:** This retrospective study included 2531 fresh IVF-SET cycles, including 277 poor-quality and 2254 top-quality embryos. The clinical pregnancy rate, miscarriage rate, live birth, implantation rate, pregnancy outcome and complication were analyzed and compared. Risk factors associated with miscarriage rate and pregnancy complication were identified using logistics regression analysis.

**Results:** Top-quality embryos resulted in higher clinical pregnancy rate (30.5% vs. 12.6%,  $P < 0.001$ ) and live birth rate (23.9% vs. 9.7%,  $P < 0.001$ ) compared with poor-quality embryos. Logistics regression analysis revealed that embryo quality was not correlated with miscarriage rate (95% CI 0.33–1.89) and pregnancy complications (95% CI 0.12–7.84). Maternal age and body mass index was a risk factor for miscarriage rate (95% CI 1.05–1.22) and pregnancy complication (95% CI 1.01–1.29), respectively.

**Conclusion:** Clinical miscarriage rate and pregnancy complication were embryo quality independent. Maternal age was the risk factor for miscarriage rate. Embryo quality did not affect miscarriage once a clinical pregnancy is achieved.

© 2020 Taiwan Association of Obstetrics & Gynecology. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Established facts

1. Pregnancy rate was significantly reduced in patients receiving poor quality embryos.
2. Maternal age, but not embryo quality was a risk factor for miscarriage rate.
3. Maternal BMI was a risk factor for pregnancy complication.

## Introduction

*In vitro* fertilization (IVF) is in increasing demand because of the rising prevalence of infertility [1]. In European countries with high fertility rates, IVF is an integral technique in women aged over 30 years [2]. Single-embryo transfer (SET) is recommended

and widely used for infertile families [3,4]. Many studies have reported that the quality of cleavage embryo has a key role in determining the implantation rate, successful rate of embryo implantation and pregnancy outcome [5,6]. Good-quality embryos will raise the rate of implantation and clinical pregnancy, and increase live birth rate [7]. Therefore, there are emerging technologies to improve the quality of embryo before embryo transfer, including autologous mitochondrial transfer accompanied with ICSI [6] and pretreatment with coenzyme Q10 [8]. However, for patients without good-quality embryo transfer and insufficient economic conditions, they have to transfer poor-quality embryos.

Recently, a few studies evaluated the relationship between embryo quality and adverse perinatal outcome [9,10]. Some authors demonstrated that the miscarriage rate is higher in women received the poor-quality embryos than women received good-quality embryos [9,11]. Others reported that there was no difference in the implantation rate, miscarriage rate, perinatal and pregnancy outcomes between the two groups [10,12]. The poor-quality embryos also have

\* Corresponding author. Department of Reproductive Endocrinology, Women's Hospital, School of Medicine, Zhejiang University, No. 2 Xueshi Road, Hangzhou 310006, China.

E-mail address: [zhuyim@zju.edu.cn](mailto:zhuyim@zju.edu.cn) (Y. Zhu).

the potential for a successful pregnancy [10,11]. For a fairly large number of women with ovarian failure, the SET of poor-quality embryos is the only way for them to choose. Thus, it is important to determine whether the SET of poor-quality embryos would result in a higher miscarriage rate, and worse pregnancy outcome compared with SET of with good quality embryos.

This study was conducted to assess the differences in pregnancy outcomes in IVF-SET with good-quality and poor-quality cleavage embryos at the Center for Reproductive Medicine of Women's Hospital School of Medicine Zhejiang University between January 2011 and January 2017. Our study would provide helpful advice for couples when they only have poor-quality embryos for IVF-SET.

## Materials and methods

### Experimental design

This study is a retrospective cohort study including 2531 IVF-SET fresh cycles at the Center for Reproductive Medicine of Women's Hospital, School of Medicine, Zhejiang University, Hangzhou, China, between January 2011 and January 2017. Inclusion criterion was women undergoing their first fresh SET cycle using autologous oocytes. Women who had uterine malformation and/or received a genetic diagnosis before implantation were excluded. On the basis of embryo quality, patients were sub-categorized into two groups (poor-quality embryo and top-quality embryo groups) according to the quality of embryos that they received on SET. Subsequently, the demographic characteristics including patients' age, body mass index (BMI; kg/m<sup>2</sup>), dose of gonadotrophin, main cause of infertility, the number of oocytes retrieved and fertilization rate were recorded. Causes of infertility, including polycystic ovarian syndrome (PCOS), male infertility, poor ovarian reserve, endometriosis, tubal factor infertility or unexplained infertility were recorded.

