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Influence of Different Quality Sperm on Early Embryo Morphokinetic Parameters and Cleavage Patterns: A Retrospective Time-lapse Study*

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Summary: To investigate whether sperm with low concentration and motility can impact preimplantation embryos and to analyze how the effects present under a time-lapse incubation system, 2905 oocytes were collected from 219 couples between January 2014 and December 2015. Patients were divided into three groups according to sperm quality. Morphokinetic parameters and six cleavage patterns in the initial three cleavages were evaluated using the Primo Vision system. Embryo quality and clinic outcomes such as implantation rate, pregnancy rate and live birth rate were measured. The results showed that the concentration and motility of sperm correlated strongly with the rate of 2PN embryos, good-quality embryos on D3, blastocysts on D5/6 and good-quality embryos on D5/6. The time-lapse system recordings showed that compromised sperm quality could result in a significant delay in cc1 and a decrease in cc2, and impact embryo developmental potential mainly through large fragments or/and blastomere fragmentation in the initial three cleavages. In conclusion, sperm with low concentration and motility can have paternal effects on preimplantation embryos. These paternal effects present both as changes in morphokinetic parameters and cleavage patterns, which occur as early as fertilization and may cause severe damage to the preimplantation embryos.
Key words: embryo development; paternal effect; time-lapse; morphokinetic parameters; cleavage patterns

Approximately 12%–15% of couples of reproductive age suffer from infertility[1]. Both male and female factors can cause infertility, and the male partner is responsible for half of all infertility cases. Researches have shown that hormonal disturbances and abnormalities in the production of semen are the main causes of male infertility[2]. With the advent of assisted reproductive technology, especially intracytoplasmic sperm injection (ICSI) and percutaneous epididymal sperm aspiration followed by intracytoplasmic injection, many men with compromised sperm quality can now have their own children. However, studies have shown that sperm quality can influence fertilization rates, embryo morphology and the implantation rates, both after conventional IVF and ICSI[3, 4], which is widely referred to as the paternal effect.

Evidence suggests that paternal effects can occur as early as fertilization, and the terms “early” and “late” have been used to clarify different conditions. Early paternal effects are observed before embryonic genome activation (EGA), which begin at the 4-cell stage in humans. This effect may be related to the abnormal release of a putative oocyte-activating factor and/or dysfunctions in the centrosome and cytoskeletal apparatus[3, 4]. On the other hand, late paternal effects can influence the embryo’s genome activation, cause implantation failure or result in pregnancy loss[5]. Sperm nuclear/chromatin defects such as the presence of aneuploidy, DNA damage and genetic anomalies may be the cause of late paternal effects[6, 7].

Although many studies have examined the cause and effect of paternal effects, it remains to be determined whether different qualities of sperm can result in certain paternal effects on early embryo

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development. A systemic and holistic research is required to clarify the relationships between low sperm quality and embryo development, cleavage patterns and clinical outcomes. In addition, illustrating the influence of different quality sperm on early embryo development is well needed for embryo selection and reason identification.

Time-lapse monitoring is a new method for studying the kinetics of early embryo development *in vitro*. By photographing the embryos sequentially with cameras, short movies of developing embryos are generated, and the characteristics of embryonic preimplantation development can be studied. Previous studies have suggested that morphokinetic parameters and cleavage patterns observed using time-lapse monitoring can be used to predict embryo quality, blastocyst formation and clinical outcomes[8–11]. This new monitoring system can provide objective and accurate information for investigating the impacts of different factors on preimplantation embryos.

The objective of this study is to investigate the patterns of the paternal effect that may be caused by low sperm quality. To accomplish this goal, we used the time-lapse incubation system to record the morphokinetic parameters and cleavage patterns of embryos and analyze when paternal effects began and how they presented.

1 MATERIALS AND METHODS

1.1 Study Design and Patient Selection

The study was designed as a retrospective time-lapse analysis of 219 IVF/ICSI cycles conducted between January 2014 and December 2015 at the Reproductive Medicine Center of Tongji Hospital. Inclusion criteria were as follows: (1) female age ≤35 years; (2) basal FSH ≤10 mIU/mL; and (3) absence of disorders that could affect oocyte competence such as polycystic ovary syndrome (PCOS), pelvic endometriosis or metabolic and quality autoimmune syndromes. Oocyte donors and semen donors were excluded from the study.

