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Association between sperm morphology and embryo cleavage rate in in vitro fertilization: a retrospective study

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Abstract

Introduction. Infertility is a major reproductive health problem, and in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) is widely used to improve conception in infertile couples. Embryo cleavage rate is an important laboratory indicator of early embryo development, while sperm morphology may reflect sperm structural and genetic integrity.

Aim: to analyze the association between sperm morphology and embryo cleavage rate in patients undergoing IVF/ICSI.

Materials and Methods. This analytical retrospective study used medical records of infertile couples undergoing IVF/ICSI at Dr. Hasan Sadikin Central General Hospital (Bandung) from January 2020 to December 2024. Patients with complete semen analysis based on World Health Organization (WHO) 2021 criteria and embryo development data up to day 2 post-fertilization were included. Sperm morphology was classified as normospermia or teratozoospermia, and cleavage rate as rapid, normal, or slow. Statistical analysis was performed using the Chi-square test, with odds ratios (ORs) and 95 % confidence intervals (CIs).

Results. A total of 159 records were analyzed. Sperm morphology was significantly associated with cleavage rate (p = 0.001). Teratozoospermia was found in 97.0 % of rapid cleavage cases, 47.3 % of normal cleavage cases, and 93.9 % of slow cleavage cases. Teratozoospermia was associated with higher odds of rapid cleavage (OR = 35.636; 95 % CI = 4.673–271.760) and slow cleavage (OR = 17.261; 95 % CI = 3.903–76.340).

Conclusion. Sperm morphology is significantly associated with embryo cleavage rate in IVF/ICSI. Teratozoospermia may be related to altered timing of early embryo cleavage and may serve as an additional prognostic parameter in IVF/ICSI counseling and laboratory assessment.

Keywords: sperm morphology, teratozoospermia, cleavage rate, in vitro fertilization, IVF, intracytoplasmic sperm injection, ICSI, infertility

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Взаимосвязь между морфологией сперматозоидов и скоростью дробления эмбрионов при экстракорпоральном оплодотворении: ретроспективное исследование

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Резюме

Введение. Бесплодие является серьезной проблемой репродуктивного здоровья, и для улучшения возможности зачатия у бесплодных пар широко используется экстракорпоральное оплодотворение (ЭКО) с интрацитоплазматической инъекцией сперматозоидов (ИКСИ). Скорость дробления эмбриона является важным лабораторным индикатором его раннего развития, в то время как морфология сперматозоидов может отражать структурную и генетическую целостность сперматозоидов.

Цель: проанализировать взаимосвязь между морфологией сперматозоидов и скоростью дробления эмбрионов у пациентов, проходящих ЭКО/ИКСИ.

Материалы и методы. В аналитическом ретроспективном исследовании использовали медицинские карты бесплодных пар, проходивших ЭКО/ИКСИ в Центральной больнице общего профиля имени доктора Хасана Садикина (Бандунг) с января 2020 г. по декабрь 2024 г. В исследование были включены пациенты с полным анализом спермы на основе критериев Всемирной организации здравоохранения (ВОЗ) от 2021 г. и данными о развитии эмбрионов

до 2-го дня после оплодотворения. По морфологии сперматозоидов выделяли нормоспермию или тератозооспермию, а скорость дробления классифицировали как быструю, нормальную или медленную. Статистический анализ проводили с использованием критерия χ^2 с расчетом отношения шансов (ОШ) и 95 % доверительных интервалов (ДИ).

Результаты. Всего было проанализировано 159 записей. Морфология сперматозоидов была значимо связана со скоростью дробления (p = 0,001). Тератозооспермия была обнаружена в 97,0% случаев быстрого дробления, в 47,3% случаев нормального дробления и в 93,9% случаев медленного дробления. Тератозооспермия была связана с более высокими шансами быстрого дробления (ОШ = 35,636; 95 % ДИ = 4,673–271,760) и медленного дробления (ОШ = 17,261; 95 % ДИ = 3,903–76,340).

Заключение. Морфология сперматозоидов значимо связана со скоростью дробления эмбрионов при ЭКО/ИКСИ. Тератозооспермия может быть связана с изменением сроков раннего дробления эмбрионов и может служить дополнительным прогностическим параметром при консультировании по поводу ЭКО/ИКСИ и при лабораторной оценке.

