31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Classification of Hyperactivated Spermatozoa using a Robust Minimum Bounding Square Ratio Algorithm

Norbert Kaula, Anneliese Andrews, Catherine Durso, Christopher Dixon and James K. Graham

Abstract— **A method for automatically identifying and classifying hyperactivated spermatozoa trajectories is described. This physiologically-based computerized algorithm captures the motion behavior of sperm during hyperactivation. A novel Minimum Bounding Square Ratio (MBSR) algorithm classifies spermatozoa as hyperactivated, transitional or progressive. Classification boundaries were established on selected trajectory data from a single stallion and then tested on random trajectories of sperm from other stallions. MBSR classified sperm in a robust and effective manner.**

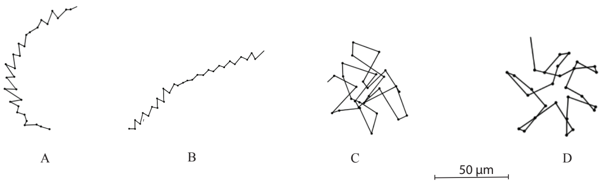

Fig. 1. Sperm Trajectories Classification examples, progressive (A,B), hyperactivated (C,D)

I. INTRODUCTION

An analysis of semen motility parameters is readily accessible and widely used assay for male infertility in human andrology clinics and in veterinary practices [4]. A prerequisite for sperm to fertilize an oocyte, a spermatozoon must undergo several biochemical changes, called capacitation [3], [7]. As part of the capacitation process the sperm motility pattern changes from a linear progressive motion to an erratic star-spin swimming pattern, called hyperactive motility. (Fig.1)[9]. Computer-assisted sperm analysis (CASA) devices can quantitatively assess sperm motility parameters as defined by the World Health Organization (WHO)[8], [1], including the percentage of motile cells in a sample as well as evaluation of the motions of individual cells, including sperm curvilinear velocity (VCL), average velocity (VAP) and straight path velocity (VSL). However these parameters fall short in determining what hyperactive motility is or how many sperm in a sample possess this type of motility. Previously, investigators have attempted to classify hyperactivated sperm using WHO parameters and reported that hyperactivated sperm exhibited increased VCL [2]. The main drawback to this method is the absence of effective thresholds between classes. Therefore, a new method is proposed to identify and classify hyperactive sperm based on describing the change in their motion from progressive to erratic sperm movement. The classification is based on interpreting changes in the sperm trajectory as a search pattern.

II. OBJECTIVES

The objective of this research was to develop and apply a robust algorithm which is capable of detecting changes in spermatozoa motility patterns and then to classify the sperm movement as being either hyperactive, transitional or progressive.

III. MATERIALS AND METHODS

A. Specimen Collection and Data Sets

Specimen from fresh stallion ejaculates were used to obtain motility data. The seminal plasma and dead cells in each ejaculate were removed by centrifugation and the sperm suspended in a diluent which maintains sperm viability to approximately $20 \cdot 10^6$ $sperm/ml$. This sperm concentration was chosen to minimize the number trajectories that would overlap with each other. Subsamples of 6 μl were analyzed using a Hamilton Thorne IVOS system for 0.5 s at 60 Hz. The resulting output file consisted of the x, y coordinate data pairs $(x_0,\ y_0)$ ... $(x_n,\ y_n)$ for each trajectory.

B. MBSR algorithm

Rather than attempting to explain and computationally identify the infinite number of different shapes of hyperactivated sperm tracks, we interpret the erratic movements as a search pattern of the spermatozoa to reach the oocyte for fertilization. We define a search area of the trajectory A_{hull} as the envelope of the set of trajectory points. Contrary to the convex or concave contour shape of a set of points, a sperm trajectory is more $snake-like$ and the trajectory can overlap itself. We decided to represent the trajectory area as a set of joint convex hulls to preserve as much as possible of the motility pattern. If the trajectory search area is A_{hull} and A_{MBS} the minimum bounding square $exploration\ region$ enveloping the trajectory (Fig.2), A_{hull} is computed as the sum of joint convex hulls, as a reasonable approximation of the are covered area by the sperm trajectory,

N. Kaula, A. Andrews and C. Durso are with the Department of Computer Science, University of Denver, Denver, CO, USA. `nkaula@cs.du.edu, andrews@cs.du.edu and cdurso@cs.du.edu`

