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Developmental Potential and Clinical Value of Embryos with Abnormal Cleavage Rate*

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Summary: To investigate the developmental potential and clinical value of embryos with abnormal cleavage rate, a retrospective analysis was performed on 66 635 2-prokaryotic (2PN) and 1-pronuclear (1PN) embryos. The embryos were given conventionally *in vitro* fertilization (IVF) treatment and continuously cultured on the day 3 (D3) at the Reproductive Medicine Center, Tongji Medical College, Huazhong University of Science and Technology from January 2016 to December 2017. The embryos were separated into the day-2 (D2) undivided group with 106 cases, the arrested development group with 3482 cases, the blastomere reduction group with 541 cases, and the control group with 62 506 cases, respectively. The blastocyst utilization rates of these three abnormal groups were 2.83%, 10.86% and 6.84%, respectively, which were significantly different from that in control group (39.46%). Furthermore, 2 cases of anabiosis and 1 case of live birth were found in D2 undivided group. In arrested development group, there were 55 cases of anabiosis, 11 cases of clinical pregnancy in single-embryo transplantation (including 6 cases of live birth), and 25 cases of clinical pregnancy in combination with one normal embryo transplantation (including 23 cases of live births and 15 cases of dizygotic twins under B-ultrasound). There were 13 case of anabiosis in blastomere reduction group: there was 1 case of single embryo transplantation and clinical pregnancy was obtained; there were also 6 cases of clinical pregnancy in combination with one single normal embryo transplantation (including 5 cases of live births and 2 cases of dizygotic twins under B-ultrasound). In conclusion, embryos with abnormal cleavage rate still have the potential to continue to develop, and have certain blastocyst utilization rate and live birth.
Key words: abnormal cleavage rate; blastocyt culture; blastocyst utilization rate; live birth

Embryonic development is a dynamic process, and cleavage rate is currently recognized as the primary factor in the selection of embryos transplantation. However, the number of embryo blastomeres reflects the cleavage rate of embryos[1–4]. 4-cell at 44±1 h and 8-cell at 68±1 h are found when observing the pronucleus of the embryos with normal developmental rate at 17±1 h after normal insemination. The non-cleavage on the day 2 (D_2), the arrested development during D_2 to D_3, and the blastomere reduction on the D_3 are the main manifestations of cleavage abnormality. This study aims to investigate the developmental potential and pregnancy outcome of these abnormal embryos by continuously culturing them into blastocysts, and thus provide a basis for their clinical applications.

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1 OBJECTS AND METHODS

1.1 Objects and Grouping

This study retrospectively analyzed 66 635 2-prokaryotic (2PN) and 1-pronuclear (1PN) embryos that were treated with *in vitro* fertilization (IVF) and continuously cultured on the D_3 in the Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology from January 2016 to December 2017. The embryos were divided into D_2 undivided group (referring to the embryos that didn't divid on the D_2 and continued to develop on the D_3 after embryo fertilization), arrested development group (referring to one or more blastomeres stopped cleavage within 24 h and resulted in the development termination of embryos from D_2 to D_3), blastomere reduction group (referring to the number of embryo blastomeres on the D_3 is less than that on the D_2) and the control group

(referring to embryos with normal developmental speed while excluding from the above three groups). Then, the blastocyst utilization rate of embryos in the three groups with abnormal cleavage rate was compared with that of the control group. Thereafter, the pregnancy live births of the thawed embryos with abnormal cleavage rate were followed up to observe their clinical values.

All the patients gave the written informed consent. Institutional review board approval was not required because all patients in this study underwent routine IVF-ET clinical treatment at the center, and no additional intervention was performed.

1.2 Treatment Methods

According to the routine treatment strategy of the Reproductive Medicine Center of our hospital, different ovulation induction protocols (long protocol, antagonist protocol, micro-stimulation protocol, artificial corpus luteum protocol, etc.) were selected in consideration of female age, the basic follicles and the basic endocrine, etc. In terms of the factors related to infertility of patients, the routine IVF or intra-cytoplasmic sperm injection (ICSI) was implemented as fertilization method based on Human Assisted Reproductive Technology Operation Manual specified by the National Health and Family Planning Commission, China.

1.3 Evaluation Time Table of Embryos

The pronucleus was observed at 17±1 h after normal insemination, followed by 2-cell at 26±1 h, 4-cell at 44±1 h, 8-cell at 68±1 h, and blastocysts at 116±2 h, respectively.

