The Impact of Blastocyst Grade on Singleton Birthweight in Fresh Transplanted Cycles

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Abstract

Background: A retrospectively cohort study was performed to compare the birth weight of different blastocyst grades in fresh transplantation cycle and explore the related factors affecting the birth weight.

Methods: The 1301 fresh cycles of single blastocyst transplantation and single live birth profile were analyzed, four groups were divided according to the grade of transplanted blastocyst. There are 170 cycles in group A with AA blastocyst grade, 312 cycles in group B with AB/BA blastocyst grade, 559 cycles in group C with BB/CA/AC blastocyst grade and 260 cycles in group D with BC/CB blastocyst grade. Comparison were made among four groups of birth weight, general conditions, fertilization rate, embryo rate, cleavage rate and D5, D6 blastocyst formation rate and other laboratory indicators. And then comparison were performed among the birth weight of different groups which were divided by the degree of blastocyst expansion, and the classification of inner cell mass(ICM) and the trophoctoderm(TE).

Results: The study shows that the birth weight of group A is significantly higher than that of the other three groups ($P < 0.05$). And the high quality embryo rate and blastocyst rate of group A are significantly higher than those of the other three groups ($P < 0.05$). What's more, the clinical pregnancy rate, implantation rate and live birth rate of high grade blastocyst are higher, but there are no significant difference in abortion rate. The birth weight of the degree of blastocyst expansion in grade 3 and below is significantly lower than that of those with grade 3 and above ($P < 0.05$). The birth weight of grade A of ICM is significantly higher than that of grade B ($P < 0.05$). The birth weight of grade B of TE is significantly heavier than that of grade C ($P < 0.05$).

Conclusions: Our results indicate that high grade blastocyst transplantation can achieve better pregnancy outcome. Different blastocyst grades affect birth weight, and low grade blastocyst transplantation is associated with a single birth weight loss.

Background

With the development of assisted reproductive technology(ART), there are more than 5 million babies born by this way. However, in the process of in vitro fertilization and embryo transplantation(IVF-ET), even singletons live births can also increase the risk of adverse perinatal outcomes, including premature delivery, low birth weight and small gestational age problems, compared to their naturally conceived peer(1–3). In previous studies, more attention has been paid to the complications of infants born with ART, such as neonatal asphyxia, respiratory distress syndrome, intracranial hemorrhage, sepsis, etc(4), but there is a lack of information on birth weight around the world. Although the positive correlation between embryo quality and pregnancy outcomes has been well confirmed in many studies(5, 6), few studies research the impact of embryo quality on neonatal weight, especially the fresh transplanted blastocysts. Therefore, this study aims to compare the birth weight of different blastocyst grades in the fresh transplantation cycle of ART to explore the related factors affecting the birth weight of infants.

Methods

1. Study object

The patients who received single blastocyst transplantation in the reproductive center of the First Affiliated Hospital of Zhengzhou University from January 2016 to December 2019 were selected as the research subjects, both couples have normal chromosomes; Exclusion of female uterine malformation, endometriosis, ovarian dysfunction and other related factors of female infertility. According to the level of transplanted blastocyst, it was divided into four groups: 170 cycles in group A with AA blastocyst grade, 312 cycles in group B with AB/BA blastocyst grade, 559 cycles in group C with BB/CA/AC blastocyst grade and 260 cycles in group D with BC/CB blastocyst grade. Four groups were compared with laboratory indexes such as birth weight, general conditions, fertilization rate, embryo rate, cleavage rate, D5 blastocyst formation rate and D6 blastocyst formation rate. Then blastocysts were divided into different groups according to the degree of blastocyst expansion, inner cell mass(ICM) development and trophoctoderm(TE) appearance to compare the difference in birth weight.