C. Dixon is with the Department of Urology, New York Medical College, New York, NY, USA `cdixon@svcmcny.org`

J, K. Graham is with the Department of Biomedical Sciences, Colorado State University, Fort Collins, CO, USA `James.K.Graham@colostate.edu`

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$$A_{hull} = \sum_{i=1}^{h} A_i \quad (1)$$

where h is the number convex hulls and A_i the $i-th$ hull. Due to its geometric properties, a progressive sperm track will therefore return a small A_{hull} whereas A_{hull} from the trajectory of a hyperactivated sperm will have a larger value. The optimal number of hulls h was experimentally determined by plotting the average of five typical star-shaped hyperactive and linear progressive trajectories, respectively (fig. 1 A, B, D). The goal was to maximize the area for hyperactive tracks, without including the center area of a star-shaped trajectory. For progressive tracks, the goal is to minimize A_{hull}. Fig. 3 shows the relationship of trajectory area and number of connected hulls. At $hull = 1$, a single convex hull, the area of the hyperactivated tracks is over-estimated by including the center portion. With increasing $h \leq 4$ the trajectory area is better described, however $h > 4$ produces increasing voids in the chain of convex hulls. Similarly for $1 < h < 4$, the progressive half-moon shaped tracks are overestimated until $h \geq 4$, where the trajectory area changes little. Given the goal of maximizing A_{hull} for hyperactive sperm while minimizing A_{hull} for progressive sperm and given that h can only be an integer, $h = 4$ is the best candidate to describe the trajectory area for both hyperactivated or progressive sperm.

Next we can calculate A_{MBS} by taking the largest side of the bounding rectangle of the trajectory to form a square.

$$A_{MBS} = \left(max_{(\Theta)}\left\{x_{\max}\left(T_\Theta\right) - x_{\min}\left(T_\Theta\right), y_{\max}\left(T_\Theta\right) - y_{\min}\left(T_\Theta\right)\right\}\right)^2 \quad (2)$$

Where $(x_{min}, y_{min}, x_{max}, y_{max})$ are the extreme $x-$ and $y-$ coordinates of a trajectory and T_Θ is the trajectory T rotated through the angle $0 \leq \Theta \leq \frac{\pi}{2}$. Choose Θ to maximize the larger side of the rectangle. The consideration of rotated trajectories makes A_{MBS} independent of the trajectories' orientation relative to the artificial CASA axis. Finally, to express the search efficiency of a spermatoza, take the ratio of the trajectory hull area A_{hull} and exploration region, A_{MBS}.

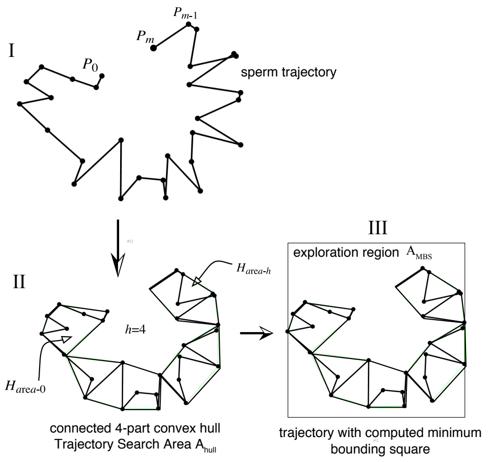


Fig. 2. MBSR calculation. I: original trajectory; II: Calculation of A_{hull}; III: A_{hull} within A_{MBS}.

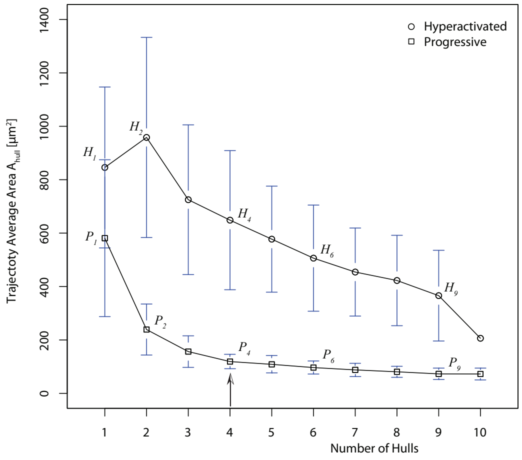


Fig. 3. The dependency of the number of convex hulls and resulting trajectory area. Plotted are the average of five typical 'star-shaped' hyperactivated and five progressive trajectories. The arrow at $hull = 4$ denotes the optimal number of hulls.