1.4 Assessment of Embryos

The grading index of embryos at D_3 cleavage stage in our reproductive center was as follows: grade Ⅰ (7–10 blastomeres, blastomeres symmetry and fragments ≤5%, no abnormal cytoplasm); grade Ⅱ (7–10 blastomeres, blastomeres uniform or slightly uneven in size and fragments <20%, no abnormal cytoplasm); grade Ⅲ (blastomeres severely uneven in size and fragments <50%, or blastomeres not severely uneven in size and the fragments between 20%–50%); grade Ⅳ (fragments >50%). High-quality blastocysts were scored according to Gardner grading standards. Blastocysts were staged by the degree of embryonic expansion and hatching and then classified according to inner cell mass (ICM) and trophoblast cell (TE) scores. The blastocysts with levels higher than 3BC were regarded as utilizable blastocysts.

1.5 Treatment Plan of Embryos

The grade Ⅰ, Ⅱ embryos transplanted and frozen on the D_3 were further cultured to D_6. Then, their developmental potential was observed and further screening was done. The blastocysts with levels of 3BC and above 3BC on the D_5 and D_6 were vitrified and preserved as utilizable embryos. The embryos were frozen according to Kato vitrification operating instructions (Japan).

1.6 Embryo Thawing

During the thawing cycle, the embryos were re-warmed according to Kato vitrification operating instructions. The endometrium was prepared in a natural cycle or artificial cycle, and the progesterone or human choionic gonadotophin (hCG) was given after transplantation in order to support the corpus luteum.

1.7 Diagnostic Criteria

After 14 days of embryo transfer, the blood β-hCG levels were detected. The β-hCG levels higher than 25 mIU/mL were regarded as positive. Twenty-eight days later, vaginal B-ultrasound examination was done to observe the gestational sac, which suggested the clinical pregnancy.

1.8 Statistical Analysis

Statistical data analysis was performed by using the SPSS19.0 statistical software package. The data were expressed as mean±standard deviation (SD). The *t*-test was used to compare the mean between groups and the chi-square test was used to compare the ratio and composition ratio.

2 RESULTS

2.1 General Clinical Data of Embryos in Each Group

No significant difference was found in average age of the embryos and the proportion of primary infertility among groups. There was significant difference in methods of insemination between the three groups with abnormal cleavage rate and the control group (table 1).

2.2 Blastocyst Formation in Each Group

The utilizable blastocyst rate in the three groups with abnormal cleavage rate was significantly different from that in the control group (2.83%, 10.86%, 6.84% *vs.* 39.46%, $P<0.05$) (table 2).

Table 1 General clinical data of embryos in each group

Groups	Total number of successive culture on D_3	Methods of insemination (%)		Average age ($\bar{x}\pm s$)	Primary infertility (%)
		IVF	ICSI		
D_2 undivided	106	68/106 (64.15)*	38/106 (35.85)	30.84±4.32	74/106 (69.81)
Arrested development	3482	2380/3482 (68.35)*	1102/3482 (31.65)	30.67±4.78	2258/3482 (64.85)
Blastomere reduction	541	350/541 (64.70)*	191/541 (35.30)	30.39±4.69	368/541 (68.02)
Control	62 506	44 191/62 506 (70.70)	18 315/62 506 (29.30)	30.36±4.38	39 919/62 506 (63.86)

*$P<0.05$ *vs.* control group

2.3 Clinical Outcomes of Thawed Cycle

Three utilizable blastocysts were obtained in D2 undivided group (2.83%), one of which was a healthy live birth. Both the arrested development group and the blastomere reduction group had live births in pregnancy no matter it was a single embryo transfer or a combination with a normal embryo. The clinical pregnancy and live births in three groups with abnormal cleavage rate are shown in table 3.

3 DISCUSSION

The early development of the embryo is a complex dynamic process. Various physiological characteristics are shown as the development proceeds, which are related to the developmental potential of the embryo. Aneuploidy or genetic abnormalities in embryos are the main reasons for abnormal cleavage[5]. Athayde *et al*[6] reported that abnormal cleavage mode exists widely during embryonic development. In addition, some certain inappropriate culture conditions (such as pH, osmotic pressure and temperature changes) can also lead to abnormal embryonic development. For embryos with abnormal cleavage rate, embryos with more developmental potential can be screened by blastocyst culture, because embryos with poor developmental potential will arrest at the cleavage stage while the abnormality rate of chromosomes in embryos that can develop into blastocysts is significantly lower[7]. In this study, embryos with abnormal cleavage rate were screened by blastocyst culture in order to maximize the utilization of embryos.

