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Morphological analysis of metabolically dysregulated spermatozoa using Artificial Intelligence based approach

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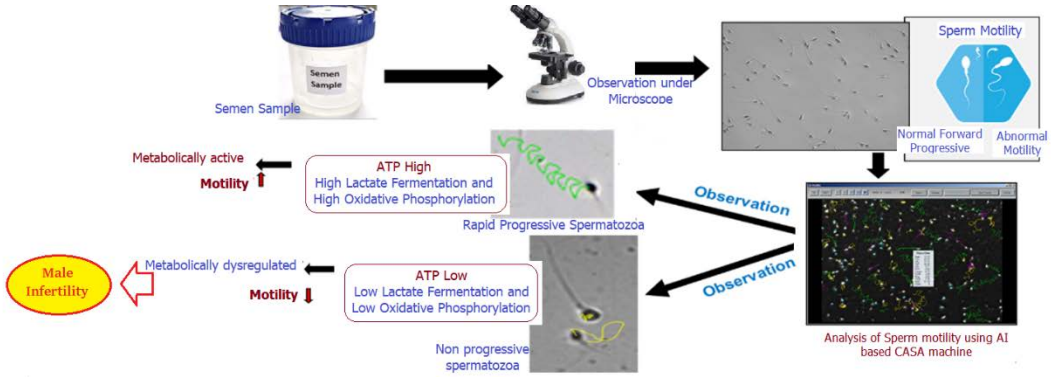
ABSTRACT

Sperm motility is an important parameter in evaluation of infertility in human semen samples and it's directly associated with the Asthenozoospermia. Poor sperm movements are often related to less production of adenosine triphosphate (ATP), or due to less lactate fermentation and oxidative phosphorylation in mitochondria. In this article, Computer Assisted Sperm Analysis (CASA) based on Convolutional Neural Network (CNN) an Artificial Intelligence (AI) approach have been reported for the understanding of flagellar waveforms and propagation of sperm movement. We also show how microscopy systems are used for evaluating spermatozoa movements and to find the difference in their movement pattern, path length and area followed by flagella of sperm and how it links to its metabolic regulation. We found that sperms covering a distance less than 40µm/sec are metabolically dysregulated or are considered to produce less amount of ATP which could be a possible reason for no fertilization of ova in women. It also suggests that the flagella of sperm are linked with the metabolic activity of the sperm and its movement which affects the rate of the fertilization.



Keywords: Metabolically dysregulated spermatozoa, Sperm movement, Sperm morphology, CASA, Male infertility

INTRODUCTION

Human sperms have a special ability to travel through the woman's reproductive tract and fertilize an oocyte. Progressive motility of sperms contributes in its ability to penetrate the oocyte and assist natural conception. However, reduction in this progressive motility has been largely observed and is contributing to infertility cases as well.[1,2] Analysis of semen is one of the basic procedures in assessing the male partner in a sub fertile couple to evaluate the condition of infertility in couples. Semen sample analysis has a standard procedure which analyses the detailed morphology of sperm. Morphology of the sperm remains the biggest parameter for analyzing the quality of spermatozoa and its effect on successful pregnancy rate.[3] As per the guidelines of the World Health Organization (WHO) the reference guidelines to analyze spermatozoa morphology has been provided to establish internal and external quality control for the measures. The guidelines also includes more commonly used tests to assess different parameters (i.e., Head, mid piece, or tail defect) of spermatozoa contributing for infertility in humans.[4,5] The electron microscopy analyses provides seven distinct sperm phenotypes in human semen samples, including spermatozoa with dysplasia of the fibrous sheath, immotile cilia, nonspecific flagellar defects, pin head, defective chromatin condensation and compaction, acrosomal hypoplasia, and even sperm cells without heads. This provides enough evidences to say that it is incorrect to think of