2. Ovulation induction program and oocyte collection

Controlled hyperovulation was performed with gonadotropin (GN), then oocyte retrieval was preformed by transvaginal ultrasound-guided puncture 36-37 hours after HCG injection, and the ovum crown-cumulus complex (OCCC) was identified under an autopsy microscope and collected in G-MOPS Plus culture medium (Vitrolife, Sweden). When OCCC of one side ovary were collected, washing three times with G-IVF Plus (Vitrolife, Sweden) medium and placing in a petri dish with G-IVF Plus. Then it was put into a three-gas incubator at 37°C, 6% CO₂ and 5% O₂ for further cultured and matured.

3. Blastocyst culture and transplantation

Petri dishes for blastocyst culture were made the day before, D3 normal fertilized embryos, except for frozen embryos, were transferred to medium containing 50ul of G-2plus (Vitrolife, Sweden) for further culture, one embryo per droplet was cultured in a three-gas incubator until blastocyst stage. The blastocyst formation was observed and recorded at day 5 and 6. Blastocyst score was made according to Gardner scoring system, which was based on the grade of blastocyst expansion, as well as morphology of ICM and TE, the number of trophoblast cells and the density of the structure. Blastocyst grading: grade 1: early blastocyst, that is, blastocyst cavity volume is less than half of the total volume of blastocyst; Grade 2: blastocyst cavity volume is more than half of total blastocyst volume; Grade 3: blastocyst in complete dilated stage, the blastocyst cavity almost occupies the whole blastocyst; Grade 4: the expanded blastocyst, the volume of the blastocyst cavity was significantly larger than that of the early blastocyst, and the zona pellucida was thinner. Grade 5:

Inhatching blastocysts, which are breaking out of the zona pellucida; Grade 6: The blastocyst has completely emerged from the zona pellucida. ICM: A indicates a large number of cells and a close bound; B indicates that the number of cells is small and the combination is loose; C means very few cells. Trophoblast cells: A denotes a large number of cells that distributed around the blastocyst; B refers to a small number of cells and a loose combination of cells; C means very few cells.

4. Blastocyst transfer

Fresh blastocysts were transferred to ET dishes (G-2 plus Vitrolife, Sweden) for transfer within day 5. Before transplantation, blastocysts were transferred to another dish filled with 1ml Embryogluue (Vitrolife, Sweden). On the 35th day after transplantation, B-ultrasonography of the gestational sac was performed to determine whether a clinical pregnancy was possible.

5. Statistical analysis

SPSS 21.0 software was used for statistical analysis. The independent sample t test was used for statistical analysis of the general data of patients, represented by means±SDs. The laboratory indicators of embryo development and pregnancy outcome were tested by chi-square test, and the datas were represented by rate. $P < 0.05$ was considered statistically significant.

Results

1. General situation and birth weight of different grades of blastocyst group

A total of 170,312,559 and 260 infants respectively with the grade of AA/AB/BA/BB/AC/CA/BC/CB blastocysts were transplanted in this study. There were no differences in female age, infertility years, Body mass index

(BMI), Follicle-stimulating hormone(FSH),Luteinizing hormone(LH), Anti-Müllerian Hormone(AMH), intimal thickness on transplantation day, gestational age and birth sex among the four groups, but the grade of blastocyst transplant to AA's birth weight of infants is significantly higher than the other three groups, and infants of low birth weight in group A and group B were significantly lower than that of group C and D, the difference is statistically significant. Relevant data are shown in Table 1.

Table 1
General situation and birth weight of different grades of blastocyst group

