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Original Research Article

A retrospective observational study on effect of sperm morphology on embryo development after intracytoplasmic sperm injection

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ABSTRACT

Background: The process of embryo development requires the contribution of both male and female gametes. However, abnormal sperm development can hinder the fertilization process, leading to infertility. To overcome these male fertility issues, intracytoplasmic sperm injection (ICSI) was developed. This technique has proven to be effective, resulting in about 80% of live births in ICSI cycles.

Methods: It is a retrospective cohort study was conducted in department of reproductive medicine, Komali fertility centre a unit of Dr. Ramesh Cardiac and multispeciality hospital P Ltd, Guntur, Andhra Pradesh, India. The study was conducted during period of January 2022 to December 2022. Total 120 subjects were included based on our inclusion and exclusion criteria.

Results: This study used the chi-square test and Pearson's correlation to evaluate the relationship between sperm morphology and various parameters, revealing that poor sperm morphology may contribute to low fertilization, cleavage, and pregnancy outcomes. Individuals with 3% sperm morphology exhibited higher mean fertility rates and lower negative pregnancy rates compared to those with 2% and 1% morphology, highlighting the importance of good sperm morphology for successful fertilization and pregnancy outcomes.

Conclusions: In summary, the study highlights a strong correlation between sperm morphology, fertilization and cleavage rates, while a weak and no correlation was observed between sperm morphology and pregnancy outcome after ICSI. The findings suggest that an improvement in sperm morphology leads to increased fertilization and cleavage rates.

Keywords: Sperm morphology, Cleavage rate, Fertility rate, Pregnancy outcome/ rate, Infertility, ICSI

INTRODUCTION

The first human sperms were identified as animalcules, or "small animals," by Antonie Van Leeuwenhoek in 1677.^{1,2} This was an incredible milestone in the history of science and medicine, as it provided a scientific explanation for how life is passed from one generation to another. By providing the female oocyte with the genetic material and proteins of the male partner during fertilisation, it performs a key purpose in sustaining life.^{2,3} The anatomy of the

human sperm tail is intricate, and each of its constituent parts, including the axonemal and peri-axonemal structures, is important for sperm motility and male fertility.

A connecting piece that attaches to the head, a mid-piece, a main piece, and an end piece make up each of the four elements of sperm.^{4,2} The head of the sperm contains DNA and other molecules that give instructions for the creation of new life. The optimum sperm morphology, according to the WHO, is as follows: Having an oval head and a clearly

marked acrosome area in the apical section (upper part). If vacuoles exist, they cannot cover more than 20% of the head's surface. The head and neck are joined at the head's axial section (opposite the acrosome region). The midpiece's length should be 1.5 times that of the head. The tail of the sperm cell should be 45-50 microns long and

thinner than the body. The sperm cell should be between 50 and 60 microns in length overall. Sperm that is aberrant or exceeds these limits is referred to as amorphous sperm.

There are three different types of sperm morphological defects. They are depicted as follows:

Table 1: Morphology of sperm classification (Table is adopted from WHO).⁵

Location	Normal (ideal/typical) appearance	Abnormal
Head	The head should be smooth, regularly contoured and generally oval in shape. There should be a well-defined acrosomal region comprising 40–70% of the head area (96). The acrosomal region should contain no large vacuoles, and not more than two small vacuoles, which should not occupy more than one fifth of the sperm head. The post-acrosomal region should not contain any vacuoles.	Acrosome less than 40% or larger than 70% of a normal head area, or Length-to-width ratio less than 1.5 (round) or larger than 2 (elongated), or Shape: pyriform (pear shaped), amorphous, asymmetrical, or non-oval shape in the apical part, or Vacuoles constitute more than one fifth of the head area or located in the post-acrosomal area, or double heads, or any combinations
Midpiece	The midpiece should be slender, regular and about the same length as the sperm head. The major axis of the midpiece should be aligned with the major axis of the sperm head.	Irregular shape, or thin or thick, or asymmetrical or angled insertion at head, or sharply bent, or any combinations
Tail	The principal piece should have a uniform calibre along its length, be thinner than the midpiece and be approximately 45 µm long (about 10 times the head length). It may be looped back on itself, provided there is no sharp angulation indicative of a broken flagellum.	Sharply angulated bends, or smooth hairpin bends, or coiled, or short (broken), or irregular width, or multiple tails, or any combinations
Cytoplasmic residue	Cytoplasmic droplets (less than one third of a normal sperm head size) are normal.	Residual cytoplasm is considered an anomaly only when it exceeds one third of normal sperm head size