### Ovulation stimulation protocol

Patients were subjected to different stimulation protocols such as short agonist protocol, midluteal long agonist protocol, fixed antagonist protocol, ultra-long protocol, microdose flare protocol or natural cycle protocol according to their conditions. IVF including intracytoplasmic sperm injection (ICSI) was performed according to the cause of infertility and semen quality on the day of oocyte harvest. Fertilization was defined when two pronuclei and two polar bodies were appeared at 16–18 h after insemination.

### Embryo culture and transfer

The embryos were cultured in G1™ cleavage medium (Vitrolife, Gothenburg, Sweden). On day 2 (43–45 h) or day 3 (67–69 h) post insemination, embryonic development was evaluated and SET was performed. On day 15 or day 16 post SET, serum beta-human chorionic gonadotropin (hCG) concentration was tested and women with hCG ≥ 5 ng/ml were suspected of pregnancy. Definite diagnosis of pregnancy was confirmed using ultrasound at 30–35 days post SET.

### Embryo quality

The cleavage-stage embryos were assessed and graded according to cell count and fragmentation percent as recommended by the Spanish Society for the Study of Reproductive Biology (ASEBIR) embryo assessment criteria with modifications [13]. The embryo quality was classified into three categories [1]: good quality: embryo had 4 or 5 cells on day 2, 7 to 9 cells on day 3, and <10% anucleated fragmentation [2]; fair quality: embryo had 3 cells on

day 2, 6 cells on day 3 and 10–25% anucleated fragmentation; and [3] poor quality: embryo had less than 2 cells on day 2, less than 5 cells on day 3, and >25% anucleated fragments. Embryos had >35% anucleated fragments with uneven blastomere symmetry, abnormal zona pellucida and multinucleation were not used in this study. Embryos with fair and good qualities were classified into the top-quality group and that with poor quality were assigned into the poor-quality group. Cleavage grading was measured by five trained embryologists, and disagreements were solved by discussion and consensus including other three authors. After SET, the other embryos with good quality and fair quality were frozen and stored according to patient requirement.

### Clinical outcomes

Clinical outcomes included ectopic pregnancies, miscarriage, and live births. Adverse perinatal outcomes included small for gestation age (SGA; below the 10th percentile for gestational age) or large for gestation age (LGA; above the 90th percentile), low birthweight (LBW, < 2500 g), very low birthweight (VLBW, <1500 g), preterm delivery (PTD, less than 37 gestational weeks) or very PTD (VPTD, less than 32 gestational weeks), and congenital malformations. Pregnancy complications included uterus rupture, pre-eclampsia, placenta previa, placental abruption, gestational diabetes, premature rupture of membranes, oligohydramnios, fetal distress, postpartum hemorrhage, and intrahepatic cholestasis of pregnancy.

### Statistical analysis

SPSS 17.0 software was used for statistical analyses. The demographic characteristics of patients were compared between two groups using *t* tests (for continuous variable) or chi-squared test (for categorical and counting variables). Risk factors for miscarriage rate were assessed using logistics regression analysis after adjustment for maternal BMI, paternal age, cause of infertility, cycle with ICSI, and the number of oocytes retrieved. Also, the correlation between embryo quality and pregnancy complication was analyzed using logistic regression analysis after adjusting for maternal age, maternal BMI, paternal age, cause of infertility, cycles with ICSI, gestation age, method of delivery, and birthweight. Odds ratio (OR) and 95% confidential interval (CI) were calculated during logistics analysis. *P* < 0.05 was defined as statistical significant.