Patients were divided into three groups according to sperm concentration and motility assessed using the World Health Organization 2016, 5th edition guidelines (>15 M/mL, >40% motility, >4% normal forms)[12]. Group A included patients with normal sperm quality who underwent standard IVF (n=62). Group B included patients who had either compromised sperm concentration or motility, but per high powered field (hpf) sperm count still >10 under an inverted microscope at 200× (n=70). Patients with severe oligo/asthenozoospermia (sperm count ≤10/HP) were classified as group C and received ICSI (n=87).

For this type of study formal consent is not required. This project was approved by the Institutional Review Board of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (No. TJ-IRB20180808).

1.2 Oocyte Retrieval, Sperm Preparation and Embryo Culture

The methods used for ovarian stimulation, sperm preparation and for IVF and embryo culture have been described previously[13]. Briefly, ovarian stimulation (GnRH agonist or GnRH antagonist protocol) was selected based on patient conditions. Recombinant follicle-stimulating hormone (Gonal F, Serono, Switzerland; or Puregon, MSD, USA) was used for ovarian stimulation. Ovulation induction was triggered using recombinant human chorionic gonadotropin (HCG, Livzon, China) when the second leading follicle was >18 mm in diameter. Semen was collected in sterile containers by masturbation after 3–5 days of sexual abstinence and kept at 37°C for 30 min. After liquefaction, samples were assessed under an inverted microscope at 200×. Patients with normal sperm quality received IVF. Patients with oligospermia received ICSI, and extremely severe male factor infertility may receive percutaneous epidymimal sperm aspiration (PESA) or testicular sperm aspiration (TESA). The fertilized oocytes were then continuously cultured in G1 medium (Vitrolife, Sweden) for 3 days inside a CO_2 incubator that contained a digital time-lapse microscope (Primo Vision; Vitrolife, Sweden).

1.3 Embryo Grading and Time-lapse Recording

Conventional grading of embryos was performed based on the Istanbul consensus in 2011[14]. On D3, good quality embryos were defined as having equally sized, mononucleated blastomeres in a three-dimensional tetrahedral arrangement, with 7–9 cells and less than 20% fragmentation. On D5/6, blastocysts were evaluated based on the criteria established by Gardner *et al*[15]. Good-quality blastocysts had scores of 3BB or higher. One or two embryos with the best grade were selected for transfer. The additional good-quality embryos were cryopreserved on D3 or cultured to D5 or D6. Implantation was confirmed if β-hCG reached values over 60 IU/L after 13 days. A clinical pregnancy was confirmed by observation of at least a fetal heartbeat via ultrasound analysis 5 weeks after the transfer.

During culture, morphokinetic parameters of each cell division in the initial three cleavages were evaluated. Time for the appearance and disappearance of pronuclei was defined as the first point when pronuclei appeared (PNA) or faded (PNF). Time for the cleavage points (T2–T8) was defined as the time when the blastomeres were completely separated. Duration of the first cell cycle (cc1; T2-PNF), second cell cycle (cc2; T3–T2), third cell cycle (cc3; T5–T3) and time to complete synchronous divisions s2 (T4–T3)

and s3 (T8–T5) were calculated. Development events were also annotated, and seven cleavage patterns were defined: (i) normal cleavage (one blastomere dividing into two even blastomeres and producing fragments less than 10%); (ii) large fragments (LF) (fragments larger than 20% after three cleavage cycles); (iii) blastomere fragmentation (BF) (blastomere turning into fragments during division); (iv) uneven (UB) (the average diameter of one blastomere was 20% larger than the another one); (v) direct cleavage (DC) (cleavage directly from 1 cell to more than 3 cells); (vi) reverse cleavage (RC) (two daughter blastomeres recombining into one cell after complete separation or incomplete separation); and (vii) developmental arrest (DA) (blastomere did not enter the next cell cycle or less than 4 cells were seen by day 3) (fig. 1).

