Investigation on the change of spermatozoa flagellar beating forces before and after capacitation

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ABSTRACT

The swimming forces exerted by mammalian spermatozoa during the pathway to the ovary and during the interaction with the oocyte are thought to play a fundamental role in the fertilization of the egg. In particular, a process named capacitation is of key relevance for its success. Capacitation enables spermatozoa to undergo the acrosome reaction and to exhibit different motility called hyperactivation with a change in the sperm cell tail motion from symmetric to a more asymmetric beating, characterized by wider flagellar bending at lower frequencies. Despite several studies about the mechanism that underlies capacitation, no quantitative information is available about the forces associated with sperm motility. Sperm cell motility has been widely studied with digital imaging tools and video microscopy, but these methodologies cannot provide information about the forces exerted by spermatozoa during the motion and the contribution of every single frequency of flagellar beating to the sperm cell movement. For this purpose, fluidic force microscopy was used to trap single swimming spermatozoa allowing to evaluate these parameters. We observe significant differences between capacitated and non-capacitated spermatozoa in terms of force exerted and beating frequencies. The description of the dynamics of this process is of great interest in the field of reproductive medicine. Such information could be useful to clarify unknown causes of male infertility or for the development of novel methods to assess the quality of semen samples.

Keywords: Spermatozoa, FluidFM, Flagellar beating frequencies, Capacitation, Swimming force, Output power

1. Introduction

In mammalian fertilization, the spermatozoa entering the female reproductive tract undergo a process of selection that significantly reduces the number of sperm cells at the site of fertilization. A key event

of this process is sperm cell capacitation, a physiological change of the plasma membrane permeability to HCO^- and Ca^{2+} that causes a change in the sperm cell motility leading to sperm cell hyperactivation [1, 2]. This process is regulated by different factors like a gradient of chemical factors released by the follicular fluid and a change in the environmental conditions of pH and ionic force. Capacitation also results in a change of the sperm cell metabolism [3] that leads to a different motility: the wider flagellar bending of capacitated sperm cells enables the detachment from the fallopian tube epithelium, a fundamental selective process occurring during the sperm cell path to the ovary [4].

A further selective process occurs once the sperm cells approach the oocyte. At this level they need to cross through the hyaluronan matrix in which the cumulus cells are embedded, to finally bind to the surface of the zona pellucida (ZP), the glycoproteic layer surrounding the oocyte. Only a few bound spermatozoa will manage to pass through the ZP. At this point, a further physiological and morphological change occurs on the sperm cells' surface: the acrosome reaction. [5].

The perfect timing of this mechanism together with the right binding on the ZP surface allows the sperm cell to cross the oocyte plasma membrane [6].

Up to this moment, sperm cell motility is thought to be one of the main factors determining its success. In particular, the swimming speed, the forces developed by the sperm flagellar motion and the different patterns of sperm flagellar bending are essential for fertilization.

In human reproduction, the development of methods to assess the quality of the semen sample is of great interest. For this reason, many different techniques have been developed to evaluate the different parameters that characterize sperm motility. The most used is computer-assisted semen analysis (CASA) in which a centroid is established for each spermatozoon and the motion of each cell is recorded as the change of centroid position in successive frames. This allows to obtain some of the motility related parameters such as curvilinear velocity (VLC), linearity of the curvilinear path (LIN) and beat-cross frequency (BCF) [4, 7]. Moreover, by high-speed video microscopy coupled to image analysis software, other parameters related to the flagellar motility (e.g. shear angle, the beat frequency and the mean curvature of the flagellar bends) can be obtained [8]. By these methods, it was possible to detect changes in the flagellar bending between the non-capacitated sperm cells and the capacitated ones, the latter being characterized by a more asymmetric beating, lower beating frequencies and higher bending curvatures [9-11]. Sperm cell manipulation in order to get motility related parameters has already been performed by optical tweezers [12, 13]. By this tool, it was also possible to investigate the forces associated with the human sperm tail beating [14, 15]. The problem with optical tweezers is related to the smaller forces applied (up to 100 pN) and normally requires the use of viscous solutions or chemicals to reduce the sperm cell movement. On the contrary the range of force applied by AFM are higher (up to 100nN) [16]. This allows to perform measurements of

sperm cells in physiological conditions. An AFM cantilever was already used as a nanomechanical sensor for the detection of sperm samples with different motility [17]. However, the quantification of forces generated by the different flagellar beating modes has not been investigated so far.

A suitable tool to achieve this goal is fluidic force microscopy (FluidFM), a recent technology which combines atomic force microscopy (AFM) with micro channelled cantilevers having micro-sized apertures, which enable to rapidly manipulate single cells under physiological conditions. Employing a pressure controller, FluidFM enables to attach cells on the cantilever tip for single-cell force measurement [18]. In this work, we used FluidFM to trap single sperm and measure the forces involved in the motility of mouse spermatozoa. The tracking of the motion of a cantilever enables the detection of the different frequencies associated with the sperm motion and to obtain the force values related to these different modes of flagellar beating. Moreover, we can also obtain the output power generated by the sperm cell movement, an additional parameter that can be useful for the sperm cell motility evaluation. Finally, through these parameters, the difference in the flagellar motion between the non-capacitated and capacitated sperm cells was evaluated showing the power of this tool to highlight changes in motility modes of different sperm cell conditions.