## Results

The 2531 fresh IVF-SET cycles included 2254 top-quality embryos and 277 poor-quality embryos. The mean age of women and their partner in the poor-quality group was both older than those in the top-quality group (*P* < 0.001 and *P* = 0.001; Table 1). There were no differences in maternal BMI, cycles with ICSI, and gonadotrophin level between the two groups. The number of retrieved oocytes (6.06 ± 5.11 vs. 11.94 ± 7.22, *P* < 0.001) and fertilized oocytes (1.74 ± 1.90 vs. 6.44 ± 5.53, *P* < 0.001), and fertilization rate (41.99 ± 30.82% vs. 52.63 ± 27.11, *P* < 0.001) in poor-quality group were significantly lower than those in top-quality group. The cause of infertility was significantly different between the two groups. Tubal factor (14.8%) and male sperm problems (4.7%) were the major cause of infertility in the top-quality embryo group. The poor-quality embryo group had a higher rate of poor ovarian reserve compared with the top-quality embryo group (1.4% vs. 0.3%, *P* = 0.025, Table 1).

The top-quality embryo group had higher pregnancy and birth rate than the poor-quality embryo group (30.5% vs. 12.6%, *P* < 0.001; 23.9% vs. 9.7%, *P* < 0.001, respectively). No statistical differences

**Table 1**  
Characteristics and outcomes of patients.

|                                   | Top-quality (n = 2254) | Poor-quality (n = 277) | P value              |
|-----------------------------------|------------------------|------------------------|----------------------|
| Maternal age (y)                  | 31.02 ± 4.83           | 32.65 ± 5.44           | <0.001 <sup>a</sup>  |
| Paternal age (y)                  | 33.20 ± 5.69           | 34.50 ± 6.12           | 0.001 <sup>a</sup>   |
| Maternal BMI (kg/m <sup>2</sup> ) | 21.83 ± 3.07           | 21.89 ± 3.16           | 0.73 <sup>a</sup>    |
| Cycles with ICSI                  | 574 (25.5%)            | 81 (29.2%)             | 0.191                |
| Dose of gonadotrophin (IU)        | 2100.49 ± 832.59       | 2124.32 ± 1058.11      | 0.718 <sup>a</sup>   |
| Number of oocytes retrieved       | 11.94 ± 7.22           | 6.06 ± 5.11            | <0.0001 <sup>b</sup> |
| Number of fertilized oocytes      | 6.44 ± 5.53            | 1.74 ± 1.90            | <0.0001 <sup>a</sup> |
| Fertilization rate (%)            | 52.63 ± 27.11          | 41.99 ± 30.82          | <0.0001 <sup>a</sup> |
| <b>Cause of infertility</b>       |                        |                        |                      |
| PCOS (%)                          | 31 (1.4%)              | 0                      | 0.042 <sup>b</sup>   |
| Male (%)                          | 105 (4.7%)             | 5 (1.8%)               | 0.020 <sup>b</sup>   |
| Tubal factor (%)                  | 333 (14.8%)            | 9 (3.3%)               | <0.0001 <sup>b</sup> |
| Endometriosis (%)                 | 37 (1.6%)              | 4 (1.4%)               | 1.000 <sup>b</sup>   |
| Poor ovarian reserve (%)          | 7 (0.3%)               | 4 (1.4%)               | 0.025 <sup>b</sup>   |
| Unexplained (%)                   | 26 (1.2%)              | 5 (1.8%)               | 0.377 <sup>b</sup>   |
| <b>Outcomes</b>                   |                        |                        |                      |
| Clinical pregnancy                | 688 (30.5%)            | 35 (12.6%)             | <0.0001 <sup>b</sup> |
| Live births                       | 539 (23.9%)            | 27 (9.7%)              | <0.0001 <sup>b</sup> |
| Pregnancy outcome                 | n = 688                | n = 35                 |                      |
| Live births                       | 539 (78.3%)            | 27 (77.1%)             | 0.867 <sup>b</sup>   |
| Miscarriage                       | 106 (15.4%)            | 8 (22.9%)              | 0.238 <sup>b</sup>   |
| First trimester                   | 97 (14.1%)             | 7 (20.0%)              | 0.332 <sup>b</sup>   |
| Second or third trimester         | 9 (1.3%)               | 1 (2.9%)               | 0.393 <sup>b</sup>   |
| Ectopic pregnancy                 | 43 (6.3%)              | 0 (0)                  | 0.247 <sup>b</sup>   |
| Birthweight (g)                   | 3232.14 ± 499.53       | 3258.52 ± 407.50       | 0.787 <sup>a</sup>   |
| Gestational age (w)               | 38.41 ± 1.92           | 38.81 ± 1.15           | 0.277 <sup>a</sup>   |
| Male gender (%)                   | 262/539 (48.6%)        | 18/27 (66.7%)          | 0.067 <sup>b</sup>   |
| <b>Mode of delivery</b>           | n = 539                | n = 27                 | 0.409 <sup>b</sup>   |
| Vaginal delivery                  | 190 (35.3%)            | 7 (25.9%)              |                      |
| Caesarean section                 | 349 (64.7%)            | 20 (74.1%)             |                      |
| Pregnancy complication (%)        | 28/539 (5.2%)          | 1/27 (3.7%)            | 1.00 <sup>b</sup>    |