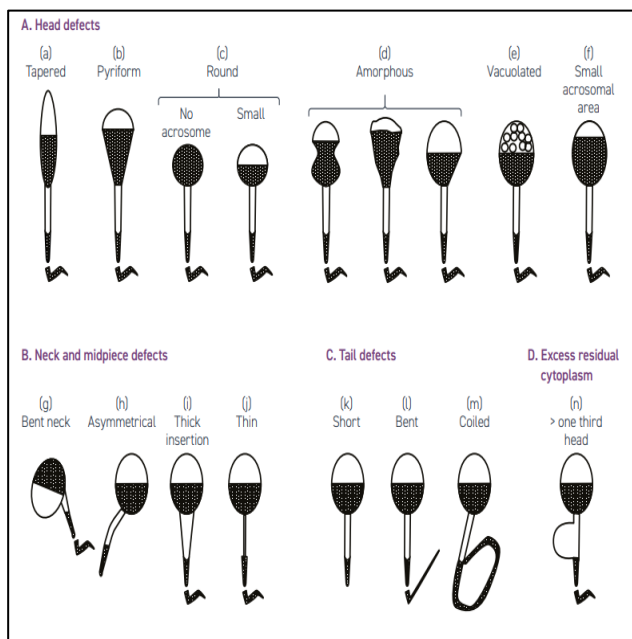


Figure 1: Schematic representations of some atypical human spermatozoa (Image is adopted from WHO).⁵

Advantages of ICSI over conventional in vitro fertilisation (IVF)

Conventional *in vitro* fertilisation and intracytoplasmic sperm injection are two popular techniques to achieve fertilization.⁶ Conventional IVF involves standard fertilization, while intracytoplasmic sperm injection involves the injection of a single sperm into a mature oocyte in order to increase the chances of successful fertilisation.⁷⁻¹³ Conventional IVF has long been used to treat infertility. Conventional IVF was much less effective when the seminal properties of concentration, morphology, or motility were significantly below standard values.^{7,15-19} In the early 1990s, research began to explore new methods of assisted reproductive technology (ART) to improve the odds of conception, in such cases intracytoplasmic sperm injection is recommended for those who are suffering from male infertility. Some common male infertile problems are: Anejaculation (inability to ejaculate). An obstruction in their male reproductive system. Sperm count is low. Poor sperm quality and retrograde ejaculation (In which the sperm flows backward into the bladder).¹⁴

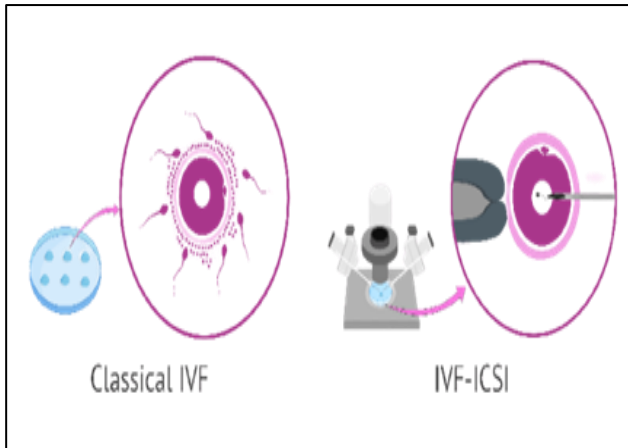


Figure 2: Classical ICSI and IVF-ICSI.

