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Impact of sperm characteristics on time-lapse embryo morphokinetic parameters and clinical outcome of conventional in vitro fertilization

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Abstract

Background: Sperm abnormalities may negatively affect embryo development.

Objectives: To determine the influence of sperm abnormalities (morphology, motility, DNA fragmentation) on embryo morphokinetic variables and clinical outcome of conventional IVF.

Materials and methods: Participants were 86 couples undergoing in vitro fertilization (IVF). Sperm morphology was evaluated according to the strict criteria proposed by Kruger/Tygerberg. CASA system was applied for sperm motility assessment. Sperm DNA fragmentation was assessed by the chromatin structure assay (SCSA). Morphokinetic parameters were determined in 223 embryos obtained from conventional IVF only and cultured in a single-step medium using time-lapse imaging technology.

Results: Time-lapse variables from the initial embryo development, such as time of pronuclei fading (tPNf) and time for two cells (t2), were those more strongly related with abnormalities of sperm motility, morphology, and DNA fragmentation. Sperm morphological abnormalities rather than sperm motility were more closely associated with embryo morphokinetics. Sperm head defects were mainly correlated with the last stages of embryonic development (t9 to tHB), sperm midpiece defects with intermediate cleaving embryos (t5-t9), and sperm tail defects with the initial stages of embryonic development (tPNa-t4). Excess residual cytoplasm was positively correlated with all embryo morphokinetic parameters except t2 and tM. Absence of acrosomes, pinheads, coiled tails, and multiple sperm morphological defects correlated negatively with time-lapse embryo morphokinetic variables.

Discussion: A large number of sperm-related variables, including frequency of specific morphological defects, morphological indexes, DNA fragmentation and motility, and time-lapse embryo variables, such as time intervals based mainly of 15 time points were recorded.

Stefka Nikolova and Dimitar Parvanov contributed equally and share first authorship credit. The authors consider that the first two authors should be regarded as joint First Authors.

Conclusion: There were strong associations between specific sperm defects of the head, midpiece, and tail with certain stages of embryonic development from observation of pronuclei to the hatched blastocyst. Coiled tail, cumulative head defects, and multiple abnormalities index (MAI) were associated both with embryo morphokinetics and the implantation success.

KEYWORDS

embryo development, embryo morphokinetics, in vitro fertilization, sperm morphology, time-lapse imaging

1 | INTRODUCTION

The introduction of time-lapse imaging technology allows continuous observation of embryo development without removal from controlled and stable incubator condition. This technology offers several potential advantages over conventional methods, including improved culture conditions with decreased frequency of handling and exposure of embryos to the risks of stress from temperature changes, high oxygen exposures, and pH changes in the culture media.[1,2] Also, by serial imaging, more information on embryo development is obtained, allowing morphokinetic monitoring (ie, assessment of the quality of embryos by tracking the timing of events and length of different intervals in embryo development), which adds another dimension to embryo selecting and grading.[3] Several predictive models based on morphokinetic parameters and time-lapse evaluation with varying outcomes have been proposed[4-7] but the general applicability of these algorithms is limited for data that do not originate from very similar clinical conditions.[8] Also, according to results of recent systematic reviews and meta-analysis, there is insufficient good-quality evidence to determine the superiority of time-lapse systems over conventional methods for human embryo incubation and selection.[9,10]

The evaluation of sperm characteristics, such as motility, DNA fragmentation, and morphological abnormalities of spermatozoa, appears to be useful for predicting successful fertilization, implantation, embryo development, and ongoing pregnancy.[11-13] Sperm morphology is correlated with sperm DNA damage since morphological abnormalities of sperm indicate elevated levels of sperm DNA fragmentation.[14] However, the influence of sperm abnormalities on embryo morphokinetics remains unclear. Previous studies assessing the relationship between sperm quality and embryo development are based on conventional sperm (concentration, motility, and morphology)[15,16] and embryo quality parameters.[17-20]

There is little information about the relationship between sperm morphology and time-lapse embryo morphokinetics. The present study was designed to assess whether sperm morphological abnormalities may have an influence on early embryo morphokinetic parameters and clinical outcome of conventional IVF. To our knowledge, this is the first study that compares sperm morphological characteristics, that is, the frequency of certain defects, with embryo morphology using time-lapse technology.