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morphology as the sole parameter to be analyzed in semen samples; rather, it should be utilized in conjunction with other factors such as motility.[6,7,8] It is crucial to understand that, in general, poor morphology scores do not exclude pregnancy, but that combinations of metrics as well as motility and morphology can be used as predictive biomarkers for infertility.[9,10] The pattern of sperm movement and velocity, distinguishes the spermatozoa as progressively motile, non-progressive and immotile. In rapid motility spermatozoa shows two type of motility pattern one fast progressive and second one is slow progressive, while in non-motile sperms they are distinguished as non-progressive or immotile. This motility pattern is evaluated according to their path length followed, divergence from the mean route, as well as the angles between nearby track points.[11,12] The heads of metabolically active spermatozoa deviate far from the direction of motion, and the angles between adjacent spots are more than 90 degrees. They are also hyperactive, and travel less forward than they would normally. Whereas metabolically dysregulated spermatozoa display sluggish movement with very little or no forward progressive movement.[13] Convolutional neural network (CNN), a form of deep learning algorithm of Artificial Intelligence (AI) approach has been widely used for processing images analyzing the morphology of sperms. The morphological categorization of sperm pictures is fully automated using distinct CNN models. The morphology analysis only deals with sperm shape and extracts shape properties in relation to physiological factors.[14, 15]

Coherent movement of sperms through uterine cervix completely depend on its rapid progressive motility (i.e., sperms with at least $\leq 25\ \mu m/s$ forward progression movement. Whereas non-progressive movement of spermatozoa can be associated with disorders of male accessory sex gland secretion of male or due to differential expression of metabolic gene that are responsible for flagellum movement.[16],[17] Routine semen analysis or functional tests fail to identify above mentioned conditions, and this makes the need for using CNN based machine to analyze semen samples to check movement and defects in spermatozoa irrespective of human errors.

Here we demonstrate the sperm movement using CASA machine and analyzed the difference between metabolically dysregulated semen samples v/s normal semen samples.[18] A high-tech electronic imaging device is employed to analyze the sperm, and AI-based software application is used to analyze the specific sperm properties. CASA systems are an effective and reliable option for the examination of the sperm parameters in clinical practice, particularly with regard to the concentration and motility of sperm. CASA devices that are based on convolutional neural networks promise to increase the trustworthiness of the findings while also offering a better level of efficiency in the examination of spermatozoa defects.[20,21]

METHODOLOGY

Sample collection and processing

The semen samples were collected from male patients that came for semen analysis at Indira IVF Hospital in Udaipur, Rajasthan. All subjects were above 18 years of age. Subjects were facing the problem of infertility even after going through unprotected sexual intercourse for more than one year. Subjects were instructed to refrain from sexual activity for 72 hours before sample retrieval. Semen was collected into sterile 50 ml vials by masturbation. For the liquefication of semen samples, the samples were incubated for 30 minutes at 37°C with 5% CO_2. Following liquefaction, a pipette was used to measure the volume of the samples, and a drop of sample was then placed on the Makler chamber. Then the Makler chamber was observed under the microscope of MMC SPERM CASA Machine for its count and motility.[22] The non-progressive or immotile spermatozoa were stained using Dip staining method for its morphological assessment.[23,24]

ImageJ analysis of sperm motility

The collected images from MMC SPERM CASA version 2.4.078.0 of spermatozoa's movements were analyzed using ImageJ software and graphs were plotted to understand the distance travel by metabolically active and dysregulated spermatozoa.[25]