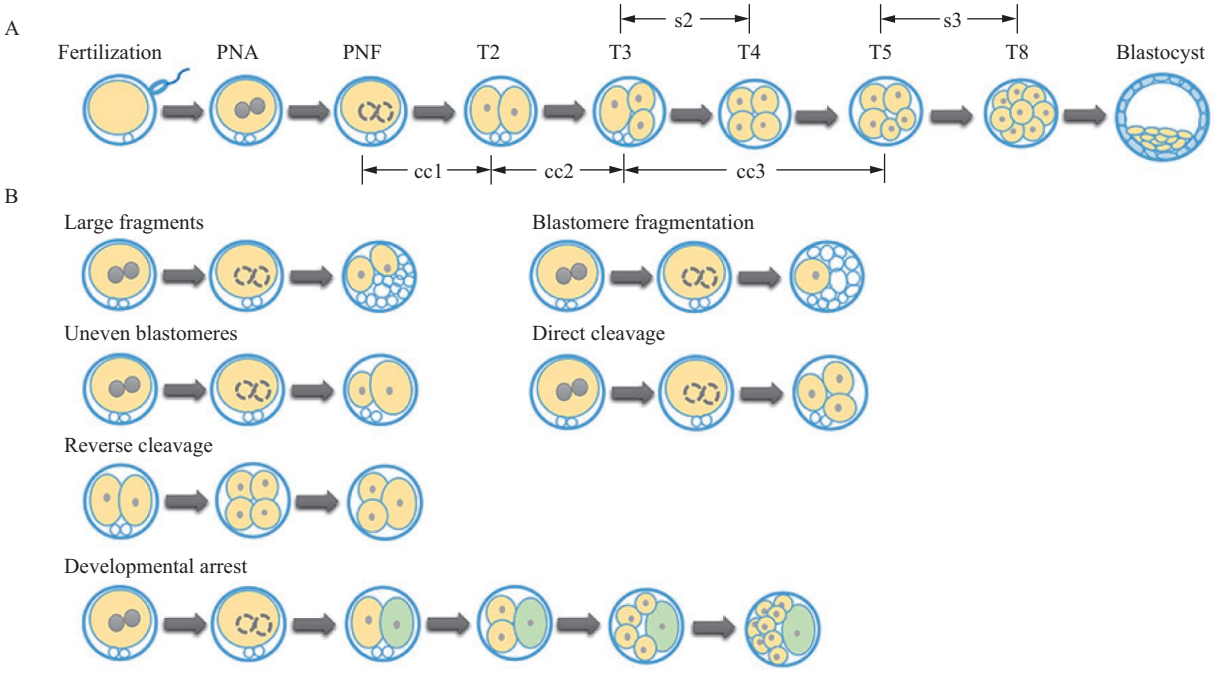

Fig. 1 The observed morphokinetic parameters and cleavage patterns (see definition in the text)
A: the description of normal cleavage and observed morphokinetic parameters in the first three days' development; B: the description of observed abnormal cleavage patterns

1.4 Statistical Analyses

Data analysis was performed using The Statistical Package for Social Sciences (SPSS) for Windows, version 18.0 (USA). An analysis of variance (ANOVA) was used to evaluate statistically significant differences in patient characteristics and time points of embryos. The chi-squared tests were performed to evaluate the biological outcomes. Kruskal-Wallis test was performed to analyze the frequencies of each cleavage pattern in the embryos. Logistic regression analysis was used to examine the relationship between abnormal cleavage patterns and blastocyst formation. $P<0.05$ was considered statistically significant.

2 RESULTS

2.1 Patient Characteristics

Overall, 219 couples were included in this study and divided into three groups as previously described. A description of sperm quality and clinical characteristics of patients of the three groups is shown in table 1. Female age decreased from group A to group C ($P<0.05$), which may be related to the rising percentage of couples with primary infertility ($P<0.05$). There were no significant differences in female basal FSH, female BMI, male age, male BMI, total gonadotropin dose or the number of oocytes retrieved between groups.