ICSI, intracytoplasmic sperm injection; BMI, body mass index. PCOS, polycystic ovarian syndrome. a and b indicates the statistical analysis is performed by *t*-test and chi-squared test, respectively.

were found in miscarriage rate, ectopic pregnancy rate, birthweight, gestational age, gender ratio, delivery mode and morbidity of pregnancy complications (Table 1). There were no significant differences in LGA, SGA, LBW, VLBW, PTD, VPTD and congenital malformations between the two groups (Table S1).

Logistic regression analysis showed maternal age was obviously related to miscarriage rate after adjusting for confounding factors including maternal BMI, paternal age, cause of infertility, cycle with ICSI, and number of oocytes retrieved ( $P = 0.002$ , 95% CI 1.05–1.22). After adjusting these confounding factors plus maternal age, embryo quality was not a risk factor for miscarriage rate ( $P = 0.60$ , 95% CI 0.33–1.89; Table 2) and pregnancy complication ( $P = 0.97$ , 95% CI 0.12–7.84; Table 3). However, maternal BMI was a key factor that associated with the pregnancy complication after adjusting for maternal age plus the above confounding factors ( $P = 0.03$ , 95% CI 1.01–1.29).

**Table 2**  
Logistic regression analysis for the factors that associate with miscarriage rate.

| Variables                   | $\beta$ | OR   | 95% CI    | P value |
|-----------------------------|---------|------|-----------|---------|
| Embryo quality              | −0.24   | 0.79 | 0.33–1.89 | 0.60    |
| Maternal age                | 0.12    | 1.13 | 1.05–1.22 | 0.002   |
| Maternal BMI                | 0.07    | 1.07 | 0.99–1.15 | 0.052   |
| Paternal age                | −0.03   | 0.97 | 0.91–1.03 | 0.36    |
| Cause of infertility        |         |      |           | 0.70    |
| Male                        | −0.27   | 0.77 | 0.20–2.90 | 0.70    |
| Tubal factor                | −0.03   | 0.97 | 0.33–2.88 | 0.96    |
| Endometriosis               | −0.04   | 0.97 | 0.38–2.46 | 0.94    |
| Poor ovarian reserve        | 0.55    | 1.73 | 0.57–5.27 | 0.34    |
| Ovulatory disorder          | −0.04   | 0.96 | 0.19–4.96 | 0.96    |
| Cycles with ICSI            | 0.14    | 1.15 | 0.63–2.10 | 0.66    |
| Number of oocytes retrieved | −0.01   | 0.99 | 0.96–1.02 | 0.53    |

ICSI, intracytoplasmic sperm injection; BMI, body mass index. OR, odds ratio. CI, confidential interval.

## Discussion

In the present study, we confirmed that the rates of clinical pregnancy and live birth were significantly increased in the top-quality embryo group compared with those in the poor-quality embryo group. Logistic regression analysis revealed that embryo quality was insignificantly associated with miscarriage rate and complications of pregnancy once a clinical pregnancy was achieved.