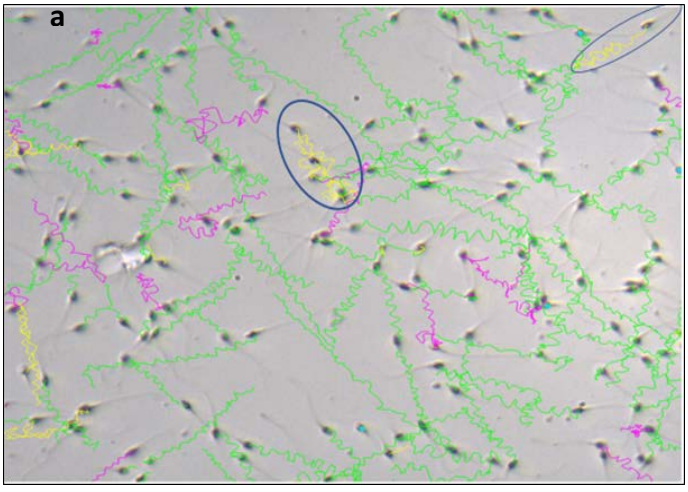
CASA

The CASA system provides information that is crucial for analyzing the quality of the sperm. The evaluation of sperm health and motility is accomplished by these systems via the use of computer vision tasks such as categorization, detection, and tracking. Convolutional Neural Network (CNN) improves the accuracy of CASA. CNN creates a probability map, which is then utilized to estimate the migration of the sperms in the most likely direction. Convolution neural networks are used to categorize human sperm heads; the design makes use of many layers and different filter sizes to increase the method's effectiveness. CNN has three layers - Convolution layer, Max pooling layer, and fully connected layer.[26,27]

RESULTS

CASA tracks of human spermatozoa

The analysis of CASA tracks for human sperm samples obtained from patient samples showed changes in motility patterns. The samples were categorized into three groups based on observations from the study. First, semen samples at a concentration between 80% – 50% having motility more than 50% (Figure 1a). Second, semen samples at a concentration between 80% – 50% having motility more than <39% (Figure 1b) and third semen sample at a concentration of 80% – 50% having no motility (Figure 1c).



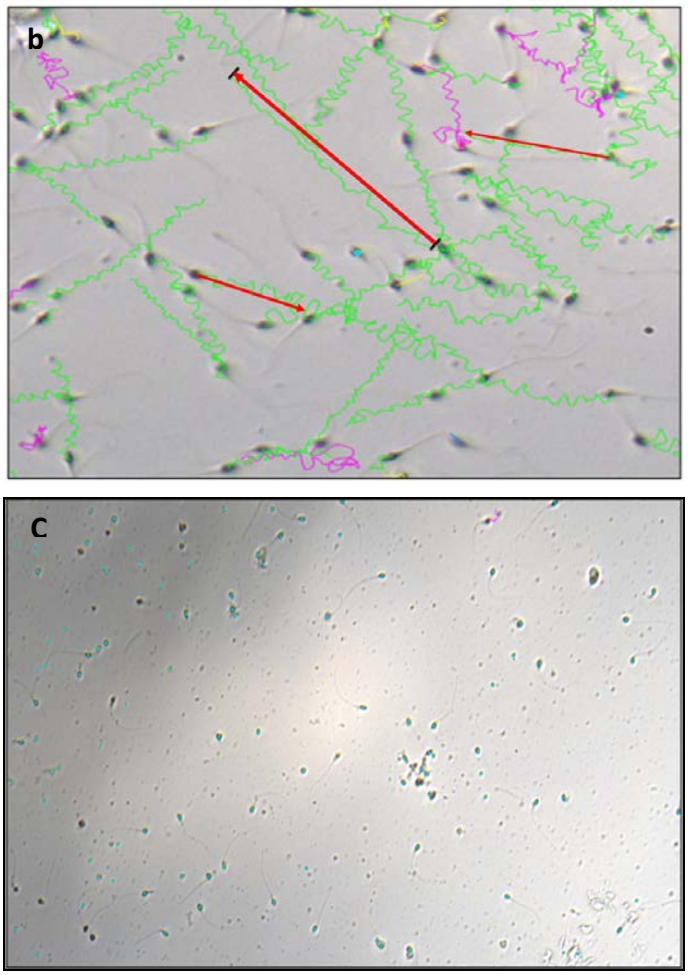


Figure 1. CASA tracks of human spermatozoa. a) Squiggly green lines represent the path traveled by metabolically active spermatozoa. b) Yellow lines represent the path traveled by metabolically dysregulated spermatozoa using backtrack method. c) Blue dots represents immotile sperms.