2 | MATERIAL AND METHODS

2.1 | Study design and participants

This was an observational study aimed to examine a potential association between the presence of sperm morphological abnormalities and embryo morphokinetics. It was hypothesized that early embryo morphology may be affected by some abnormal sperm morphological characteristics. The study was carried out at Nadezhda Women's Health Hospital in Sofia, Bulgaria. All experimental procedures and sample procurements were approved by the Ethics Committee of the hospital. Written informed consent was obtained from all participants.

A total of 86 couples undergoing in vitro fertilization (IVF) for treatment infertility at the Nadezhda Women's Health Hospital from April 2013 to July 2015 were included in the study. All women included in the study underwent oocyte retrieval after standard long stimulation protocol. Other inclusion criteria were top-quality oocytes fertilized only by conventional IVF (n = 223) and embryos cultured only in a global single-step culture medium. Exclusion criteria were as follows: men or women with known genetic disorders, men with severe oligospermia (<1 million spermatozoa/mL), women aged > 42 years, women with a body mass index (BMI) <18 kg/m^2 or > 30 kg/m^2, and women undergoing oocyte retrieval after other stimulation protocols (natural, short, or mild IVF cycles) and signs of ovarian hyperstimulation. Oocytes fertilized using intracytoplasmic sperm injection (ICSI) (n = 395) were also excluded.

2.2 | Semen handling and analysis

Semen samples were collected by masturbation after 3-5 days of abstinence on the day of oocyte retrieval for IVF. The sperm samples were prepared by simple wash and subsequent resuspension. The semen was allowed to liquefy and then washed with *AllGrad Wash® medium (LifeGlobal Europe, Brussels, Belgium)* and centrifuged

at 450 *g* for 5 min. The supernatant was discarded, and sperms were collected from the pellet. Semen analyses were performed according to the fifth edition of the WHO guidelines.[21] Semen analysis included sperm concentration, sperm motility, and sperm morphology. A Sperm Class Analyzer® (SCA) CASA system (SCA Microptic SL) was employed to assess kinetic parameters and sperm count. Sperm morphology was evaluated in a single sample counting 100 cells, according to the strict criteria proposed by Kruger/Tygerberg[22,23] and adopted by the World Health Organization[21] using the Diff-Quik® staining set (Microptic SL).

The total number of spermatozoa with morphological abnormalities of the head, midpiece, and tail were recorded. Defective conditions for heads included large, small, tapered, pyriform (pear-shaped), round, and amorphous heads (formless), heads with a small cap area (<40% of the head area), and double heads, as well as any combination of these. Midpiece defects included bent neck, asymmetrical insertion of the midpiece into the head, irregular midsection, and abnormally thin and thick midsection, cytoplasmic droplet, and excess residual cytoplasm (ERC) as well as a combination of these. Tail defects included short, multiple, hairpin, broken, or bent tails; coiled tails; and terminal droplet as well as a combination of these. The total number of head, midpiece, and tail defects found per 100 spermatozoa in a sample was expressed as cumulative head, cumulative midpiece, and cumulative tail defects, respectively. Spermatozoa that had more than one morphological abnormality were classified as having multiple defects. Spermatozoa with borderline morphologies were counted as abnormal. Normal and abnormal sperm categories were defined using the strict criteria approach as proposed in the 2010 WHO manual. The multiple abnormalities index (MAI) was calculated as the average number of abnormalities per abnormal spermatozoon.[21,24]