2.2 Biological Outcomes

Table 2 shows the biological outcomes of three groups. The 2PN rates in group C were significantly lower than those in group A or group B ($P<0.05$). Among fresh cycles, 291 embryos were transferred to 152 women, and no significant differences were seen in the implantation rate, clinical pregnancy rate or live birth rate. Up to August 2018, 208 couples had received fresh or cryopreserved embryo transfer. There were no significant differences in cumulative pregnancy rate or cumulative live birth rate.

There were obvious differences among the three groups regarding embryo quality before implantation. In group A, the rate of good-quality embryos on D3, the rate of blastocyst on D5/6 and the rate of good-

quality embryos on D5/6 were the highest among the three groups, while they were the lowest in group C (P<0.05).

2.3 Morphokinetic Parameters

To understand the impact of sperm quality on embryo development, we compared the morphokinetic parameters from PNA to T8. Surprisingly, significant differences were found at almost all time points (table 3). Using PNF as t0 to account for the artificial difference between standard IVF- and ICSI-fertilized embryos[16, 17], t2 was significantly delayed from group A to group C (table 4). For the duration of the cell cycles, cc1 was defined as T2-PNF and was equated with t2. cc2 declined from group A to group C (P<0.05). No significant differences were seen in cc3, s2 or s3.

2.4 Abnormal Cleavage Patterns

As paternal effects may present as abnormal cleavage patterns, we analyzed abnormal cleavage patterns as described in fig. 1 (table 5). In groups B and C, the proportions of embryos presenting LF were

Table 1 Clinical characteristics of patients

Predictors	Group A (n=62)	Group B (n=70)	Group C (n=87)	Total (n=219)	P value
Female age (years)	29.23±3.26[a]	28.89±3.29[ab]	27.82±3.32[b]	28.56±3.34	0.023
Female basal FSH (mIU/mL)	6.79±1.27	7.03±1.34	6.99±1.30	6.95±1.30	0.529
Female BMI (kg/m^2)	21.93±3.08	21.06±2.99	21.82±2.83	21.61±2.96	0.166
Male age (years)	31.55±3.69	31.94±4.66	30.78±4.78	31.37±4.47	0.255
Male BMI (kg/m^2)	24.45±3.28	23.07±3.19	23.61±3.42	23.68±3.34	0.059
Sperm quality					
Concentration (×10^6/mL)	63 (48–75)	35 (12–51.25)	2 (1–4)		
Motility (%)	50 (40–59.25)	10 (3–22)			
Primary infertility (%)	24 (39)[a]	49 (69)[b]	79 (92)[c]	152 (69)	<0.001
Total gonadotropin dose (IU)	2024.31±607.50	2077.11±789.45	2012.61±772.34	2036.54±732.57	0.851
No. of oocytes retrieved	13.98±3.41	12.50±3.92	13.37±4.48	13.26±4.05	0.104

Values are expressed as mean±standard deviation (SD), number (rate), or mean (25%–75%). BMI: body mass index; FSH: follicle-stimulating hormone. [a, b, c]: Different superscript in the same row indicates statistical significance (P<0.05).

Table 2 Biologic outcomes of patients

Biologic outcomes	Group A	Group B	Group C	P value
Retrieved oocytes	867	875	1163	
2PN embryos (%)	568 (65.5)[a]	547 (62.5)[a]	645 (55.5)[b]	<0.001
Good quality embryos on D3 (%)	387 (68.1)[a]	324 (59.2)[b]	375 (58.1)[b]	0.001
Embryos cultured to D5/6	473	431	507	
Blastocysts on D5/6 (%)	263 (55.6)[a]	216 (50.1)[ab]	227 (44.8)[b]	0.003
Good blastocysts on D5/6 (%)	107 (22.6)[a]	74 (17.2)[ab]	65 (12.8)[b]	<0.001
Fresh embryos transfered	90	93	108	
Fresh cycle implanted embryos (%)	35 (39)	38 (41)	35 (32)	0.426
Fresh cycles	46	48	58	
Fresh cycle pregnancy (%)	25 (54)	28 (58)	27 (47)	0.463
Fresh cycle live birth (%)	24 (52)	20 (42)	21 (36)	0.258
Cumulative cycles	61	67	80	
Cumulative pregnancy (%)	47 (77)	48 (72)	59 (74)	0.782
Cumulative live birth (%)	42 (69)	39 (58)	51 (64)	0.457

Values are expressed as number and rate. [a, b]: Different superscript in the same row indicates statistical significance (P<0.05).