Abnormal sperm morphology is referred to as teratozoospermia.

The term "sperm morphology" describes the sperm's size and shape. Deviations from the normal size and shape of sperm are called teratozoospermia. A normal-looking sperm has an oval head with a dark-coloured nucleus (DNA) and one or two empty spaces (vacuoles). The head is connected to the tail in the central part of the body with the neck. Teratozoospermia is diagnosed when more than 95% of the sperm in a man's semen sample have an abnormal morphology.²⁰ When we come to the role of ICSI in teratozoospermia, it has a vital role in fertilization.

ICSI is used in cases of severe teratozoospermia or when IUI or conventional IVF methods have failed. In ICSI, selected sperm are microinjected into the egg for fertilization, increasing the likelihood of fertilization.

Cytoplasmic morphologically selected sperm injection (IMSI) is a type of ICSI that improves sperm selection as observed under a high-magnification light microscope.²⁰

Teratozoospermia index

This measurement is used to identify the number of defects present in each sperm cell. It is possible for a sperm to have an anomaly in just one part or in multiple areas. To calculate the teratozoospermia index (TZI), we use the following formula: $TZI = (c + p + q) / x$, where each variable means:

c=head flaws, p=midpiece flaws, q=tail flaws, x denotes the total number of abnormal sperm.

The TZI score should be interpreted as follows: TZI close to 1: One affected part, TZI close to 2: Two parts affected and TZI close to 3: Affected head, midpiece, and tail.²¹

Sperm morphology can be analysed by staining techniques as per WHO guidelines. These staining techniques provide

a quick and accurate way of identifying the percentage of normal sperm present in the sample.

Aim

Aim of the study was to assess the effect of sperm morphology on fertilization, embryonic development, and pregnancy outcome.

METHODS

Study design

A non-invasive cohort retrospective observational study design was used.

Study duration

The duration of study is 3 months.

Study department and site

Department of reproductive medicine, Komali Fertility centre, a Unit of Dr. Ramesh cardiac and multispeciality hospital P Ltd, Guntur, Andhra Pradesh, India.

Study population or sample size

Sample size was 120.

Study recruitment procedure

Selection of patients based on inclusion and exclusion criteria.

Inclusion criteria

Inclusion criteria include male and female subjects between ages of 25 and 27 who are willing to give consent, have abnormal sperm morphology, and Subjects who have been married for more than a year but have not given birth.

Exclusion criteria

Exclusion criteria include subjects who are not willing to give their consent, psychiatric patients and unmarried subjects.

Study procedure

Data will be collected and followed up of individuals who have undergone semen morphology and ICSI in the past few months and are invited to participate and sign a consent if they are willing to hear an explanation of the project. Patients will be recruited based on inclusion and exclusion criteria for the study. The obtained results were categorised and tabulated. The tabulated data will be subjected to statistical analysis using SPSS software. The results so obtained will be discussed and documented for publication.

Statistical analysis

Data storage and analysis were performed on a local computer network PC in the department of reproductive medicine at Komali Fertility Centre, Guntur, A.P., India, statistical analysis done by using the SPSS V26 2019 statistical programme package. As usual, a significance level of $\alpha=0.05$ was chosen.

The patients were grouped according to sperm morphology as per WHO staining techniques. The groups were compared as follows: (i) Fertilization rate: one sample T test; (ii) Cleavage rate: one sample T test; (iii) Pregnancy outcome: percentage calculations. Chi-square test and Pearson's correlation was used to test the possible relationship between sperm morphology and fertilisation rate, pregnancy rate, and cleavage rate.²² The association between the parameters was interpreted by using Cramer's V value.²³

RESULTS

In order to improve reproductive outcomes, researchers have looked at the relationship between sperm shape, fertilisation rate, cleavage rate and possibility of pregnancy.