Although women underwent double embryo transfer (DET) achieved multiple pregnancy, they had higher miscarriage rate and poorer obstetric and perinatal outcomes than those received SET [11]. SET is generally recommended by physicians, especially when

**Table 3**  
Logistics regression analysis for the factors that associate with pregnancy complication (n = 566).

|                      | $\beta$ | OR   | 95% CI    | P value |
|----------------------|---------|------|-----------|---------|
| Maternal age         | 0.07    | 1.07 | 0.94–1.22 | 0.29    |
| Embryo quality       | −0.05   | 0.95 | 0.12–7.84 | 0.97    |
| Maternal BMI         | 0.14    | 1.14 | 1.01–1.29 | 0.03    |
| Paternal age         | 0.03    | 1.03 | 0.93–1.12 | 0.53    |
| Cause of infertility |         |      |           | 0.99    |
| Male                 | −18.58  | 0.00 | 0.000     | 0.99    |
| Tubal factor         | −0.431  | 0.65 | 0.09–4.81 | 0.67    |
| Endometriosis        | −0.323  | 0.72 | 0.15–3.53 | 0.69    |
| Poor ovarian reserve | 0.13    | 1.14 | 0.16–8.07 | 0.90    |
| Ovulatory disorder   | −19.13  | 0.00 | 0.000     | 1.00    |
| Cycles with ICSI     | −0.23   | 0.79 | 0.23–2.79 | 0.72    |
| Method of delivery   | 0.48    | 1.61 | 0.65–4.02 | 0.31    |
| Gestational age      | −0.10   | 0.91 | 0.72–1.14 | 0.41    |
| Birthweight          | −0.00   | 1.00 | 0.99–1.00 | 0.36    |

ICSI, intracytoplasmic sperm injection; BMI, body mass index. OR, odds ratio. CI, confidential interval.

there are more than one top-quality embryos [11,14,15]. In addition, DET has a higher economic cost for every family managing short-term and long-term implications of multiple pregnancy [16]. Therefore, the present study only included patients with SET, which was a major strength for this study.

Embryo quality has been proven to be strongly associated with the rates of implantation and live birth [6,17,18]. Our research displayed that the rates of clinical pregnancy and live birth in the top-quality embryo group were more than 2-fold higher than those in the poor-quality embryo group (30.5% vs. 12.6%, and 23.9% vs. 9.7%, respectively). These results were similar to that reported in earlier studies [17,18]. These studies suggested that there were close relationships between embryo quality and the success rates of implantation and live birth. Therefore, women and clinicians both give preference to high-quality cleavage-stage embryos for IVF-SET.

For cleavage embryo with poor-quality, physicians have difficulty deciding whether the SET should be performed. Available evidence shows that SET of the poor-quality cleavage-stage embryos has comparable pregnancy outcome compared with transfer for the good quality embryos, although with a lower rate of pregnancy [10–12]. Previous studies showed that poor-quality cleavage-embryo significantly increased the rate of miscarriage [9] and malformation [19]. Recently, there are more and more evidence shows that poor-quality embryos has comparable pregnancy outcome [11,20,21]. Oron et al. [10] and Akamine et al. [21] showed that there were similar live birth rates and obstetric and neonatal outcomes between patients receiving poor-quality and good-quality embryos once a clinical pregnancy was achieved. Both of them reported that the morbidity of PTD, birthweight, SGA, LGA, malformation and pregnancy complications was not different between the two groups [10,21]. The higher rate of miscarriage after embryo transfer in the study by Zhu et al. might be included by the DET [9]. Evidence shows that DET results in a lower implantation rate and a higher incidence of miscarriage and adverse perinatal outcomes compared with SET [11,22]. In our present study there was no significant difference in miscarriage rate and pregnancy outcomes and complications between the two groups. After adjusting for the confounding factors, we demonstrated that embryo quality was not associated with the miscarriage rate and pregnancy complication. Therefore, we confirmed that embryo quality was not a risk factor for miscarriage and pregnancy complications in pregnant women after IVF-SET.