Table 3 Morphokinetic parameters of early embryos (fertilization as T0)

Time points (fertilization as T0)	Group A	Group B	Group C	P value
PNA	8.06±2.82	7.96±2.68	8.41±4.55	0.065
PNF	24.52±4.18[a]	25.08±4.23[b]	25.68±5.61[c]	<0.001
T2	27.07±4.40[a]	27.82±4.80[b]	28.63±6.34[c]	<0.001
T3	36.50±5.94[a]	35.13±9.34[b]	36.84±6.99[a]	<0.001
T4	38.87±5.80[a]	37.34±11.09[b]	39.83±6.83[c]	<0.001
T5	48.95±8.32[a]	41.96±18.68[b]	48.74±8.89[a]	<0.001
T6	52.90±6.78	52.24±7.78	52.94±7.29	0.226
T7	54.84±6.35[ab]	54.34±6.76[a]	55.42±6.17[b]	0.028
T8	56.79±6.00[a]	55.95±6.10[b]	56.95±6.03[a]	0.030

Values are expressed as mean±standard deviation (SD). [a, b, c]: Different superscript in the same row indicates statistical significance (P<0.05).

significantly higher than those in group A (P<0.05). Embryos with BF significantly increased from group A to group C (P<0.05). However, embryos with DC decreased from group A to group C (P<0.05). Embryos with UB, RC or DA showed no significant differences among groups.

We further analyzed the frequency of abnormal cleavage patterns that occurred in each embryo. As LF and DA could only occur once per embryo, we did not present the results repeatedly (table 6). The frequency of BF in each embryo rose from group A to group C (P<0.05). The frequency of DC in group C was significantly lower than that in group A (P<0.05). There were no significant differences in the frequencies of UB and RC (table 6).

2.5 Logistic Regression Analysis

It seemed that sperm with low concentration and motility was associated with elevated LF and BF and decreased DC. As different division patterns have different effects on blastocyst formation, a logistic regression analysis was performed to find out which cleavage patterns had greater impact on blastocyst formation and embryo quality (table 7). Six abnormal cleavage patterns were included as independent variables and the results demonstrated that embryos with BF were 5 times as likely to fail to form blastocyst (OR: 0.18, CI: 0.11–0.31; P<0.05). LF could significantly impact blastocyst formation and embryo quality on D5 (OR: 0.28, CI: 0.19–0.43; P<0.05; OR: 0.19, CI: 0.08–0.46, P<0.05, respectively). Although

Table 4 Morphokinetic parameters of early embryos (PNF as t0)

Time points (PNF as t0)	Group A	Group B	Group C	*P* value
t2 (cc1)	2.72±1.39[a]	2.88±2.15[ab]	3.06±2.89[b]	0.037
t3	12.02±5.02	11.53±5.42	11.44±5.27	0.144
t4	14.40±4.94	14.66±5.32	14.49±4.77	0.705
t5	24.79±8.30	24.13±8.33	24.23±8.39	0.407
t6	28.63±6.79	27.97±7.12	28.48±6.45	0.328
t7	30.79±6.40	30.44±6.00	31.22±5.28	0.195
t8	33.18±6.27[a]	32.12±5.44[b]	33.24±5.62[a]	0.029
cc2	10.79±6.38[a]	9.69±6.20[b]	9.43±6.24[b]	0.001
cc3	15.75±9.64	14.62±8.32	15.16±8.82	0.151
s2	5.24±10.15	5.18±9.57	5.17±9.58	0.991
s3	10.36±10.57	12.25±12.30	12.11±12.93	0.085

Values are expressed as mean±standard deviation (SD). [a, b]: Different superscript in the same row indicates statistical significance (P<0.05).