The normal zygotes divide within a predetermined period of time with a low abnormality rate of embryonic chromosome while a high plantability[8]. In this study, 106 undivided embryos on the D_2 were further cultured and 3 utilizable blastocysts were formed, resulting in a low utilizable blastocyst rate. This result was consistent with the findings reported by Neuber *et al*[9]. Embryos with cleavage 42 h after fertilization exhibited much lower capability to develop into blastocysts. Zhao *et al*[10] observed the morphologies of nuclear/chromosomes and tubulin/spindle in zygotes without cleavage. They found that the abnormality in cell cycle transitions and the morphology of tubulin/spindle is one of the reasons why zygotes could not divide. Abnormalities in spindle and tubulin initiated spindle checkpoints, which delayed or arrested zygotes from completing the first meiosis. In addition, animal experiments showed that Polo-like kinase-1 (PLK1) played a key role in the first meiosis of zygotes. Zygote could not divide due to the lack of active PLK1[11].

The transcription and activation of embryonic genes means the transition from maternal modulation to embryonic modulation of gene expression. At this point, the embryo begins to synthesize its own mRNA and protein, which no longer relies on maternal genetic mRNA and protein. This smooth transition from maternal control to zygote control is essential for normal embryo development. However, the developmental arrest of embryos might be impeded by this transition and stopped at a specific developmental stage[12]. Studies[13] showed that embryos with developmental arrest possessed high chromosomal anomalies and most of them were chimeras and non-diploids. Our result showed that the utilizable blastocyst rate of embryos with arrested development was only 10.86%, which was consistent with the above-mentioned results. In addition, embryonic developmental arrest might be associated with mitochondrial dysfunction and production of reactive oxygen species, and the p^{66Shc} might play a key role in embryonic developmental arrest[14].

The reduction of blastomere mainly manifested as blastomere fusion or cleavage into fragments, and the utilizable blastocyst rate on D_2 and D_3 differing by one blastomere was 89.19% (33/37). This result suggested that under the condition of blastomere reduction, embryos with smaller difference in the number of blastomeres among two days always

Table 2 Blastocyst formation in each group

Groups	Total number of successive culture	Blastocyst formation number	Blastocyst formation rate (%)	Utilizable blastocysts number	Utilizable blastocysts rate (%)
D_2 undivided	106	15	14.14[a]	3	2.83*
Arrested development	3482	1079	30.99[b]	378	10.86*
Blastomere reduction	541	138	25.51[c]	37	6.84*
Control	62 506	42 452	67.92[d]	24 664	39.46

Different letters (a, b, c and d) represent significant difference among groups (P<0.05); *P<0.05 *vs.* control group

Table 3 Clinical observation of embryos in 3 groups with abnormal cleavage rate

Groups	Total transplantation (n)	Single embryo transplantation		Combined transplantation		
		Pregnancy (n)	Live birth (n)	Pregnancy (n)	Live birth (n)	Dizygotic twins (n)
D_2 undivided	2	1	1	0	0	0
Arrested development	55	11	6	25	23	15
Blastomere reduction	13	1	0	6	5	2

possessed more developmental potential. Moreover, the utilizable blastocyst rate of embryos differing by two or more blastomeres was as low as 10.81% (4/37), which exhibited poor development potential. As for blastomere fusion, some studies[15] reported that the use of antagonist programs or weak sperm motility might increase the incidence of blastomere fusion during cleavage, which was bad for embryonic development.

In this study, the embryos in blastomere reduction group and arrested development group with faster development rate on D_2 (≥6 cells) exhibited lower utilizable blastocyst rates, which were 1.11% (6/541) and 1.90% (66/3482), respectively. Magli *et al*[16] and Ambartsumyan *et al*[17] reported that excessive cleavage of embryos might indicate embryonic chromosomal abnormalities and the defects might be passed to more daughter cells. Moreover, embryos with faster cleavage might cause a decrease both in implantation rate and pregnancy rate because they might miss the implantation window.

The morphological evaluation of the embryos does not fully represent their developmental potential. Evidence from blastocyst culture verified that the embryos with abnormal cleavage rate had certain rate of blastocyst utilization and pregnancy productivity, and their clinical applications should be valued. Thus, a time-lapse camera system is recommended for medical centers in order to dynamically observe the development of embryo, which will facilitate the selection of high-quality embryos. Meanwhile, the safety will be improved if the preimplantation genetic screening (PGS) is performed before transplantation.

Conflict of Interest Statement

The authors declared no conflicts of interest to this work.

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