The only risk factor for miscarriage identified in this study was maternal age after adjusting confounding factors. Pregnancy loss is reported to be associated with endometrial factors, chromosomal abnormality and history of polypectomy [23,24]. In women undergoing IVF, serum beta-hCG concentrations at 14–16 days after embryo transfer, endometrial thickness, low anti-Müllerian hormone level, history of miscarriage, maternal age, frozen embryo transfer, uterus malformation and PCOS are reported to be independent factors that associates with spontaneous miscarriage [25,26]. In patients with PCOS, BMI and basal androstenedione are independent risk factors for miscarriage following IVF [27,28]. In view of the embryo quality, evidence shows that the  $\geq 30\%$  sperm DNA fragmentation in ICSI is correlated with poor embryo development and a higher miscarriage rate [29]. It has been reported that advanced maternal age is associated with oocyte chromosome abnormality, and then is correlated with miscarriage [25,30,31]. However, the embryos with  $>30\%$  anucleated fragments were excluded, which may eliminate the impact of embryo quality on miscarriage.

However, our present study was limited to the number of poor-quality embryos for SET. This was because of that the patients who had poor-quality embryos preferred to DET or withdrawn. This small cohort had a weak power to determine the statistical

differences in pregnancy complication and congenital malformation. There is evidence showing that obesity correlates positively with branched-chain amino acids in follicular fluid microenvironment [32], and lower serum  $\beta$ -hCG and progesterone after IVF [33]. These suggested that BMI may or may not influence the outcome of IVF-SET using a large cohort study.

Based on the present cohort, we concluded that IVF-SET with a poor-quality embryo did not result in a significant increase in adverse outcomes (such as miscarriage rate and adverse pregnancy outcome) in pregnant women compared with a good-quality embryo. Maternal age was identified as the only risk factor for miscarriage, and maternal BMI was the only risk factor for pregnancy complication.

## Statement of ethics

This retrospective cohort study was approved by the Ethics Committee of Women's Hospital, Hangzhou, China (20190008). Written informed consent was obtained from each participant. The research was conducted ethically in accordance with the Declaration of Helsinki.

## Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: National science & Technology Pillar Program during the 13th Five-year Plan Period (No. 2016YFC1000302); Natural Science Foundation of Zhejiang Province (No. LZ15H040001); the Program for Key Subjects of Zhejiang Province in Medicine and Hygiene; the Science Foundation of Health Bureau of Zhejiang Province (No. 2014KYA252).

## Authors' contributions

Conception and design of the research: Zhu YM. Acquisition, analysis and interpretation of data: Sun Y, Li M and Li ES. Statistical analysis: Fu YL, Liu XZ, and You JL. Drafting the manuscript: Sun Y. Manuscript revision for important intellectual content: Zhu YM. Obtaining funding: Zhu YM and Feng GF. All authors have read and approved the manuscript.

## Declaration of competing interest

None.

## Acknowledgments

The author(s) thank all investigators and patients who participated in this study.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tjog.2020.08.003>.

## References

- [1] Jain T, Grainger D, Ball G, Gibbons W, Rebar R, Robins J, et al. 30 years of data: impact of the United States in vitro fertilization data registry on advancing fertility care. *Fertil Steril* 2019;111.
- [2] Kocourkova J, Burcin B, Kucera T. Demographic relevancy of increased use of assisted reproduction in European countries. *Reprod Health* 2014;11(1):37.
- [3] Barash O, Ivani K, Huen N, Willman S, Weckstein L. Morphology of the blastocysts is the single most important factor affecting clinical pregnancy rates in IVF PGS cycles with single embryo transfers. *Fertil Steril* 2017;108(3):e99.