Sperm DNA fragmentation was assessed by the chromatin structure assay (SCSA) following the protocol established by Evenson et al[25] For each semen sample, a total of 10,000 acridine orange-labeled spermatozoa were evaluated using the FACSCalibur flow cytometer (BD Biosciences) and SCSA data analyzed using BD CellQuest™ Pro software (BD Biosciences). Results were expressed as sperm %DFI (an index of DNA damage) and sperm %HDS (a measure of nuclear chromatin compaction).[26]

2.3 | In vitro fertilization (IVF) procedure and embryo culture

All patients underwent controlled ovarian stimulation by a standard long protocol following pituitary downregulation with GnRH analog (3.75 mg) and subsequent addition of rFSH (300–375 IU/day) until at least three or more follicles had attained a mean diameter of 18 mm. Oocyte retrieval was performed up to 36 h after hCG trigger injection was administered (5000 IU). Metaphase-II oocytes were graded, and only those without any irregularities, defined as top-quality oocytes, were included in the next stages of the study. The criteria applied for estimation of the oocyte quality included morphological assessment of zona pellucida, cytoplasm, and first polar body (PB1) using conventional phase-contrast microscopy. Good quality oocytes showed clear, colorless zona pellucida, clear cytoplasm with uniform texture and homogeneous fine granularity, a round or ovoid non-fragmented PB1, and perivitelline space of normal size.

All matured oocytes included in this study were fertilized by conventional IVF, and embryos were cultured individually according to standard procedures. Fertilization check was performed 16-18 h after insemination by the presence of two pronuclei. The embryos were individually cultured under mineral oil, in 20-μL droplets of single-step medium (Global Total, IVFonline, Guelph, Ontario, Canada) at 37°C in an atmosphere of 6% CO_2, 5% O_2.

High-quality cleavage stage (day 3) embryos were defined as those with equal size 6-8 cells, no multinucleation, and ≤ 10% of fragmentation. High-quality blastocysts (day 5) were defined as having an inner cell mass and trophoectoderm with many cells, tightly packed or several cells, loosely grouped.

2.4 | Embryo morphokinetic assessment

All embryos were cultured in single-step culture medium (Global®, LifeGlobal) and EmbryoscopeTM time-lapse incubator (Vitrolife). During embryo cultivation, 15 morphokinetic parameters were recorded, including time of pronuclei appearance (tPNa), time of pronuclei fading (tPNf), cleavage times (t2, t3, t4, t5, t6, t7, t8, t9), morulae formation time (tM), starting blastulation (tSB), full blastocyst stage (tB), expansion (tEB), and hatching timing (tHB). All events were counted from t0 as the time of insemination. Definitions included tPNa, time of first visualization of both pronuclei; tPNf, time to pronuclear fading; t2, t3, t4, t5, t6, t7, t8, and t9, times for the corresponding number of cells (t2 for 2 cells, t3 for 3 cells, etc); tM, first frame in which the embryos were compacting into the morula stage; tB, the frame in which a crescent-shaped area began to emerge from the morula; and tEB, the frame of expanded blastocyst consistent with the increase of the overall volume of the embryo and expansion of the blastocoel cavity. In order to minimize the operator-dependent variation, especially in blastocyst annotation, two embryologists were specifically trained and performed the annotation together according to the published guidelines.[27]

2.5 | Statistical analysis

Quantitative data are expressed as mean and standard deviation (± SD). Data were checked for normal distribution using the Kolmogorov-Smirnov test and analyzed with the Student's t test or the Wilcoxon rank-sum test or the Mann-Whitney *U* test according to conditions of application based on normal or not normal distribution of data. The relationship between sperm morphological variables, sperm motility, sperm DNA fragmentation, and embryo morphokinetic parameters was analyzed with the Spearman's

rank-order correlation coefficient (ρ). Statistical significance was set at $P < .05$. Statistical analyses were performed with SPSS statistical software for Windows, version 21.0 (SPSS).