This study establishes a connection between sperm morphology, fertilisation rate, cleavage rate and pregnancy outcomes. The mean fertilization rate is low in subjects with sperm morphology of 1% (69.81) when compared with subjects with sperm morphology of 2% (70.16). where the mean fertilization rate is high in subjects with sperm morphology of 3% (71.06), compared to sperm morphology of 2% (70.16). The results indicate that sperm morphology quality plays a significant role in influencing fertilisation rate, with higher mean fertilisation rates often corresponding to a higher percentage of normal sperm morphology (Table 2) (Figure 3).

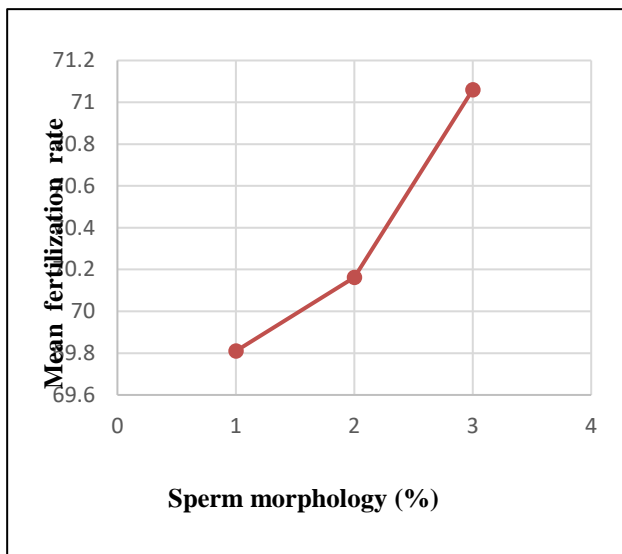


Figure 3: Sperm morphology vs fertilization rate.

The mean cleavage rate was high in sperm morphology of 3% (67.94) subjects when compared with 2% (60.87) and 1% (54.38). The findings of this study indicate a substantial correlation between sperm morphology and fertility rate, which is reinforced by the finding that a higher proportion of normal sperm morphology correlated with a higher mean cleavage rate (Table 2) (Figure 4).

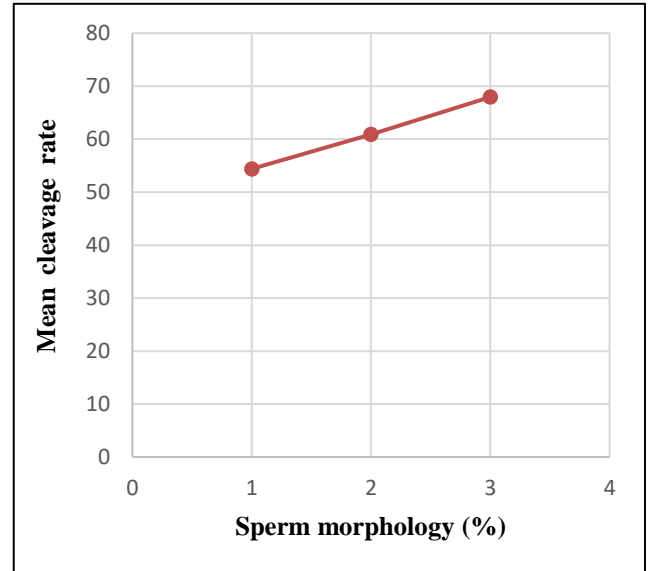


Figure 4: Sperm morphology vs cleavage rate.

Subjects with sperm morphology of 3% (71.42%) had the highest pregnancy-positive rates, compared to 2% (67.74%) and 1% (46.15%) respectively. Our results indicate that sperm morphology quality plays a significant role in influencing fertilisation rate and that a higher proportion of normal sperm morphology may be associated with higher rates of fertilisation and pregnancy-positives (Table 2) (Figure 5).