$$MBSR = \frac{A_{hull} * 100\%}{A_{MBS}} \quad (3)$$

C. Model Parameter Calibration

We utilize three sperm motion classifications: hyperactivated, transitional and progressive sperm. The MBSR model parameters were calibrated on a training set of 40 randomly selected hyperactivated trajectories and 40 progressive sperm trajectories that were visually classified by experts in the field. Transitional sperm trajectories were not included, because the identification guidelines of transitional sperm trajectories vary substantially from investigator to investigator [6]. Therefore, we decided to define our own margins for this classification group. Each training set was run through the MBSR algorithm with an incrementally increasing MBSR threshold. The number of detected tracks were noted. Fig. 4 shows the detection sensitivity as a function of MBSR. The MBSR was increased until all trajectories of the progressive training set were detected, at 11%. The reverse was observed on the hyperactivated training set, where initially all tracks were identified and a declining detection rate was seen at approximately 10.5%. It is reasonable to use the intersection of both curves with a small margin of 5% for transitional trajectories. This small margin is justified, although neither training set contained transitional trajectories, the existence of this classification has been described by investigators [6], [5]. According to the training data, we defined the margins as: Progressive trajectories: $MBSR_p < 8.5$; Transitional: $8.5 \leq MBSR_t \leq 13.5$; Hyperactive tracks: $MBSR_h > 13.5$.

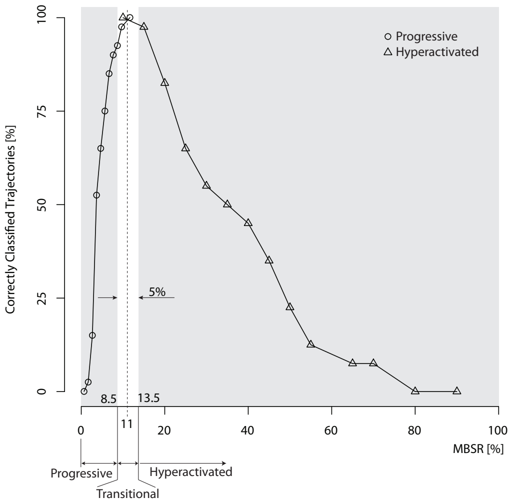


Fig. 4. Normalized MBSR Classification Boundaries. Based on a progressive ($n = 40$) and hyperactivated ($n = 40$) training data set. Margin were set to $\pm 2.5\%$ around the curve's intersection at $\sim 8\%$ MBSR. Progressive trajectories: $MBSR_p < 8.5$; Transitional: $8.5 \leq MBSR_t \leq 13.5$, Hyperactive tracks: $MBSR_h > 13.5$; Units are in $\%$.

D. Test Data

The training data consisted of 40 selected hyperactive and 40 progressive tracks, while the test data set was composed of four typical CASA laboratory recordings from one sperm droplet from each of two stallions (different from the stallion used for the training data). The droplets contained 18, 21, 52 and 47 trajectories (138 total). To keep the test data realistic, no further sub-selection was performed. This set was classified visually by experts to contain 29 hyperactivated, 25 transitional and 71 progressive trajectories. Thirteen tracks were either too small or considered unclassifiable (table I).

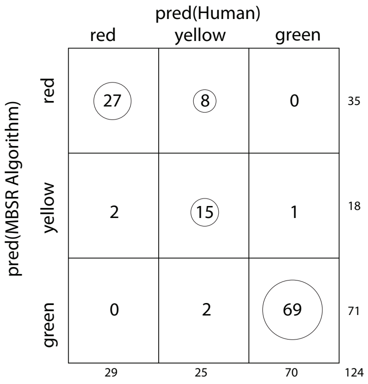
hyperactivated	transitional	progressive
29	25	71

classified total	rejects	Total Tracks
125	13	138

TABLE I
EXPERT CLASSIFIED TEST DATA

E. Evaluation

In addition to evaluating the MBSR algorithm with the test data set, we also compared the data retrospectively to the VCL thresholds that have been used to identifiy hyperactivated sperm previously.