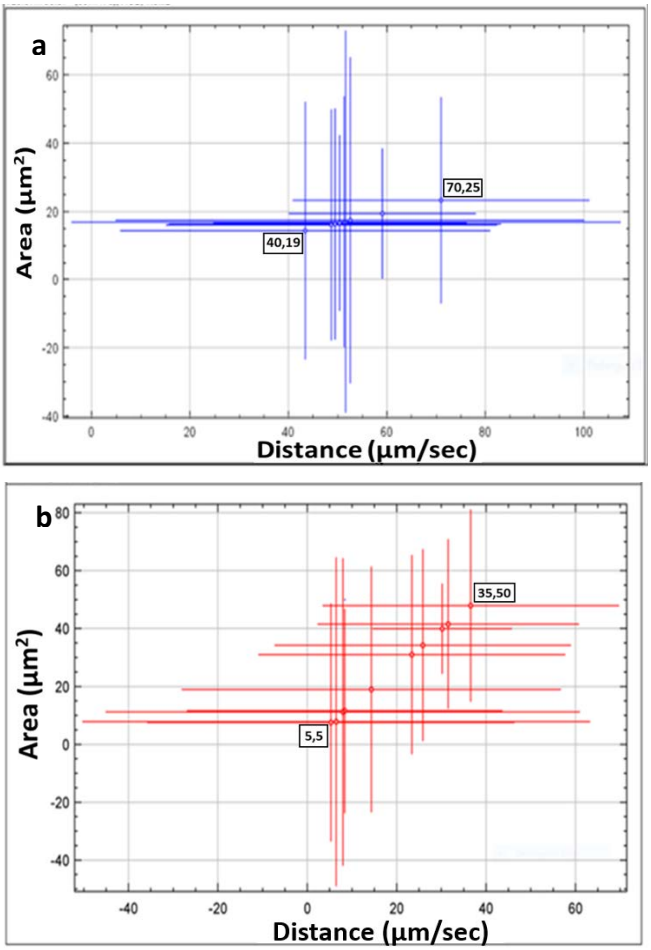


Figure 2. Sperm motility track in semen samples using ImageJ. a) The graph represents the path length and area covered by highly motile/hyperactive semen sample (Motility = more than 50%) to be more than 40µm/sec. b) The graph represents the path length and area covered by low motile/progressive semen sample (Motility = less than 30% or Rapid Progressive = less than 15%) to be between 0 to 40µm/sec.

CASA derived changes in sperm motility track

WHO has revised the standard limits for semen analysis,[29] in which progressive motility in a semen sample has to be 32%. The efficient passage of spermatozoa through the cervical mucus completely depends on progressive motility of at least 25 um/s. Here our study reveals motility rate of progressive spermatozoa in semen sample to be more than 40um/s i.e., (motility >50%). (supplementary data 1) (Figure 2a). whereas, non-progressive spermatozoa meant to be less motile in semen Sample shows a motility rate of 0- 40um/s (i.e., motility <39%) (Figure 2b).

Morphological Assessment of metabolically dysregulated spermatozoa

About 40% men in the reproductive age has reported to have a decline in sperm quality. Males with abnormal sperm morphology has also been in study as an important parameter causing male infertility. Assessment of sperm morphology is considered as a hallmark to determine the quality of sperm in semen sample. We analyzed the semen samples with less motile sperms and found tail

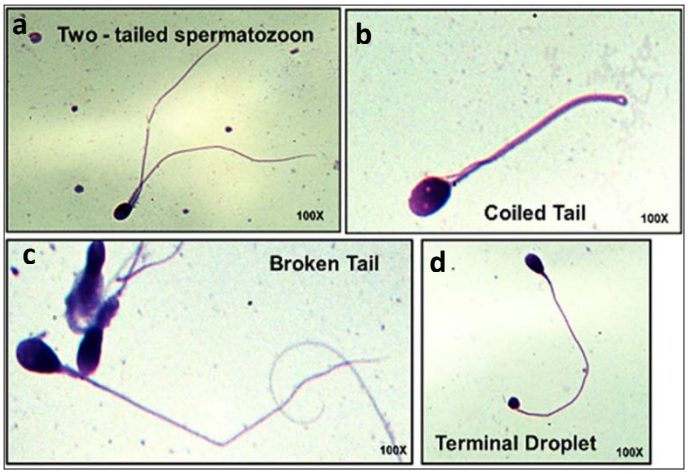


Figure 3. Morphological analysis of semen samples containing immotile sperms. (a) two-tailed (b) coiled tail (c) broken tail (d) terminal droplet. Images obtained in 100x resolution.

defects in sperms with broken tail, double tail, coiled tail, and some even had terminal droplet spermatozoa (Figure 3).