Fresh	A	B	C	D	χ^2	P
cycle number(n)	170	312	559	260		
Female age(years)	29.14 ± 3.78	29.40 ± 3.36	29.25 ± 3.92	29.43 ± 3.87	1.597	0.188
Infertility duration(years)	3.73 ± 2.78	3.41 ± 2.67	3.53 ± 2.38	3.83 ± 2.85	1.240	0.294
Female BMI(kg/m2)	23.24 ± 3.29	23.25 ± 3.24	23.45 ± 3.31	23.43 ± 3.39	1.060	0.365
Female FSH(mIU/ml)	6.13 ± 1.46	5.77 ± 1.57	6.11 ± 1.57	6.16 ± 1.86	2.508	0.057
Female LH(mIU/ml)	6.57 ± 4.54	6.66 ± 4.79	6.42 ± 4.25	7.68 ± 3.96	2.368	0.069
Female AMH(ng/ml)	5.64 ± 3.37	5.86 ± 3.13	5.48 ± 3.29	5.45 ± 3.50	0.475	0.700
Endometrial thickness(mm)	12.40 ± 2.69	12.54 ± 2.33	12.45 ± 2.55	12.47 ± 2.41	0.120	0.948
Gestational age(weeks)	38.94 ± 1.45	38.82 ± 1.37	38.83 ± 1.33	38.63 ± 1.42	1.976	0.116
Percentage of male births(%)	57.65(98/170)	59.94(187/312)	57.96(324/559)	59.23(154/260)	0.113	0.990
Birthweight(g)	3512.69 ± 506.76 ^{B,C,D}	3401.24 ± 521.99	3395.26 ± 528.62	3343.58 ± 522.09	5.584	0.001
Birthweight≤2500g(%)	1.18(2/170) ^{C,D}	1.92(6/312) ^{C,D}	5.90(33/559)	6.15(16/260)	13.710	0.003
Note: B,C,D indicate that difference between the two groups is statistically significant, $P < 0.05$.						

2. Comparison of pregnancy outcomes in different grades of blastocyst

The number of embryo transplantation(ET) cycles at AA/AB/BA/BB/AC/CA/BC/CB blastocyst grade are 300,581,1073 and 567 respectively, there are significant difference in clinical pregnancy rate, implant rate, live birth rate among four groups, $P < 0.05$; while no difference observed on abortion rate between the four groups, $P > 0.05$. Datas are shown in Table 2. After abortion, Single-nucleotide polymorphism (SNP) detection cycle was counted, a total of 75 cycles, and 42 cycles is abnormal. Statistical analysis showed that there was no significant difference in the distribution of abnormal embryonic karyotype between

different groups. The data showed that 30 cases were trisomy, 2 cases were triploid, 6 cases were monomers and 4 cases were structural abnormality. Details were shown in Table 3.

Table 2
Comparison of pregnancy outcomes between different levels of blastocyst

Fresh	A	B	C	D	χ^2	P
<i>ET cycles(n)</i>	300	581	1073	567	-	-
<i>Clinical pregnancy rate[n(%)]</i>	196(65.33)	377(64.89)	675(62.91)	319(56.26)	11.766	0.008
<i>Planting rate[n(%)]</i>	192(64)	369(63.51)	665(61.98)	313(55.20)	11.117	0.011
<i>Live birth rate[n(%)]</i>	170(56.67)	312(53.70)	559(52.10)	260(45.86)	11.709	0.008
<i>Abortion rate[n(%)]</i>	20(10.2)	54(14.32)	89(13.19)	48(15.05)	2.758	0.431

Table 3
Analysis of abnormal SNP of different levels of blastocyst abortion

Abortion	A	B	C	D
<i>cycles[n(%)]</i>	8	21	30	16
<i>Trisome [n(%)]</i>	3(37.5)	10(47.6)	12(40.0)	5(31.3)
<i>Triploid[n(%)]</i>	0(0.0)	1(4.8)	0(0.0)	1(6.3)
<i>Monosome[n(%)]</i>	1(12.5)	0(0.0)	5(16.7)	0(0.0)

3. Comparison of laboratory conditions of different grades of blastocysts groups

2PN fertilization rate in group A is significantly higher than that in group D ($P < 0.000$); 2PN cleavage rate in group A is significantly higher than that in group D ($P < 0.001$); The high quality embryo rate and D6 blastocyst formation rate in group A are significantly higher than those in the other three groups ($P < 0.000$). The blastocyst formation rate of group A, B and C are significantly higher than that of group D ($P < 0.000$). The ratio of high quality blastocysts and D5 high quality blastocysts are significantly different between the groups ($P < 0.000$). Dates are shown in Table 4.