- [4] Reljić M, Knez J, Kovač V, Kovačić B. Endometrial injury, the quality of embryos, and blastocyst transfer are the most important prognostic factors for in vitro fertilization success after previous repeated unsuccessful attempts. *J Assist Reprod Genet* 2017;34(6):775–9.
- [5] Juan Q, Jin B, Shi Y, Wang L, Shao X. Effect of post-thaw embryo quality on clinical outcome of pregnancy. *Cryobiology* 2018;80:186.
- [6] Labarta E, de los Santos MJ, Herraiz S, Escribá MJ, Marzal A, Buigues A, et al. Autologous mitochondrial transfer as a complementary technique to intracytoplasmic sperm injection to improve embryo quality in patients undergoing in vitro fertilization—a randomized pilot study. *Obstet Gynecol Surv* 2019;111(1):86–94. <https://doi.org/10.1016/j.fertnstert.2018.09.023>.
- [7] Barash O, Ivani K, Hinckley M, Willman S, Rabara F, Huen N, et al. Impact of embryo morphology on clinical pregnancy rates in IVF PGS cycles with single embryo transfer. *Fertil Steril* 2017;107(3):e18–9.
- [8] Xu Y, Nisenblat V, Lu C, Li R, Qiao J, Zhen X, et al. Pretreatment with coenzyme Q10 improves ovarian response and embryo quality in low-prognosis young women with decreased ovarian reserve: a randomized controlled trial. *Reprod Biol Endocrinol* 2018;16(1):29. <https://doi.org/10.1186/s12958-018-0343-0>.
- [9] Zhu J, Lian Y, Li M, Chen L, Liu P, Qiao J. Does IVF cleavage stage embryo quality affect pregnancy complications and neonatal outcomes in singleton gestations after double embryo transfers? *J Assist Reprod Genet* 2014;31(12):1635–41.
- [10] Oron G, Son W-Y, Buckett W, Tulandi T, Holzer H. The association between embryo quality and perinatal outcome of singletons born after single embryo transfers: a pilot study. *Hum Reprod* 2014;29(7):1444–51.
- [11] Wintner EM, Hershko-Klement A, Tzadikévitch K, Ghetler Y, Gonen O, Wintner O, et al. Does the transfer of a poor quality embryo together with a good quality embryo affect the in Vitro Fertilization (IVF) outcome? *J Ovarian Res* 2017;10(1):2.
- [12] Li X, Huang R, Fang C, Wang Y, Liang X. Logistic regression analysis of risk factors associated with spontaneous abortion after in vitro fertilization/intracytoplasmic sperm injection-embryo transfer in polycystic ovary syndrome patients. *Reprod Dev Med* 2018;2(2):105–10.
- [13] Cuevas Saiz I, Carme Pons Gatell M, Vargas MC, Delgado Mendive A, Rives Enedáguila N, Moragas Solanes M, et al. The Embryology Interest Group: updating ASEBIR's morphological scoring system for early embryos, morulae and blastocysts. *Med Reprod Embriol Clin* 2018;5(1):42–54.
- [14] Sundhararaj UM, Madne MV, Biliangady R, Gurunath S, Swamy AG, Gopal IS. Single blastocyst transfer: the key to reduce multiple pregnancy rates without compromising the live birth rate. *J Hum Reprod Sci* 2017;10(3):201.
- [15] Clua E, Roca-Feliu M, Tresánchez M, Latre L, Rodríguez I, Martínez F, et al. Single or double embryo transfer? Decision-making process in patients participating in an oocyte donation program. *Gynecol Endocrinol* 2020;36(4):365–9.
- [16] Le KD, Vuong LN, Ho TM, Dang VQ, Pham TD, Pham CT, et al. A cost-effectiveness analysis of freeze-only or fresh embryo transfer in IVF of non-PCOS women. *Hum Reprod* 2018;33(10):1907–14.
- [17] Adamson GD, Abusief ME, Palao L, Witmer J, Palao LM, Gvakharina M. Improved implantation rates of day 3 embryo transfers with the use of an automated time-lapse-enabled test to aid in embryo selection. *Fertil Steril* 2016;105(2):369–375.e6.
- [18] Motato Y, de los Santos MJ, Escriba MJ, Ruiz BA, Remohí J, Meseguer M. Morphokinetic analysis and embryonic prediction for blastocyst formation through an integrated time-lapse system. *Fertil Steril* 2016;105(2):376–384.e9.