Table 5 The number of embryos with abnormal cleavage patterns

Embryos with abnormal cleavage patterns	Group A (n=575)	Group B (n=539)	Group C (n=648)	*P* value
Large fragment (LF)	42 (7.3)[a]	66 (12.2)[b]	89 (13.7)[b]	0.001
Blastomere fragmentation (BF)	57 (9.9)[a]	75 (13.9)[ab]	111 (17.1)[b]	0.001
Uneven blastomeres (UB)	96 (16.7)	107 (19.9)	129 (19.9)	0.276
Direct cleavage (DC)	107 (18.6)[a]	90 (16.7)[a]	79 (12.2)[b]	0.006
Reverse cleavage (RC)	38 (6.6)	40 (7.4)	63 (9.7)	0.113
Developmental arrest (DA)	49 (8.5)	31 (5.8)	55 (8.5)	0.135

Values are expressed as number and rate. [a, b]: Different superscript in the same row indicates statistical significance (P<0.05).

Table 6 The frequency of embryos with abnormal cleavage patterns

Frequencies of abnormal cleavage patterns	Group A (n=575)	Group B (n=539)	Group C (n=648)	Kruskal-Wallis test
	Mean rank	Mean rank	Mean rank	
Blastomere fragmentation (BF)	847.21[a]	881.62[a]	911.82[b]	0.001
Uneven blastomeres (UB)	863.02	890.35	890.53	0.294
Direct cleavage (DC)	908.45[a]	890.35[a]	850.23[b]	0.005
Reverse cleavage (RC)	869.38	876.12	896.73	0.112

[a, b]: Different superscript in the same row indicates statistical significance (P<0.05).

Table 7 Logistic regression analysis of relationship between cleavage patterns and embryo development

Logistic regression analysis	Blastocyst formation on D5/6		Embryo quality on D5/6	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Large fragment (LF)	0.18 (0.11–0.31)	<0.001	0.24 (0.08–0.68)	0.007
Blastomere fragmentation (BF)	0.28 (0.19–0.43)	<0.001	0.19 (0.08–0.46)	<0.001
Uneven blastomeres (UB)	0.61 (0.46–0.81)	0.001	0.70 (0.48–1.02)	0.064
Direct cleavage (DC)	0.78 (0.59–1.05)	0.098	0.61 (0.41–0.90)	0.013
Reverse cleavage (RC)	0.40 (0.26–0.61)	<0.001	0.17 (0.07–0.41)	<0.001
Developmental arrest (DA)	0.03 (0.01–0.08)	<0.001	0.05 (0.01–0.39)	0.004

decreased DC had a positive influence on embryo quality on D5/6 (OR: 0.61; CI: 0.41–0.90, $P<0.05$), this positive effect was low as compared with significant impacts induced by LF and BF.

3 DISCUSSION

Previous studies have shown that some sperm deficiencies such as malformations and DNA damage can cause paternal effects in preimplantation embryos and induce repeated failure of ART attempts[4]. With the integration of time-lapse monitoring into IVF/ICSI cycles, quantitative and deep assessments of paternal factors on embryo development are able to be implemented. It has been reported that sperm morphology has some correlation with early morphokinetic parameters of embryos[18]. Different sperm origin (testicular, epididymal or ejaculated sperm) can significantly influence some kinetics of embryo development[12, 19, 20]. However, little is known about whether sperm with low concentration and motility can cause alterations in preimplantation embryo kinetics. This study investigated the influence of sperm concentration and motility on embryo morphokinetic parameters and abnormal cleavage patterns. The comprehensive and extensive recordings of our study enable us to have an integrated and completed analysis of this paternal effects on preimplantation embryos. It also provides us some guidance for embryo selection.

The analysis of biological outcomes of normal and compromised sperm quality groups indicates that low sperm quality can cause declines in 2PN rates and reduce good quality embryos on D3 and D5. While the clinical outcomes of fresh and cumulative cycles showed no statistically significant differences in the implantation rate, pregnancy rate or live birth rate. The reason may be that only the relatively good embryos were chosen for implantation in clinic, of which the impact from low sperm quality was not obvious. Another reason may be that the low concentration and motility mainly manifested as early paternal effects and influenced the early embryo development before implantation, while the genome of embryos was less damaged so the pregnancy rate and live birth rate were not influenced.