Ключевые слова: морфология сперматозоидов, тератозооспермия, скорость дробления, экстракорпоральное оплодотворение, ЭКО, интрацитоплазматическая инъекция сперматозоидов, ИКСИ, бесплодие

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Highlights	Основные моменты
What is already known about this subject?	Что уже известно об этой теме?
Infertility affects many couples worldwide, and in vitro fertilization with intracytoplasmic sperm injection (IVF/ICSI) is a widely used assisted reproductive technology for managing infertility.	Бесплодие затрагивает значительное число супружеских пар во всем мире, и экстракорпоральное оплодотворение с интрацитоплазматической инъекцией сперматозоидов (ЭКО/ИКСИ) широко применяется как метод вспомогательных репродуктивных технологий.
IVF success is influenced by multiple factors, including sperm quality, oocyte quality, embryo development, endometrial receptivity, and patient characteristics.	Успешность ЭКО зависит от многих факторов, включая качество спермы, качество ооцитов, развитие эмбриона, рецептивность эндометрия и характеристики пациентов.
Sperm morphology may influence fertilization and embryo development through its association with oocyte penetration, DNA integrity, oxidative stress, and early embryonic cleavage.	Морфология сперматозоидов может влиять на оплодотворение и развитие эмбриона через связь с пенетрацией ооцита, целостностью ДНК, оксидативным стрессом и ранним дроблением эмбриона.
What are the new findings?	Что нового дает статья?
This study evaluates the association between sperm morphology and cleavage rate among IVF patients	В исследовании оценивается связь между морфологией сперматозоидов и частотой дробления эмбрионов у пациентов, проходящих

undergoing ICSI at Dr. Hasan Sadikin Central General Hospital, Bandung.	ЭКО с применением ИКСИ в Центральной больнице общего профиля имени доктора Хасана Садикина в Бандунге.
The study classifies sperm morphology as normal or abnormal and cleavage rate as high or low, allowing focused analysis of their relationship.	В работе морфология сперматозоидов классифицируется как нормальная или патологическая, а частота дробления – как высокая или низкая, что позволяет провести целенаправленный анализ их взаимосвязи.
The findings may clarify whether sperm morphology remains clinically relevant for predicting early embryo cleavage after ICSI.	Полученные результаты могут уточнить, сохраняет ли морфология сперматозоидов клиническую значимость как предиктор раннего дробления эмбриона после ИКСИ.
How might it impact on clinical practice in the foreseeable future?	Как это может повлиять на клиническую практику в обозримом будущем?
The results may help clinicians consider sperm morphology as an additional prognostic parameter in IVF/ICSI counseling and treatment planning.	Результаты исследования могут помочь клиницистам рассматривать морфологию сперматозоидов как дополнительный прогностический параметр при консультировании и планировании ЭКО/ИКСИ.
Improved understanding of sperm morphology and cleavage rate may support better embryo assessment and optimization of laboratory sperm selection strategies.	Более глубокое понимание связи между морфологией сперматозоидов и частотой дробления может способствовать улучшению оценки эмбрионов и оптимизации лабораторных стратегий отбора сперматозоидов.
The study may provide local evidence to guide reproductive specialists in improving IVF outcomes among infertile couples.	Исследование может предоставить локальные данные, полезные для репродуктологов при разработке подходов к повышению эффективности ЭКО у бесплодных пар.

Introduction / Введение

Infertility is a reproductive health issue affecting approximately 8–12 % of couples worldwide. One of the commonly employed methods to address infertility is assisted reproductive technology, such as in vitro fertilization (IVF) [1]. Several indicators can determine the success of IVF, including sperm motility, blastocyst development rate, fertilization rate, embryo development rate, implantation rate, and cleavage rate [2]. According to the European Society of Human Reproduction and Embryology (ESHRE), cleavage rate is an important indicator because it reflects how well the culture system supports cell division, assesses the embryo's growth potential, and detects contamination within the culture [2]. One factor that can influence cleavage rate is sperm quality, particularly sperm morphology [3].