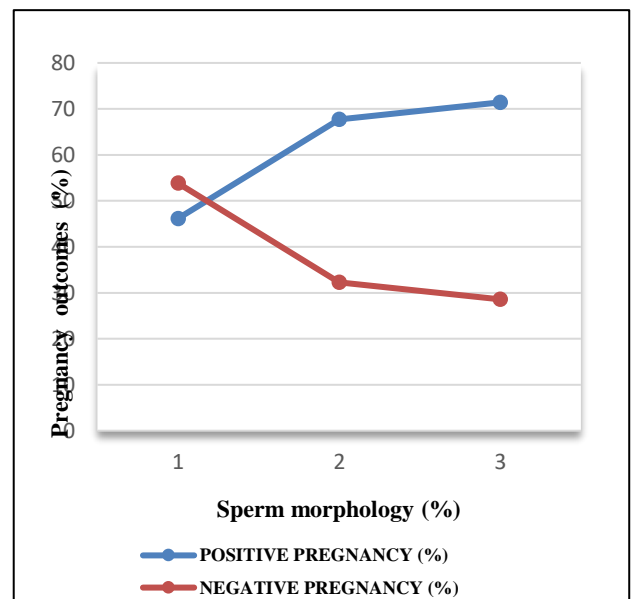


Figure 5: Sperm morphology vs pregnancy outcome.

The lowest percentage of the negative pregnancy rate was observed in subjects with sperm morphology of 3% (28.57%) when compared to 2 (32.25%) and 1 (53.84%). These findings suggest that successful fertilisation and pregnancy may be significantly influenced by the sperm's morphological quality (Table 2) (Figure 6).

There is a high significance between sperm morphology and fertilization rate (p=0.004) as well as cleavage rate

(p=0.000) The data suggests that couples with higher rates of normal sperm morphology have a higher rate of fertilization and positive pregnancies. but there was no significance between sperm morphology and pregnancy outcome (p=0.070). Considering the strong relationships between sperm morphology as well as the rates of fertilisation and cleavage, it seems that other parameters may have a greater impact on the success of the conception (Table 3).

Table 2: Effect of morphology on fertilization, cleavage rate and pregnancy outcomes in ICSI patients.

Sperm morphology (%)	Mean fertilization rate		Mean cleavage rate		Pregnancy outcome (%)	
	Mean value	Significance value	Mean value	Significance value	Positive (%)	Negative (%)
1	69.81	0.002	54.38	0.001	46.15	53.84
2	70.16	0.001	60.87	0.001	67.74	32.25
3	71.06	0.001	67.94	0.002	71.42	28.57

Table 3: Statistical analysis of the correlation between sperm morphology and fertilization rate, cleavage and pregnancy outcomes after ICSI.

Groups	Significance value (2 tailed)	Summary	Crammrs V value	Interpretation of association
Fertilization rate*	0.004	hs	0.720	Strong relationship
Cleavage rate**	0.000	hs	0.714	Strong relationship
Pregnancy outcome*	0.070	ns	0.210	Moderate relationship

*Chi-square Test; **Pearson's Correlation; hs: highly significant; ns: not significant; s: significant.

According to the Crammrs V rule, there is a strong association between sperm morphology and fertilization rate (Crammrs V=0.720) and cleavage rate (Crammrs V=0.714), indicating that sperm morphology can indeed be used as a predictor of positive fertilization and cleavage. where a moderate association between sperm morphology and pregnancy outcome (Crammrs V=0.210) was observed. However, the correlation between sperm morphology and pregnancy outcome is not as strong (Table 3).