DISCUSSION

The male infertility arises due to a number of different physiological factors.[30-33] Functional spermatozoa are important in male fertility but presence of spermatozoa that are metabolically active majorly contributes towards high quality sperms in semen sample. Spermatozoa are highly specialized cells and perform many distinguish functions, therefore the expression of key genes is required to regulate its metabolic activity. Also, the management of sperm energy is required to perform its overall functions. Therefore, it is mandatory for us to understand the mechanisms through which sperm regulate its metabolism and manage their energy.[34,35] Here, we utilize artificial intelligence[36] based CASA systems to precisely evaluate the important factors responsible for the sperm quality. Primarily we assess for two predominant factors sperm motility and morphology. The different movement patterns followed by metabolically active and metabolically dysregulated spermatozoa using back track method provides us the insight of the path travelled by motile and immotile sperms in the semen sample. The highly motile sperms have been noted to have a motility rate of above 40um/s. The morphological analysis of semen samples reveal the presence of defects mostly in the tail regions of the sperm with different morphological patterns such as broken tail, double tail, coiled tail, and some even had terminal droplet in the tail region.

CONCLUSION

Infertility is an increasing global health issue affecting 8-12% of couples worldwide. Male infertility accounts for a considerable proportion of these cases. Defects in tail has been seen as a major reason for its low motility/non progressive motility or immotility. Sperm are propelled by bending waves traveling along their flagellum. So, studying flagellar defects in spermatozoa is highly recommended to improve the diagnosis and treatment of infertility conditions. The high motile samples are often taken for ICSI, but fails to provide a permanent solution for conceiving. These samples however need further investigation at their molecular level. Investigation for drug development against specific targets responsible for functioning the motility and morphology of sperms has to be explored as to stop the inheriting of such conditions to next generation. CASA tracks the different movement of spermatozoa's and provides us the image of different paths followed by the spermatozoa of highly motile and low motile sperm sample using backtrack method. Concentration, motility, morphology, leukocyte count and acrosome response might all be accurately, repeatedly, and automatically evaluated with the use of CASA system. Images of sperms that resemble a strobe light may be captured by modern CASA systems when they automatically examine numerous fields contained inside a shallow specimen container. The most recent iterations of the CASA system have the capability to automatically examine several fields contained inside a shallow specimen chamber and record images of sperms. The analysis of predominant factors such as morphological defects and motility based defects in sperm samples can provide a detailed insights in better understanding of factors responsible for male infertility.

FUTURE PROSPECT

This study further needs to be investigated at molecular level by gene analysis in motile as well as non-motile semen samples. Exploring and finding differential expression and correlation of metabolic genes which are responsible for ATP generation and spermatozoa motility has to be done to find novel targets to improve sperm motility.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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SUPPLEMENTARY INFORMATION

Data 1.

Result obtained from ImageJ of Progressive spermatozoa in Highly Motile Semen Sample

Spermatozoa	Area	StdDev	Angle	Length
1	18.049	33.783	-94.792	54.767
2	18.476	31.512	56.125	56.286
3	16.34	41.132	68.827	49.764
4	16.019	45.293	70.346	48.582
5	16.233	44.72	-7.97	49.498
6	16.981	48.461	47.311	51.573
7	23.602	33.505	-23.883	71.837
8	16.874	43.395	152.292	51.308
9	20.398	40.81	-175.764	61.934
10	17.942	39.799	-35.02	54.669

Data 2

	Area	StdDev	Angle	Length
1	7.583	40.991	29.745	5.269
2	7.796	56.718	-45	6.47
3	11.214	52.992	99.462	7.951
4	11.534	35.243	-20.556	8.376
5	19.01	42.36	-34.778	14.323
6	30.971	34.247	20.462	23.37
7	34.282	33.092	110.726	25.856
8	39.942	15.494	170.028	30.195
9	41.544	29.204	124.019	31.542
10	47.952	33.093	48.621	36.584