Normal sperm morphology indicates a well-structured sperm suitable for fertilization, whereas abnormal sperm morphology is often associated with impairments in fertilization and embryo development processes [4]. Good sperm morphology is linked to a reduced risk of genetic material damage, as sperm genetic integrity plays a crucial role in embryo cleavage [5]. Several studies have demonstrated that sperm with abnormal morphology tend to have difficulties penetrating the oocyte, which can adversely affect cell division and the quality of resulting embryos [6, 7]. Poor sperm morphology is associated with DNA damage that leads to chromosomal misdistribution,

resulting in aneuploidy and cleavage arrest at the 8-cell stage [8]. Additionally, sperm with abnormal morphology exhibit reactive oxygen species (ROS) levels 2.5 times higher, which can reduce blastocyst formation by 34 % [9].

Sperm quality can be influenced by several factors, including motility, sperm count, as well as external factors such as lifestyle and medical conditions of the male partner [4]. In IVF procedures, the selection of the best sperm is performed using specific techniques such as swim-up or density gradient centrifugation to enhance the likelihood of successful fertilization [10]. A previous study conducted in Cleveland in 2011 found no significant difference in cleavage rates between patients with poor sperm morphology (98 %) and those with normal sperm morphology (99 %) undergoing intracytoplasmic sperm injection (ICSI) [3]. Conversely, a study conducted in India in 2023 reported different findings, where subjects with normal sperm morphology had a higher average cleavage rate (68 %) compared to those with abnormal morphology (54.4 %) [11]. Therefore, it remains a subject of debate whether selecting sperm with optimal morphology during IVF procedures significantly improves cleavage rates.

Aim: to analyze the relationship between sperm morphology and cleavage rate in patients undergoing IVF, serving as a reference for determining management strategies in infertility cases.

Materials and Methods / Материалы и методы

Research tasks / Задачи исследования

The research tasks of this study are as follows:

1. to describe the distribution of cleavage rates (rapid, normal, and slow) among embryos produced from IVF-ICSI procedures at Dr. Hasan Sadikin Central General Hospital;
2. to analyze the statistical association between sperm morphology categories and cleavage rate groups using the Chi-Square test;
3. to determine the odds ratio (OR) of experiencing rapid or slow cleavage in patients with teratozoospermia compared to those with normospermia, with a 95 % confidence interval (CI).

Study design / Дизайн исследования

This study is an analytical retrospective study. The research was conducted to analyze the relationship between sperm morphology and cleavage rate in infertile couples undergoing IVF programs at Dr. Hasan Sadikin Central General Hospital. Data collection was performed using medical records obtained from the period of January 2020 to December 2024.

The study population consisted of all infertile couples undergoing IVF treatment using ICSI method at Dr. Hasan Sadikin Central General Hospital who met the inclusion and exclusion criteria. Data were collected using a whole sampling method, followed by selection based on inclusion and exclusion criteria to obtain a sample with the desired characteristics.

Inclusion and exclusion criteria / Критерии включения и исключения

Inclusion criteria: infertile couples undergoing IVF with the ICSI method, availability of complete sperm analysis data including morphology parameters based on the manual laboratory examination according to WHO 2021 guidelines, and availability of embryo development data up to day 2 post-fertilization (cleavage rate).

Exclusion criteria: patients with medical conditions that could affect IVF outcomes, such as genetic disorders or chromosomal abnormalities.

Study methods / Методы исследования

Samples were selected using consecutive sampling according to inclusion criteria: complete semen analysis including sperm morphology based on WHO 2021 criteria and available embryo development data to assess cleavage rate; patients with genetic or chromosomal disorders will be excluded. Sperm morphology, categorized as normal or abnormal, is the independent variable, while cleavage rate, categorized as high (≥ 80 %) or low (< 80 %), is the dependent variable.

Statistical analysis / Статистический анализ

Data were analyzed using SPSS version 26.0. Descriptive statistics were used to summarize the baseline characteristics. Numerical variables were presented as mean ± standard deviation, median, and range, while categorical variables were presented as frequencies and precentages. The association between sperm morphology and cleavage rate was analyzed using the Chi-square test or Fisher's exact test when the assumptions of the Chi-square test were not met. Binary logistic regression was performed to control for potential confounders. A p-value < 0.05 was considered statistically significant.