3 | RESULTS

The study population comprised 86 couples undergoing IVF during the study period, with a total of 86 semen samples and 223 embryos fertilized through conventional IVF. Main baseline characteristics and clinical outcome data of the study patients are shown in Table 1. As shown in Table 2, immotile sperm cells were observed in more than half of the samples, and small acrosomes, acrosome vacuoles, large head, amorphous head, thick midsection, and short and coiled tails were the most common sperm abnormalities. Multiple defects were found in a mean of 27.2% of samples, with cumulative head defects as the most remarkable Figure 1. The mean MSI was 1.9. The mean %DFI was 25.1 and the mean %HDS 19.7%. Data of time-lapse embryo morphokinetic parameters are shown in Table 3.

TABLE 1 Baseline characteristics and clinical outcome data of 86 couples included in the study

Baseline characteristics	Mean ± SD	Range (min-max)
Men (n = 86)		
Age, years	38.3 ± 7.1	27-65
Body mass index, kg/m^2	21.5 ± 6.2	18.4-27.1
Duration of abstinence, days	3.6 ± 1.7	1-10
Semen pH	8.0 ± 0.3	7.0-9.0
Sperm count, × 10^6	52.8 ± 53.8	6.7-310.0
Round cells, × 10^6	1.6 ± 2.5	0-16
Women (n = 86)		
Age, years	36.9 ± 5.0	25-42
Body mass index, kg/m^2	23.8 ± 4.9	18-29
Baseline FSH, mIU/mL	8.9 ± 4.3	2.3-21.5
Oocytes retrieved	12.9 ± 3.1	2-16
Mature oocytes	10.6 ± 4.2	2-13
Oocytes fertilized	6 ± 2	2-9
Fertilization rate	67.8%	-
Clinical outcome[a]	Number	%
Biochemical pregnancy (implantation)	42	48.8
Clinical pregnancy	38	44.2
Clinical pregnancy loss	12	13.9
Live births	26	30.2

Abbreviations: FSH, follicle stimulating hormone; SD, standard deviation.

[a]After the first (single) embryo transfer.

The association between the main types of sperm morphology abnormalities (head, midpiece, and tail defects) and embryo morphokinetics is shown in Figure 2. Table 4 shows the correlations

TABLE 2 Sperm characteristics

Characteristics	Mean ± SD	Range (min-max)
Motility, grading, %		
"a" (rapidly progressing > 20 µm/s)	16.6 ± 8.7	0-42
"b" (slowly progressive 5-20 µm/s)	19.5 ± 6.9	2-34
"c" (non-progressive < 5 µm/s)	9.9 ± 3.2	1-24
"d" (immotile)	53.9 ± 15.0	24-96
Morphology		
Normal forms, %		
Non-strict criteria	7.6 ± 4.1	2-19
Strict criteria	4.8 ± 3.4	1-15
Round cells, × 10^6/mL	1.6 ± 2.5	0.1-16
Morphological abnormalities, %		
Head defects		
Long head	12.8 ± 9.0	0-34
Macrocephalic head	15.4 ± 11.3	0-52
Pyriform head	12.1 ± 9.5	0-41
Microcephalic head	12.7 ± 11.5	0-52
Round head	7.7 ± 5.1	0-26
Amorphous head	13.5 ± 7.6	0-36
Double head	0.3 ± 0.9	0-5
Pinheads	2.8 ± 2.5	0-8
Acrosome vacuoles	20.6 ± 14.0	4-61
Nuclear vacuoles	5.4 ± 5.2	0-30
Small acrosomes	21.7 ± 12.9	0-70
Large acrosomes	3.9 ± 3.9	0-14
Absence of acrosomes	9.1 ± 7.0	0-27
Midpiece defects		
Asymmetrical insertion	3.3 ± 3.0	0-11
Bent neck	5.5 ± 4.7	0-20
Thick midsection	8.4 ± 6.5	0-29
Thin midsection	3.3 ± 2.9	0-13
Excess residual cytoplasm (ERC)	5.1 ± 4.8	0-24
Tail defects		
Short tail	6.6 ± 7.6	0-37
Coiled tail	5.4 ± 6.0	0-27
Double tail	0.7 ± 1.8	0-10
Multiple anomalies index (MAI)	1.9 ± 0.3	1-3
DNA fragmentation		
%DFI	25.1 ± 16.1	5-71
% HDS	19.7 ± 11.9	3-66

Abbreviation: SD, standard deviation.