Table 4
Comparison of laboratory conditions of different grades of blastocysts groups

Fresh	A	B	C	D	χ^2	P
cycle number(n)	170	312	559	260		
2PN fertilization rate [n(%)]	2378(82.54) ^D	3972(81.79)	6880(80.94)	2889(78.99)	15.982	0.001
2PN cleavage rate [n(%)]	2362(99.33) ^d	3919(98.67)	6804(98.90)	2841(98.34)	11.921	0.008
Quality embryo rate [n(%)]	1920(81.29) ^{B,C,D}	3062(77.09)	5205(76.50)	1948(68.57)	125.528	0.000
blastocyst formation rate[n(%)]	1359(71.64)	2188(71.36)	3601(68.37)	1297(61.53) ^{A,B,C}	67.774	0.000
D5 blastocyst formation rate[n(%)]	920(48.50)	1928(62.88)	3138(59.58)	1023(48.53)	125.378	0.000
D6 blastocyst formation rate[n(%)]	439(23.14)	260(8.48)	463(8.79)	274(13.00)	319.152	0.000
Quality embryo ratio [n(%)]	756(39.85)	1056(34.44)	1334(25.33)	80(3.80)	841.012	0.000*
D5 quality embryo ratio [n(%)]	715(37.69)	996(32.49)	1255(23.83)	0	156.139	0.000*
D6 quality embryo ratio [n(%)]	41(2.16)	60(1.96)	79(1.50)	80(3.80) ^{A,B,C}	38.891	0.000
Note: *: There were significant differences between groups, after correction $P < 0.000$; "A","B","C","D" indicate that significant differences were detected between the two groups,after correction $P < 0.000$; "d" indicate that significant differences were detected between the two groups,after correction $P < 0.001$;						

4. Relationship between the grade of blastocyst expansion, ICM, TE level and birth weight

The birth weight of infants with blastocysts grade 3 and below is significantly lower than that of infants with blastocysts grade 3 and above ($P < 0.05$), while the former is significantly higher than the later in low birth weight, ($P < 0.05$). The birth weight of infants with level A of ICM is significantly higher than that of blastocysts with level B, and the incidence rate of low birth weight infants of group A is significantly lower than that of group B, with statistical significance ($P < 0.05$). The birth weight of infants with level B of TE is significantly higher than that of level C, $P < 0.05$. Seen Table 5.

Table 5
Relationship between cystic cavity expansion, endocytic cell mass, ectodermal trophoblast cell level and birth weight

Fresh	blastocyst level		χ^2	P	ICM		χ^2	P	TE	
	≤ 3	>3			A	B			A	B
n	289	1012	-	-	476	825	-	-	153	866
Birthweight(g)	3365.10 ± 571.65	3427.96 ± 533.57	1.738	0.041	3470.21 ± 515.02	3381.37 ± 526.74	8.727	0.003	3402.68 ± 492.13	3441.52 ± 526.13 ^C
Birthweight≤2500g	6.57(19/289)	2.87(29/1010)	8.659	0.003	1.26(6/476)	4.48(37/825)	9.819	0.002	2.61(4/153)	3.0(26/866)
Note : C: There was significant difference between group B and group C. $P < 0.05$.										

Discussion

Our study suggests that the grade of transplanted blastocysts is related to the birth weight of infants, and the birth weight of the grade AA blastocysts is significantly higher than that of other grades, and the incidence of low birth weight infants is significantly lower than that of other grades of blastocysts. At the same time, the grade of blastocyst expansion, ICM development and TE appearance all have a certain influence on the birth weight of infants, especially the blastocyst level above 3, the ICM level of the A blastocyst can better avoid the birth of low birth weight infants, this is consistent with previous research

results(7). However, Ebner et al. believe that TE is the only relevant parameter that significantly affects the outcome of pregnancy, and is related to the birth weight by affecting the growth and development of the placenta(8). We believe that this difference may be due to the different grouping standards used in different studies, and the differences in the ratings of embryos by operators of different proficiency. In addition, the effect of transplanted blastocyst quality on pregnancy outcomes is different(5, 9). The results of this study suggests that high-grade blastocyst transplantation can obtain better pregnancy outcome, but it has no significant effect on abortion rate.