2. Materials and methods

2.1. Collection and preparation of spermatozoa

Spermatozoa are obtained from the caudal epididymis of five 7-weeks-old male mice (C57BL/6). All experiments are performed under the project #509LAZ19, which was approved by the Administrative Panel of Laboratory Animal Care at the University of Trieste and by the Italian Health Ministry. To optimize the number of sacrificed mice, sperm cells are subjected to a freezing-thawing procedure as described elsewhere [19, 20]. For thawing, 50μl of sperm cell solution are diluted into 4 ml of sperm preincubation medium (c-TYH) with or without the addition of 0.75 mM metil-β-cyclodextrin (MBCD, Sigma Aldrich), a molecule that interacts with the membrane cholesterol inducing the sperm cell capacitation [21]. The composition of the medium is described by Choi et al. [22]. All the chemicals were purchased from Sigma Aldrich. The concentration of the sperm cells during the measurement was maintained at around 3000 per ml.

2.2. FluidFM measurements

Sperm cell trapping is performed with a JPK Nanowizard III AFM (JPK instruments, Berlin) with FluidFM micropipettes with a 2 μm aperture and a nominal spring constant of 0.3 N/m (Cytosurge, Zurich). Before each measurement calibration of the probe was performed by thermal noise method

[23] to detect the change in the force signal associated with the oscillations of the vertical deflection during the time of the measurement. In Figure 1 the experimental setup is showed.

To trap a single sperm cell, the pressure inside the cantilever is maintained at 0 Pa, until we approach a sperm cell inside the medium. Once sufficiently close, we start to apply a negative pressure of -800 Pa. When the sperm cell is trapped on the cantilever tip, we immediately lower the pressure to -200 Pa to avoid any negative interference of the applied force with the spermatozoon motion. We generally capture sperm cells by the tail, to maintain constant the trapping region over the analysed sperm cells, and to have comparable data.

The sperm cell motion is recorded for a time interval of about two minutes at a sampling rate of 1kHz. Most of the time the trapped sperm cells strongly adhere to the cantilever tip during the measurement. In these cases we need to apply an additional force to the sperm cell by rapidly moving the cantilever up and down inside the medium. This has a negative effect on the sperm cell motility after the release that is however not detected before and after the trapping of the sperm cell and during the time of the measurement. All the measurements were carried out at room temperature. After every trapping, the sample is transferred into the incubator and a cantilever cleaning procedure, as described below is performed.

The cantilever is immersed sequentially in a warm milliQ solution (50-60°C), a warm bleach 5% solution and again in Milli-Q water. Every step takes more or less 2 min, each time a positive and negative pressure is applied to remove any cell debris attached to the probe.

2.3. Maximum amplitude (Fmax) and mean output power of the single oscillation frequencies

Fast Fourier transform (FFT) of the oscillation trace, separation of the single frequencies and inverse Fourier transform (IFFT) are performed with Igor Pro 6.37. Briefly, different frequencies were isolated from the FFT plot taking into account both the imaginary and the real part of the data. These FFT plots were inverse transformed to come back to the real space where the contribution of the frequencies to the original deflection signal can be detected (Figure S1). In the case of the comparison between capacitated and non-capacitated sperm cells, we select directly from the FFT plot different ranges of frequencies up to about 20 Hz, for a constant interval width of 3Hz. This is the smaller frequency that allows to include an integer number of peaks for each interval.

Then we perform an IFFT of the selected ranges and we obtain the contribution of the single frequencies to the force signal. As we cannot control the moving direction of the sperm cell during the measurement and this affects the amplitude of the force signal, we chose for each sperm cell the maximum force as an informative parameter.

The instantaneous output power (P) generated by the different range of frequencies is given by

$$P = \frac{d(FS)}{dt} = \frac{1}{k}\frac{dF^2}{dt}.$$

where k is the spring constant of the cantilever, F is the detected force, and the displacement (S) is obtained from hook law for the cantilever deflection, considered as an ideal spring. To obtain the value of the mean power we calculate the mean value around the peak of the maximum force identified in the iFFT plot for each different frequency. The mean value and the standard deviations of the Fmax and the mean output power for the different ranges of frequencies are calculated and plotted with GraphPad Prism 9.

2.4. Evaluation of the sperm cell distance from the cantilever

The distance of the sperm cell from the cantilever in the z-axis for each frame of the movie is calculated on the base of an optical calibration (Figure S2). A dead sperm cell is deposited on the bottom of a petri dish and different images are acquired by changing the focus of a known distance (every 50 nm). Then for each image, we obtain the width in pixel evaluated at the first region of the sperm cell midpiece. The pixel calculation was performed manually with imageJ. We use the equation of the line interpolating the data to convert the number of pixels of the movie frames into the spermatozoon-cantilever distance.