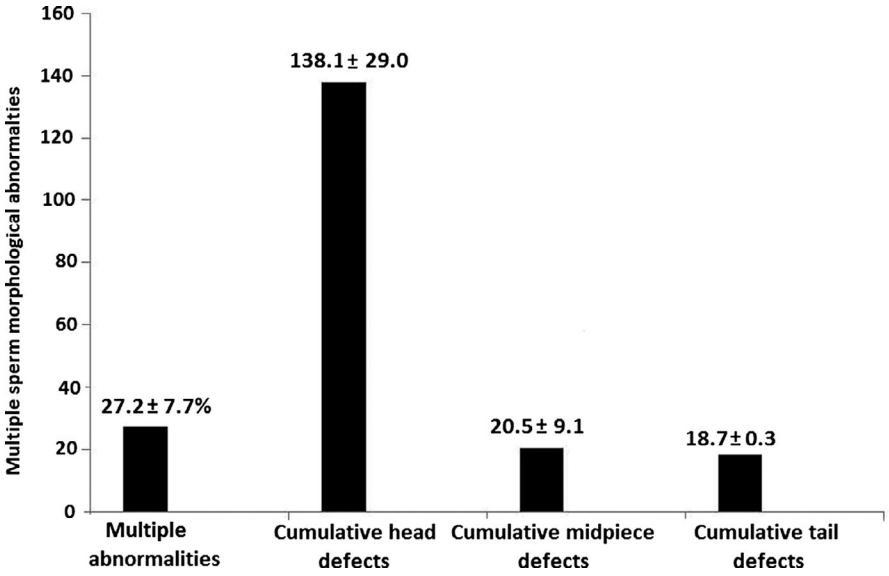

FIGURE 1 Multiple sperm morphological abnormalities. Data expressed as mean ± standard deviation of percentages. Cumulative defects are expressed as (mean ± standard deviation) number of certain type defects (head, midpiece, or tail) per 100 spermatozoa

TABLE 3 Results of time-lapse embryo morphokinetic parameters

Morphokinetic variables	Mean ± SD (h)	Range (Min-Max)
tPNa, pronuclei appearance	8.0 ± 2.6	5-26
tPNf, pronuclei fading	24.5 ± 3.7	17-42
t2 (two cells)	27.6 ± 4.3	20-43
t3 (three cells)	38.0 ± 5.0	23-58
t4 (four cells)	39.8 ± 5.1	23-61
t5 (five cells)	51.0 ± 7.6	23-76
t6 (six cells)	53.3 ± 7.3	24-89
t7 (seven cells)	55.5 ± 7.7	34-89
t8 (eight cells)	58.1 ± 9.6	39-112
t9 (nine cells)	72.1 ± 11.0	49-113
tM (morula formation)	90.6 ± 10.3	65-121
tSB (starting blastulation)	102.7 ± 7.7	89-123
tB (blastocyte stage)	107.2 ± 6.0	92-125
tEB (expansion)	111.4 ± 5.9	95-126
tHB (hatching timing)	116.8 ± 4.6	109-128

between sperm-related variables and time-lapse embryo morphokinetic variables with statistical significance ($P < .05$). Time-lapse variables from the initial embryo development, such as time of pronuclei fading (tPNf) and time for two cells (t2), were those more strongly correlated with abnormalities of sperm motility, morphology, and DNA fragmentation. In general, sperm morphological abnormalities were more closely associated with embryo morphokinetics as compared to sperm motility. Sperm head defects were mainly correlated with the last stages of embryonic development (t9 to tHB), sperm midpiece defects with intermediate cleaving embryos (t5-t9), and sperm tail defects with the initial stages of embryonic development (tPNa-t4). Excess residual cytoplasm (ERC) was positively correlated with all embryo morphokinetic parameters except t2 and tM. Negative correlations between sperm abnormalities and time-lapse embryo morphokinetic variables were found for absence of acrosomes, pinheads, coiled tails, and multiple sperm morphological defects Table 4.