Data confidentiality / Конфиденциальность данных

To ensure data confidentiality, all patient data were anonymized. Access to the dataset was restricted to the research team, and electronic data were deleted from the researchers' repository within five years after the completion of the study.

Results / Результаты

There are 159 data recorded and has been screened from the patient's registry at Dr. Hasan Sadikin General Hospital, the characteristic is shown in **Table 1**. The mean age for the female patients is 33.56 ± 4.153 years and the mean age for male patients is 36.29 ± 51.62 years. The mean for body weight is 60.05 ± 6.301 kg, the mean height is 1.55 ± 0.047 sm, and the mean body mass index is 24.97 ± 3.235.

Table 1. Research patient's general characteristic.

Таблица 1. Общая характеристика пациентов, участвовавших в исследовании.

Variable / Показатель	N = 159
Female age, years / Возраст женщин, лет	

Mean ± SD	33.56 ± 4.153
Median / Медиана	33.00
Range / Диапазон (min–max)	27.00–42.00
Male age, years / Возраст мужчин, лет	
Mean ± SD	36.29 ± 5.16
Median / Медиана	35.00
Range / Диапазон (min–max)	30.00–55.00
Weight, kg / Масса тела, кг	
Mean ± SD	60.05 ± 6.301
Median / Медиана	59.00
Range / Диапазон (min–max)	48.00–71.00
Height, m / Рост, м	
Mean ± SD	1.55 ± 0.047
Median / Медиана	1.55
Range / Диапазон (min–max)	1.48–1.67
Body mass index / Индекс массы тела	
Mean ± SD	24.97 ± 3.235
Median / Медиана	24.14
Range / Диапазон (min–max)	19.57–32.41

The comparison of association between sperm morphology and cleavage rate is shown in **Table 2**. In the rapid cleavage group, patients with normal sperm morphology accounted for 1 case (3.0 %), while those with Teratozoospermia comprised 32 cases (97.0 %). In the normal cleavage group, patients with normospermia numbered 49 cases (52.7 %), and those with teratozoospermia totaled 44 cases (47.3 %). In the slow cleavage group, patients with normospermia were 2 cases (6.1 %), whereas teratozoospermia cases amounted to 31 (93.9 %).

Table 2. Comparison or association between sperm morphology and cleavage rate.

Таблица 2. Сравнение или взаимосвязь между морфологией сперматозоидов и скоростью дробления.

Variable **Переменная**	**Cleavage rate, n (%) / Скорость дробления, n (%)**			**p value**
	Rapid **Быстрая** **n = 33**	**Normal** **Нормальная** **n = 93**	**Slow** **Медленная** **n = 33**	**Значение p**
Sperm morphology / Морфология сперматозоидов				
Normospermia / Нормоспермия	1 (3.0)	49 (52.7)	2 (6.1)	0.001
Teratozoospermia / Тератозооспермия	32 (97.0)	44 (47.3)	31 (93.9)	

Note: for categorical data, the p-value is calculated based on the Chi-Square test with alternative tests of Kolmogorov-Smirnov and Fisher's Exact test if the assumptions of the Chi-Square test are not met. The significance is determined based on a p-value < 0.05.

Примечание: для категориальных данных значение p рассчитано на основе критерия χ^2, а в случае несоблюдения предположений критерия χ^2 – с помощью альтернативных критериев Колмогорова-Смирнова и точного критерия Фишера. Значимость различий определена при значении p < 0,05.

For categorical data analysis in the above table, the Chi-square statistical test was applied to evaluate sperm morphology. The results revealed a statistically significant $p < 0.05$ for the sperm morphology variable. This indicates a significant difference in proportions between sperm morphology categories across the cleavage rate groups. Thus, it can be concluded that sperm morphology distribution varies significantly among the cleavage rate cohorts.