- [19] Ebner T, Yaman C, Moser M, Sommergruber M, Pölz W, Tews G. Embryo fragmentation in vitro and its impact on treatment and pregnancy outcome. *Fertil Steril* 2001;76(2):281–5.
- [20] Oron G, Sokal-Arnon T, Son W-Y, Demirtas E, Buckett W, Zeadna A, et al. Extended embryo culture is not associated with increased adverse obstetric or perinatal outcome. *Am J Obstet Gynecol* 2014;211(2):165.e1–7.
- [21] Akamine K, Mekaru K, Gibo K, Nagata C, Oishi S, Miyagi M, et al. Comparative study of obstetric and neonatal outcomes of live births between poor- and good-quality embryo transfers. *Reprod Med Biol* 2018;17(2):188–94.
- [22] Martin AS, Chang J, Zhang Y, Kawwass JF, Boulet SL, McKane P, et al. Perinatal outcomes among singletons after assisted reproductive technology with single-embryo or double-embryo transfer versus no assisted reproductive technology. *Fertil Steril* 2017;107(4):954–60.
- [23] Fukuta K, Yoneda S, Yoneda N, Shiozaki A, Nakashima A, Minamisaka T, et al. Risk factors for spontaneous miscarriage above 12 weeks or premature delivery in patients undergoing cervical polypectomy during pregnancy. *BMC Pregnancy Childbirth* 2020;20(1):27.
- [24] San Lazaro Campillo I, Meaney S, Sheehan J, Rice R, O'Donoghue K. University students' awareness of causes and risk factors of miscarriage: a cross-sectional study. *BMC Wom Health* 2018;18(1):188.
- [25] Bu Z, Hu L, Su Y, Guo Y, Zhai J, Sun Y-P. Factors related to early spontaneous miscarriage during IVF/ICSI treatment: an analysis of 21,485 clinical pregnancies. *Reprod Biomed Online* 2020;40(2):201–6.
- [26] Peuranpää P, Hautamäki H, Halttunen-Nieminen M, Hydén-Granskog C, Tiitinen A. Low anti-Müllerian hormone level is not a risk factor for early pregnancy loss in IVF/ICSI treatment. *Hum Reprod* 2020;35(3):504–15.
- [27] Li X, Huang R, Fang C, Wang Y, Xy L. Logistic regression analysis of risk factors associated with spontaneous abortion after in vitro fertilization/intracytoplasmic sperm injection-embryo transfer in polycystic ovary syndrome patients. *Reprod Dev Med* 2018;2:105–10.
- [28] Yang W, Yang R, Lin M, Yang Y, Song X, Zhang J, et al. Body mass index and basal androstenedione are independent risk factors for miscarriage in polycystic ovary syndrome. *Reprod Biol Endocrinol* 2018;16(1):119.
- [29] Borges E, Zanetti BF, Setti AS, Braga DPdAF, Provenza RR, Iaconelli A. Sperm DNA fragmentation is correlated with poor embryo development, lower implantation rate, and higher miscarriage rate in reproductive cycles of non-male factor infertility. *Fertil Steril* 2019;112(3):483–90.
- [30] Bingol B, Abike F, Gedikbasi A, Tapisiz OL, Gunenc Z. Comparison of chromosomal abnormality rates in ICSI for non-male factor and spontaneous conception. *J Assist Reprod Genet* 2012;29(1):25–30.
- [31] Vaiarelli A, Cimadomo D, Patrizio P, Venturella R, Orlando G, Soscia D, et al. Biochemical pregnancy loss after frozen embryo transfer seems independent of embryo developmental stage and chromosomal status. *Reprod Biomed Online* 2018;37(3):349–57.
- [32] Bou Nemer L, Shi H, Carr BR, Word RA, Bukulmez O. Effect of body weight on metabolic hormones and fatty acid metabolism in follicular fluid of women undergoing in vitro fertilization: a pilot study. *Reprod Sci* 2019;26(3):404–11.
- [33] Mejia RB, Cox TW, Nguyen EB, Summers KM, Eyck PT, Sparks AE, et al. Effect of body weight on early hormone levels in singleton pregnancies resulting in delivery after in vitro fertilization. *Fertil Steril* 2018;110(7):1311–7.