DISCUSSION

Male infertility is primarily brought on by low sperm counts. The sperm's size and shape, however, might also be significant.²⁴ Morphology can affect fertility because sperm need a particular shape to enter the outer layers of the egg. Abnormalities in the size and shape of sperm can interfere with this process and make it difficult for successful fertilization to take place. Therefore, it is important to take both sperm count and morphology into consideration when diagnosing male infertility.²⁵ Abnormal sperm morphology may also be caused by environmental factors, such as exposure to certain toxins, drug or alcohol use, or excessive stress. These environmental factors, if not addressed, can have a major impact on a man's fertility. Moreover, because lifestyle factors such as poor diet, a lack of exercise, smoking, and obesity can also affect sperm morphology, it is important for men who are trying to conceive to maintain a healthy

lifestyle and avoid environmental toxins that may hurt sperm morphology.²⁶ These lifestyle changes may help to improve the quality of the sperm and potentially increase a man's chances of conceiving a child. Additionally, several treatments can be used to improve sperm morphology. They include hormone therapy, nutritional supplements, and ART such as intrauterine insemination or in vitro fertilization.²⁷

ICSI is used in nearly half of all IVF procedures.²⁸ ICSI, is a form of in-vitro fertilization that allows an infertility specialist to directly inject a single sperm into an egg. Only one sperm is required for ICSI, which is injected directly into the egg.²⁹ The fertilized embryo is then transferred to the uterus. All of these treatments are designed to improve a man's chances of conceiving a healthy baby.³⁰ ICSI is an effective treatment for male infertility and should be considered as a viable option when dealing with cases of male factor infertility, especially if there are concerns about sperm morphology.³¹

ICSI can help couples where the man's sperm either can't get to the egg at all or can reach the egg but not fertilise it for some reason. ICSI has helped many couples where the man's sperm has been unable to fertilise the egg by natural means.³²

ICSI is likely to be recommended in the following circumstances: If they have a very low sperm count, a high percentage of abnormally shaped or slow sperm, or no

sperm showing up in the fresh sample. Additionally, if the male partner has had a vasectomy or previous failed conventional IVF.³³

A retrospective observational study was carried out for 3 months in the department of reproductive medicine. In our study, 120 members were followed up and are included in the study. Several studies have determined a substantial correlation between sperm morphology and fertility outcomes. The current study discovered that when sperm morphological percentages increased, so did fertilisation, cleavage rate, and pregnancy rate.

A study published by Baker and colleagues also found a significantly reduced incidence of fertilisation and implantation with morphologically abnormal sperm. This confirms the findings of our study.³⁴ Similarly, a study conducted by Watson and Collins also showed that higher sperm morphology percentages corresponded to increased fertilization, with morphologically normal sperm resulting in higher rates of fertilisation, cleavage, and live births.³⁵ These results indicate that sperm morphology is an important factor in successful fertilization. Taken together, these studies suggest that increased sperm morphology percentages are correlated with improved fertilisation rates, cleavage, and live births.

Subjects in this study are divided into three groups based on sperm morphology. Subjects with lower sperm morphology had a lower fertilization and cleavage rate when compared to subjects with higher sperm morphology; however, the negative pregnancy outcome was higher in subjects with lower sperm morphology. This study reveals that low sperm morphology might be one of the reasons for low fertility, cleavage, and pregnancy outcomes. The implications of this study are clear. Sperm morphology is an important factor in successful fertilization and pregnancy.

Limitations

The route cause for abnormal sperm morphology like excess BMI, occupational hazards and sedentary lifestyle, history of alcohol and smoking, sperm DNA fragmentation index were not taken in to consideration.

CONCLUSION

In this study highlights a strong correlation between sperm morphology, fertilization, and cleavage rates, while a weak and no correlation between sperm morphology and pregnancy outcome following ICSI. Further investigation is warranted to determine the significance of the relationship between sperm morphology and pregnancy outcome after ICSI. Future research should focus on elucidating the potential correlation between these factors and how this correlation could be leveraged to enhance the likelihood of pregnancy. Such inquiry could prove invaluable for individuals struggling with infertility, providing them with enhanced insights into how sperm

morphology can impact pregnancy outcomes following ICSI and paving the way for more effective treatments.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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