3.1 | Sperm characteristics, embryo morphokinetics, and clinical outcome

Clinical outcome of the first (single) embryo transfer in the 86 couples included in the study is shown in Table 1. All embryos were obtained from conventional IVF, and successful implantation was recorded in 42 (48.8%) out of 86 cases. Sperm characteristics and time-lapse variables were compared between cases with successful and unsuccessful implantation. Significant differences were found regarding specific sperm tail (coilded tail) and sperm head abnormalities (cumulative head defects, nuclear vacuoles, and large acrosomes) and MAI Table 5. Among the studied embryo time-lapse variables, the mean values of tPNf, t2, and tSB were significantly lower in the embryos with successful implantation Table 5.

4 | DISCUSSION

Our hypothesis that early embryo morphology may be affected by some abnormal sperm morphological characteristics was confirmed. It is noteworthy that this is the first study that compares the frequency and the presence of certain sperm morphological abnormalities with embryo morphology using time-lapse imaging. Of note, the complexity of the study in which a large number of sperm-related variables, including frequency of specific morphological defects, morphological indexes, DNA fragmentation and motility, and time-lapse embryo variables, such as time intervals based mainly of 15 time points were recorded. This is a novel and salient characteristic of the study.

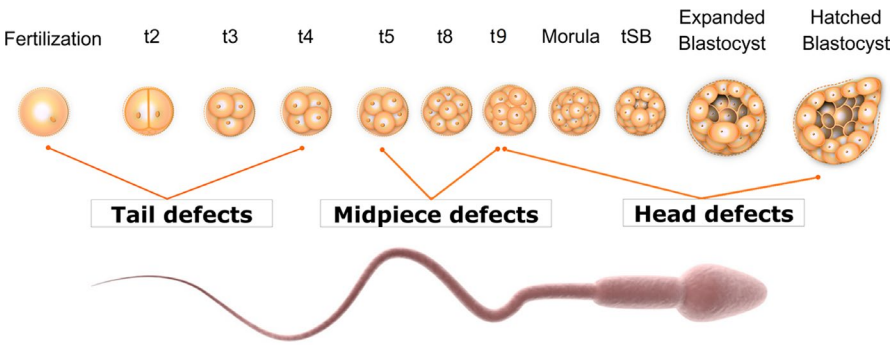

FIGURE 2 Association between the main types of sperm morphological abnormalities and embryo morphokinetic parameters

Sperm defects in the current group of male subjects were quite expectable and comparable to data reported in other studies.[28-31] Abnormal sperm morphologies are associated with fertilization failure in couples undergoing IVF programs, and the percentage of normal sperm morphology is an important predictor of time to pregnancy.[32,33] Also, there is evidence that the percentage of normal sperm morphology may indeed decrease in men with recurrent spontaneous abortion partners compared with normal controls.[34] On the other hand, in recent years, gene defects have been demonstrated as the cause of specific sperm abnormalities[35] and deleterious effects that affect the chromatin condensation and DNA integrity could occur during spermatogenesis.[36]

We found statistically significant relationships between certain sperm defects and embryo morphokinetic parameters. Overall, an interesting finding was a positive correlation between sperm characteristics and time-lapse kinetic markers from the early stages of embryonic development (tPNf and t2). Other authors have shown that these early markers are strongly associated with successful embryo implantation and clinical pregnancy.[37,38]

On the other hand, one of the most tightly bound variables with all stages of 5-day embryo development was ERC, which is a specific sperm midpiece abnormality. ERC contains elevated levels of enzymes that produce pathological amounts of reactive oxygen species.[39] ERC is known to be associated with oxidative stress-mediated sperm DNA fragmentation,[40] and in our study, ERC also showed a significant correlation with DFI (r = .28, P = .05). It has been shown that ERC affects sperm motility,[41] morphology,[42] and fertilization potential.[43] Our study reveals that this specific type of midpiece defect has a considerable influence on embryo morphokinetics as ERC was positively correlated with almost all kinetic variables.