pred(MBSR Algorithm)	pred(Human) red	yellow	green	
red	27	8	0	35
yellow	2	15	1	18
green	0	2	69	71
	29	25	70	124

Fig. 5. Diffusion matrix of the MBSR classification effectiveness. Hyperactive=red, transitional=yellow and progressive=green

IV. EXPERIMENTAL RESULTS

We evaluated the effectiveness of the MBSR algorithm by determining the number of correct classifications, false positives and false negatives on the set of 138 trajectories. Tracks were excluded if either the human experts or the MBSR algorithm threshold filter of $VCL \geq 50\ \mu m/s$, rejected it.

The MBSR algorithm correctly classified 93.1% (27) of the hyperactive trajectories, 60.0% (15) of the transitional trajectories and 98.57% (70) of the progressive trajectories (Fig. 5). MBSR produced 32.0% (8/25) false positives, where MBSR classified transitional sperm trajectories as hyperactive and two false negatives 6.9% (2/29) for the same category. More importantly, no hyperactivated trajectories were misclassified as progressive, or vice versa.

Using VCL values to classify hyperactive motility, the method used currently to indicate hyperactivity, did not perform as well as MBSR (Fig. 6). The threshold to match the MBSR performance (95 percentile) results in a loss of almost half of the progressive tracks. Also, the threshold to match the performance for progressive tracks, results in a loss of almost 50% of the hyperactivated trajectories when using VCL.

V. CONCLUSION AND DISCUSSION

The goal of this study was to develop an algorithm that would automatically classify spermatoza trajectories with comparable or better results than the current VCL thresholding or human expert classification. MBSR proved to perform better than VCL hyperactivity classification. At the same time the problem of classifying transitional sperm trajectories crystallized. While MBSR produced a high classification rate of $> 93\%$ and $> 98\%$ for hyperactive and progressive sperm, respectively, 32% of transitional sperm were falsely

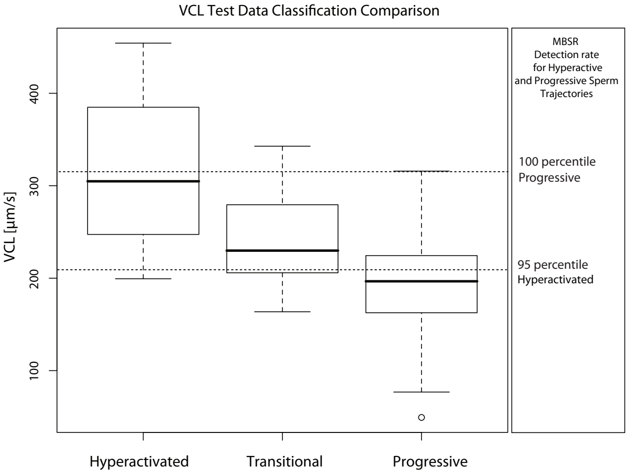


Fig. 6. VCL of Test data in hyperactive, transitional and progressive sections. The horizontal lines denote the detection rate of the MBSR algorithm. Note: There is no possible VCL threshold setting to achieve a similar or better result. To capture as many hyperactivated trajectories as the MBSR algorithm would immediately exclude one quantile of the transitional tracks and almost half of the progressive tracks.

classified as hyperactive. A retrospective examination of these misclassified transitional sperm trajectories uncovered a limitation of classifying transitional sperm trajectories by the experts in the field. Recognizing the difficulty of transitional sperm trajectory classification and to minimize ambiguity of the training data set, the experts in the field were instructed to classify only into hyperactive and progressive sperm. Remaining sperm trajectories fell into the transitional category. Retrospectively, these 8 false positive sperm trajectories could well have been classified by the experts as hyperactive instead of transitional. This raises the question about the validity of the current guidelines for experts for transitional sperm classification. Even with these difficulties, MBSR generated better results also for the transitional sperm trajectories than VCL (Fig. 6). Moreover, interpreting the shape of the motility trajectories as a search pattern does not restrict the algorithm to a limited number of hyperactivity patterns and mimics human behavior during sperm classification. Since each individual sperm sets its own exploration region, MBSR is scale invariant and works even on samples with lower motility.

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