In the Rapid Cleavage group, patients with sperm morphology categorized as teratozoospermia accounted for 32 cases or 97 %, while those with normospermia accounted for only 1 case or 3 %. In the Normal Cleavage group, patients with teratozoospermia were 44 cases or 47.3 %, and those with normospermia were 49 cases or 52.7 %. To analyze the categorical data in the table above, a Chi-Square statistical test was employed to assess sperm morphology. The statistical analysis revealed that the p-value for the sperm morphology variable was less than 0.05 ($p < 0.05$), indicating statistical significance. Therefore, it can be concluded that there is a statistically significant difference in the proportion of sperm morphology between the Rapid Cleavage and Normal Cleavage groups (**Table 3**). The odds ratio further suggests that patients with teratozoospermia are 35.64 times more likely to experience rapid cleavage compared to patients with normospermia, with a 95 % confidence interval of 4.67 to 271.76.

Table 3. Comparison and odds ratio (OR) of sperm morphology in rapid and normal cleavage rate.

Таблица 3. Сравнение и отношение шансов (ОШ) морфологии сперматозоидов при быстром и нормальном дроблении.

Variable Переменная	**Cleavage rate, n (%)** Скорость дробления, n (%)		**OR (95 % CI)** ОШ (95 % ДИ)	**p value** Значение p
	Rapid Быстрая **n = 33**	**Normal** Нормальная **n = 93**		
Sperm morphology / Морфология сперматозоидов			35.636 (4.673–271.760)	**0.001**
Normospermia / Нормоспермия	1 (3.0)	49 (52.7)		
Teratozoospermia / Тератозооспермия	32 (97.0)	44 (47.3)		

Note: for categorical data, the p-value was calculated using the Chi-Square test, with the Kolmogorov-Smirnov test or Fisher's Exact test as alternatives when the Chi-Square test's assumptions were not met. Statistical significance was determined at a threshold of $p < 0.05$.

Примечание: для категориальных данных значение p рассчитано на основе критерия χ^2, а в случае несоблюдения предположений критерия χ^2 – с помощью альтернативных критериев Колмогорова-Смирнова и точного критерия Фишера. Значимость различий определена при значении $p < 0,05$.

In the Slow Cleavage group, patients with sperm morphology categorized as teratozoospermia accounted for 31 cases or 93.9 %, while those with normospermia accounted for 2 cases or 6.1 %. In the Normal Cleavage group, patients with teratozoospermia were 44 cases or 47.3 %, and those with normospermia were 49 cases or 52.7 %. To analyze the categorical data presented in the table above, a Chi-Square statistical test was employed to assess the variable of sperm morphology. The statistical analysis indicated that the p-value for the sperm morphology variable was less than 0.05 ($p < 0.05$), signifying statistical significance. Accordingly, it can be concluded that there is a statistically significant difference in the proportion of sperm morphology between the Slow Cleavage and Normal Cleavage groups (**Table 4**). The odds ratio further indicates that patients with teratozoospermia are 17.26 times more likely to experience slow cleavage compared to patients with normospermia, with a 95 % CI of 3.90 to 76.34.

Table 4. Comparison and odds ratio (OR) of sperm morphology in slow and normal cleavage rate.

Таблица 4. Сравнение и отношение шансов (ОШ) морфологии сперматозоидов при медленном и нормальном дроблении.

Variable	Cleavage rate, n (%) Скорость дробления, n (%)		OR CI 95 % ОШ (95 % ДИ)	p value Значение p
	Slow Медленная n = 33	Normal Нормальная n = 93		
Sperm morphology / Морфология сперматозоидов			17.261 (3.903–76.340)	**0.001**
Normospermia / Нормоспермия	31 (93.9)	44 (47.3)		
Teratozoospermia / Тератозооспермия	2 (6.1)	49 (52.7)		

Note: for categorical data, the p-value was calculated using the Chi-Square test, with the Kolmogorov-Smirnov test or Fisher's Exact test as alternatives when the Chi-Square test's assumptions were not met. Statistical significance was determined at a threshold of $p < 0.05$.

Примечание: для категориальных данных значение p рассчитано на основе критерия χ^2, а в случае несоблюдения предположений критерия χ^2 – с помощью альтернативных критериев Колмогорова-Смирнова и точного критерия Фишера. Значимость различий определена при значении $p < 0,05$.