The observed correlation coefficients between time-lapse embryo variables and sperm motility parameters were low as compared to those between embryo characteristics and sperm morphological abnormalities. Although there is a relationship between sperm motility and sperm morphology, these two types of parameters reflect different types of problems in the process of spermatogenesis (eg, environmental/lifestyle impacts)[44] and could result in specific effects on embryo morphokinetics.

Sperm head defects were mainly correlated with the last stages of embryonic development, sperm midpiece defects with intermediate cleaving embryos, and tail defects with the initial stages of embryonic development. In a prospective study of semen samples from non-azoospermic men, an inverse relationship between normal head morphology forms and the percentage of cells with high DNA stainability was found, suggesting that head abnormalities may, in part, be due to incomplete sperm chromatin condensation.[45] In another study, DNA sperm fragmentation led to delayed cell division in donated oocytes.[46] In our study, sperm characteristics were analyzed in sperm samples before each IVF procedure, and the data obtained has external validity for fresh sperm used for IVF. In a retrospective study in which the impact of sperm origin (fresh ejaculate or surgically retrieved) on embryo morphokinetics was assessed, clinically relevant differences between the sperm groups were not found.[20]

Nowadays, embryonic time-lapse parameters are becoming an essential tool in the process of embryo selection for embryo transfer. There is an increasing data about the predictive ability of these parameters for clinical pregnancy and live birth.[47-50] Predictive models based on different time-lapse variables have been proposed and tested in retrospective analyses.[50-53] Most of these models are based on t2 to t5, as well as intervals between these time points. However, other models use tPNf and t8[8] or even later events of embryo development such as tEB.[54] Our study showed that the most discussed time-lapse biomarkers for successful implantation, which are predominantly early events, were mainly correlated with sperm tail abnormalities. However, certain head defects such as acrosomal abnormalities also showed association with early stages of embryo development.

Analyzing the clinical outcome of the performed embryo transfers, we found out that unsuccessfully implanted embryos had significant delay in pronuclei fading (tPNf), division to two cells (t2) but also delay in a later event—starting of blastocyst (tSB). These results confirmed the impact of specific time points of embryo development on clinical outcome. Moreover, our data revealed that successfully implanted embryos usually originated from spermatozoa with significantly lower MAI, lower number of cumulative head defects, and decreased percentage of nuclear vacuoles, large acrosomes, and coiled tails. In agreement with our findings, another recent study has shown that sperm abnormalities such as acrosome defects and coiled tails have relatively high frequency of occurrence in sperm population.[55] Furthermore, the authors found similar decrease in abovementioned sperm defects in infertile men, compared to the fertile ones.

Interestingly, sperm head defects such as nuclear vacuoles and large acrosomes which do not have a significant correlation with

TABLE 4 Correlation between sperm-related variables and time-lapse embryo morphokinetic parameters

	Time – lapse embryo morphokinetic parameters														
Sperm-related variables	**tPNa**	**tPNf**	**t2**	**t3**	**t4**	**t5**	**t6**	**t7**	**t8**	**t9**	**tM**	**tSB**	**tB**	**tEB**	**tHB**
Round cells, × 10^6/mL		−0.23	−0.17		−0.15			−0.23	−0.24			−0.31	−0.34	−0.25	
Motility grading, %															
"a"			0.17					0.17			0.189	0.21	0.21	0.31	
"b"			0.15												
"c"			0.15												
"d"		−0.14	−0.18												
Head defects, %															
Macrocephalic											−0.33				
Amorphous	0.26	0.24								0.26				0.41	
Pinheads	−0.35	−0.23			−0.26						−0.33	−0.35	−0.36		−0.61
Acrosome vacuoles														0.35	
Small acrosomes					−0.31		−0.37	−0.35	−0.37						
Absence of acrosomes		−0.20	−0.24	−0.27	−0.32	−0.27	−0.36	−0.35	−0.38						
Midpiece defects															
Asymmetrical insertion						0.28	0.26								
Bent neck						0.29	0.29	0.24	0.26	0.28					
Excess residual cytoplasm (ERC)		0.31		0.3	0.36	0.36	0.41	0.41	0.44	0.48		0.36	0.43	0.53	
Tail defects		−0.37													
Short		−0.30	−0.34	−0.29											
Coiled				−0.23	−0.25										
Multiple defects		−0.38	−0.34	−0.29	−0.32										
Cumulative head defects							−0.30		−0.27	−0.26					
Cumulative tail defects													−0.34		
Multiple anomalies index (MAI)			−0.25												
DNA fragmentation, %															
%DFI															
%HDS			−0.36	−0.25											