Previous studies have shown that multiple pregnancy rate was obviously reduced by single blastocyst transplant(5, 10), thereby complications during pregnancy and the negative impacts of perinatal outcome were reduced(11), meanwhile, the clinical pregnancy rate and singletons live birth rate were improved(12), therefore, the proportion of blastocyst embryo transfer is increasing. Many studies have shown that high quality blastocyst transfer can achieve a better clinical pregnancy rate and live birth rate(13). Akamine Kozue et al, however, think that blastocyst quality has no significant effect on neonatal outcome, including birth weight(14). This difference may be related to its small sample size and single. Studies have suggested that the selection of transplanted blastocysts is very important for single blastocyst transplantation without affecting the embryo implantation rate, clinical pregnancy rate, live birth rate and other indicators(15, 16).

The causes of low grade blastocysts leading to birth weight loss are unclear. Some studies believe that the changes in deoxyribonucleic acid(DNA) methylation of poor quality embryos are uncontrollable(17), so epigenetic changes may play an important role. In addition, multiple exposures were involved in the in vitro culture stage of ART, such as changes in DNA methylation caused by embryo operation, culture and implantation, which may also had adverse effects on placenta development and fetal growth(17, 18). Studies have also shown that when embryos are damaged in development, they are more active in metabolism, stress and other aspects than normal embryos, producing more reactive oxygen species and adenosine triphosphate(ATP). Therefore, metabolic disorder and imbalance in vivo may also be the reason why low grade blastocyst transplantation leads to lower birth weight(19). Another cause of birth weight loss due to low grade blastocyst transplantation may be related to maternal uterine factors, In vitro studies have shown that the endometrium has the function of a biosensor to identify embryo quality(20), that is, after embryo implantation, it can actively transmit information to facilitate implantation by improving the internal uterine environment, decidualized endometrium stromal cells can receive and identify high quality and inferior embryos, and respond to them by producing different growth factors or cytokines, but the response to inferior embryos is greater than that of high quality embryos, more energy is required to participate in the repair of developmental damage. These reactions and changes may have an impact on the uterine environment of embryo implantation, thus affecting placenta formation and subsequent embryo development and fetal growth(21).

Conclusions

this study believes that in the fresh transfer cycle, the blastocyst level has a greater impact on the pregnancy outcome and the birth weight. The blastocyst grade is also related to laboratory indicators such as the rate of high quality embryos and the rate of high quality blastocysts. In the selection process of blastocyst transplant, paying attention to the size of the blastocyst cavity and ICM is beneficial to reduce the birth of low birth weight infants.

Abbreviations

ICM: Inner cell mass

TE: Trophectoderm

ART: Assisted reproductive technology

IVF-ET: In vitro fertilization and embryo transplantation

GN: Gonadotropin

OCCC: Ovum crown-cumulus complex

G-IVF:

CO₂:

O₂:

BMI: body mass index

FSH: Follicle-stimulating hormone

LH: Luteinizing hormone

AMH: Anti-Mullerian Hormone

ET: embryo transplantation

SNP: Single-nucleotide polymorphism

DNA: Deoxyribonucleic acid

Declarations

Ethics approval and content to participate

All study participants gave verbal informed consent, this study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University as it was a retrospective study and no interventions was given to the participants. Approval Number: 2019-KY-39. This manuscript reported adherence to Declaration of Helsinki.

Consent for publication

All authors gave their consent for publication.

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Author Contributions

SSL and GYH designed the study. SWY, ZFF, JHX, YGD and LY were responsible for the study subjects' enrollment and the collection of data. SQY, and SH analyzed the data and SQY was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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