In the last few years, morphokinetic parameters have been found to be associated with the biological competence and developmental potential of the embryo[21, 22]. So we compared the morphokinetic parameters of the initial three cleavages to determine the influence of sperm quality on embryos. However, our retrospective study has certain limitations. Couples with normal sperm quality did not receive ICSI in our center. Therefore, we had to compare groups who received different ART methods. It's indicated in the references that during early cleavage stages, embryos grow more rapidly following ICSI than standard IVF when insemination is used as the starting point (T0). However, normalization to a common time point of PNF can eliminate the artificial difference between ART methods and permit the joint analysis of IVF- and ICSI-fertilized embryos[16, 17]. In our study, timing of PNF increased from group A to group C (24.52±4.18 *vs.* 25.08±4.23 *vs.* 25.68±5.61 h), which is probably due to a combined influence of sperm quality and ART methods. After adjusting all morphokinetic parameters to the time of PNF (t0), t2 (T2-PNF) still showed an increase from group A to group C. It is reasonable to consider t2 to be the kinetic parameter most affected by low sperm quality. Additionally, with declining sperm quality, the duration of the first cycle (cc1; t2) increased, but the duration of the second cycle (cc2; T3–T2) decreased. Many time-lapse studies have indicated the relationship between duration of cell cycle and developmental potential of the embryo. Prolonged cc1 and cc2 can adversely affect pregnancy outcomes after fresh embryo transfer on Day 2[23]. Several researches reported that cc2 can be used for predicting blastocyst formation, implantation or ploidy[21, 24]. It is reasonable to infer that the sperm quality can influence the cell cycle division and then influence the developmental potential of embryo.

Studies have shown that abnormal cleavage patterns can negatively affect the development of embryos[10, 25]. In our study, we discovered a higher occurrence and frequency of LF and BF in severe asthenospermia/oligospermia. A logistic regression analysis further indicated that LF and BF could cause tremendous damage in early developmental embryos. Mechanistically, large fragments and blastomere fragmentation have been found to be correlated with apoptosis and altered gene expression[25], which may be the causes of decreased developmental potency of embryos in asthenospermia/oligospermia groups.

The declines in 2PN rates, the morphokinetic changes in t2 and cc2, and the non-difference clinical outcomes all indicate that sperm with low concentration and motility mainly produces early paternal effects in preimplantation embryos, rather than causes implantation failure or pregnancy loss defined as late paternal effects. Early paternal effects are said to be related to the abnormal release of oocyte-activating factor (OAF) and/or dysfunctions of the centrosome and cytoskeletal apparatus. Although the mechanism of oocyte activation by the spermatozoa is unclear, it has been suggested that nearly 40% of failed fertilizations are caused by disordered oocyte activation after ICSI[26]. The declines in 2PN rates in our study may be a failure from the abnormal release of OAF. In our research, t2 (T2-PNF) represents the anaphase and telophase stages of the first mitotic cell cycle. cc2 is defined as T3-T2 and represents all stages of the second mitotic cell

cycle. The changes in these parameters may represent paternal effects caused by dysfunctional centrosomes or microtubules, which play vital roles in the mitotic cell cycle[4, 27].

In conclusion, the present study has demonstrated that (1) sperm with low concentration and motility can cause paternal effects in preimplantation embryos; (2) these paternal effects can present as various morphokinetic parameters, mainly in cc1 and cc2; (3) low-quality sperm causes developmental failure in embryos mainly by creating LF and BF; and (4) these effects mainly manifested as early paternal effects that occur as early as fertilization and may severely damage the preimplantation embryos. Our conclusion is helpful for embryo selection and patients' consulting. However, as a retrospective study, our research has some limitations. Due to its small sample size, it is uncertain whether compromised sperm quality will produce failures in implantation and pregnancy. Further large scale study is still needed.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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