Discussion / Обсуждение

The findings of this study demonstrate a statistically significant association between sperm morphology and cleavage rate in patients undergoing IVF, as evidenced by the Chi-square test results ($p < 0.05$). The predominance of teratozoospermia in both the rapid cleavage rate (97,0 %) and slow cleavage rate (93.9 %) groups, compared to the more balanced distribution in the normal cleavage

rate group (52.7 % normospermia vs. 47.3 % teratozoospermia), suggests that abnormal sperm morphology may compromise the timing and quality of embryo division.

The results from this study partially contradict earlier studies, such as the 2011 Cleveland trial reporting no significant cleavage rate differences between normal and abnormal sperm morphology groups. In this study, they analyze 235 women and found no difference in fertilization rate and pregnancy rate in group A (0–2 % normal morphology) and group B (5–13 % normal morphology). They also found that significantly fewer embryos from Group A had undergone compaction compared to Group B embryos ($p = 0.0007$). But in Day 5, Group A blastulation was 34,0 % compared to group B 38,0 % blastulation ($p > 0.05$). This study concluded that blastulation rates were not significantly different even though compaction rates showed a delay in Group A [3]. In contrast, this study resonates with findings from the 2023 Indian study, which observed superior cleavage rates in normospermic samples. In this study, it was found that the cleavage rate was higher in subjects with normal sperm morphology at 3,0 % (67.94) compared to 2,0 % (60.87) and 1,0 % (54.38). The study also employed the Cramér's V test to assess the strength of the correlation between sperm morphology and cleavage rate. A Cramér's V value of 0.714 was obtained, indicating a strong correlation between sperm morphology and fertility rate. Furthermore, this correlation was reinforced by the finding that a higher proportion of normal sperm morphology was associated with an increased average cleavage rate [11].

Several factors may explain why this phenomenon occurs. The first is the morphology of the acrosome structure. The acrosome plays a crucial role in the structural changes that occur when sperm encounters the oocyte. These processes include capacitation, the acrosome reaction, and the release of enzymes. If the acrosome is improperly formed, these processes cannot proceed effectively, thereby preventing fertilization. The shape of the sperm head is also important for recognition and binding to the zona pellucida of the oocyte. Sperm with abnormal head morphology are less likely to be recognized by the oocyte, hindering the fusion between the acrosome and the zona pellucida [12, 13]. Poor sperm morphology may also reflect DNA fragmentation within the sperm nucleus. This occurs due to failure in the repair of nuclear DNA during the spermatid stage. Sperm exhibiting large residual cytoplasm are associated with increased DNA strand breaks and elevated ROS production [14]. A study conducted in Bulgaria found that defects in the sperm head are associated with late-stage embryo development, defects in the neck (midpiece) correlate with intermediate embryo cleavage, and tail defects are related to early stages of embryo development. Additionally, sperm with head defects have been linked to delayed blastocyst formation (t9–tHB) [15].

This study's retrospective design limits causal inference, and confounding variables such as female age, ovarian response, or embryological techniques were not controlled for. The unusually high standard deviation in male age (36.29 ± 51.62 years) warrants scrutiny, as it may reflect data

entry errors or sampling bias. Future prospective studies with larger cohorts and standardized protocols are needed to isolate the impact of sperm morphology while accounting for female and procedural factors. Additionally, extending follow-up to later developmental stages (e.g., blastocyst formation or live birth rates) could clarify whether cleavage rate discrepancies translate to clinically meaningful outcomes.

Conclusion / Заключение

There is a statistically significant association between sperm morphology and cleavage rate in patients undergoing IVF treatment. Specifically, the distribution of sperm morphology categories (normospermia vs. teratozoospermia) differs significantly across the rapid, normal, and slow cleavage rate groups. The rapid cleavage rate group exclusively consisted of patients with teratozoospermia, while the normal cleavage group had a more balanced distribution, and the slow Cleavage rate group was predominantly teratozoospermia as well. This suggests that abnormal sperm morphology (teratozoospermia) may be linked to impaired or delayed embryo cleavage, which could potentially affect IVF outcomes.

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