Note: Data in the table are Spearman's rank-order correlation coefficient, rho (ρ). Only statistically significant correlations ($P < .05$) are shown.

TABLE 5 Sperm morphological characteristics and embryonic morphokinetic parameters with statistically significant difference ($P \leq .05$) between cases with successful and unsuccessful implantation

Characteristics	Successful implantation (n = 42)	Unsuccessful implantation (n = 44)	*P* value
Morphological abnormalities, %	Mean ± SD	Mean ± SD	
Spermatozoa			
Head defects			
Nuclear vacuoles	4.26 ± 4.02	6.33 ± 4.83	.05
Large acrosomes	2.97 ± 2.99	4.67 ± 3.42	.05
Cumulative head defects	131.60 ± 23.44	143.02 ± 24.35	.05
Tail defects			
Coiled tail	4.06 ± 3.70	6.37 ± 3.15	.04
Multiple anomalies index (MAI)	1.77 ± 0.35	1.93 ± 0.26	.02
Embryos			
Morphokinetic variables, hours			
tPNf, pronuclei fading	23.93 ± 2.26	24.65 ± 4.14	.05
t2 (two cells)	26.65 ± 2.45	27.71 ± 4.33	.01
tSB (starting blastulation)	100.63 ± 6.47	104.36 ± 8.14	.03

embryo morphokinetics were associated with implantation success, and contrary to expectations, other sperm abnormalities that have an essential impact on many embryo time-lapse variables (ERC, pinheads, absence of acrosomes, etc), including on tPNf, t2, and tSB, were not related to the studied clinical outcome. Sperm parameters which were both associated with the predictor's time-lapse variables and the implantation success were coiled tail, cumulative head defects, and MAI. Finally, the observed diverse impact (multilevel effect) makes these variables good potential predictors for the clinical outcome following conventional IVF.

5 | CONCLUSION

The present findings based on a single-center experience using time-lapse imaging and exclusively high-quality embryos add evidence of the relationship between sperm morphological abnormalities and embryo morphokinetic parameters. The study highlights the presence of significant correlations between specific sperm defects of the head, midpiece, and tail with stages of embryonic development from observation of both pronuclei to the hatched blastocyst. The implantation rate was 48.8%, the clinical pregnancy rate 44.2%, and live births 30.2%. Certain sperm defects (coiled tail, cumulative head defects, and MAI) have impact not only on the embryo morphokinetics but also on the implantation success. For this reason, they could be considered as good potential predictors for the clinical outcome.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors qualify for authorship by contributing substantially to this article. SN, DP, and GS developed the original concept and design of the study. SN, DP, VG, II, and RG collected the data, DP performed the statistical analysis, and DP, SN, and GS provided input to the interpretation of the data. All authors have contributed to critical discussion and reviewed the final version of the article and approve it for publication.

HUMAN RIGHTS STATEMENT AND INFORMED CONSENT

All the procedures were followed in accordance with the ethical standards of the responsible committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and its later amendments. Informed consent was obtained from all the patients to be included in the study.

ANIMAL STUDIES

This article does not contain any study with animal participants that were performed by any of the authors.

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