2.5. Statistical analysis

The statistical difference between the output power and the forces related to the different frequency ranges between capacitated and non-capacitated samples was evaluated by using the Mixed Effects Model statistical test with GraphPad Prism 9.0. A p value < 0.05 was considered to be statistically significant.

3. Results

3.1. Detection of flagellar beating frequencies and forces

A scheme of the measurement setup is illustrated in Figure 1. FluidFM consists of a cantilever with a microfluidic channel inside that is connected with the external environment by a hole on the tip. From the other side, the microchannel is connected to a reservoir, linked to a tubing system that allows to control the pressure inside the channel by means of a pressure controller. By the application of a negative pressure the medium inside the petri dish is aspirated, by the application of a positive pressure the medium is thrown out. We exploited this working principle to trap the sperm cell on the cantilever tip and to detect the oscillations of the cantilever caused by its movements.

We record the cantilever oscillations as a change in the AFM vertical deflection signal vs time. By a proper calibration procedure, it is possible to get the values of the force related to these oscillations.
In Figure 2, we show the change in the vertical deflection signal and the corresponding change in the variance around the mean value when no sperm cell is bound to the cantilever. When we look at the motion of the cantilever in the medium without sperm cells, the change in the vertical deflection signal is very small and also the variance is close to zero (Figure 2a, b). When sperm cells are introduced in the medium, we can detect an increase in the cantilever fluctuation highlighted by a change in the vertical deflection signal and a slight increase in the corresponding variance from zero to a maximum value of 1,5 nN^2 (Figure 2 c,d). These force variations are caused by fluid perturbations induced by the sperm cell motion inside the medium, which may either induce the oscillation of the cantilever itself or perturb the laser beam path through the creation of medium inhomogeneities.
When a vital and active sperm cell is trapped on the cantilever tip the change in the cantilever vertical deflection and the corresponding variance is shown in Figure 3.
We notice an increase in the cantilever response (Figure 3a) that is also highlighted by a sharper increase in the variance at specific times (Figure 3b). The increase in the variance is higher in this case and reaches maximum values of 3.5-4 nN^2. If we observe in detail the evolution of the force signal in these time intervals, the presence of some pattern of periodic oscillation can be detected (Figure 3c). These sharp peaks can only be detected when a sperm cell is actively trapped on the cantilever tip, so we assume they are related to the tail bending of the attached sperm cell. In order to minimize the background signal coming from the free swimming sperm cells in the medium, we maintain low the concentration of sperm cells during the measurement. 3000 per ml, that still allows to easily trap the single swimming spermatozoa.
To separate the contribution of the different frequencies, we perform a fast Fourier transform (FFT) of the force-time plot. As the aspiration at a pressure of -200Pa is maintained constant during the sperm cell trapping and the signal detection, we first checked for the presence of some specific frequency when no sperm cell is captured. In this case, the only frequency detected is the one at 40Hz, which is associated with the aspiration of the liquid inside the fluidic probe, indeed its amplitude changes according to the aspiration pressure (Figure S3a-d). On the other hand, when the sperm cell is trapped on the tip of the cantilever, different frequencies can be distinguished as shown in Figure 4. To detect the contribution of the single oscillation frequencies in the original oscillation plot, we select and separate the specific frequencies from the FFT plot and we perform an inverse fast Fourier transform (IFFT) (as described in Figure S1).
Here we show the force-time plots (Figure 4a, d), the magnitude of the fast Fourier transform (FFT) (Figure 4b,e) and the inverse Fourier transform (iFFT) (Figure 4c, f) for the selected frequencies for

two sperm cells recorded over 2.5 minutes. A few sperm cells (2 cells out of 15 measured in these conditions) are characterized by the contribution of two low-frequencies only (< 5Hz, Figure 4a, b, c). However, most of the analysed sperm cells (85%) are characterized by a higher number of frequencies rising up to 20 Hz (Figure 4d, e, f).

If we look at the iFFT we can notice that the amplitude of each frequency changes independently during the time of the measure, reaching a maximum at times that may differ depending on the specific frequency. In this way the contribution of the different frequencies to the overall oscillation trace changes in time.

3.2. Different swimming directions influence the sperm cell force recording

As we are measuring the vertical displacement of the cantilever, we suppose that the change in the transmitted force may depend on the swimming direction of the spermatozoon when captured on the cantilever. To test this hypothesis, we evaluate the change in the sperm cell position along the z-axis with respect to the cantilever by looking at the defocusing of the first region of the sperm cell midpiece, during the movie acquisition. The measured values are converted in distance (nm) on the base of a dedicated optical calibration (Figure S2 and Methods paragraph 2.4).

In Figure 5 we show different frames of the movie (Figure 5a) together with the simultaneous oscillation trace of a non-capacitated sperm cell trapped on the cantilever tip for 90 s (Figure 5b) and the change in the corresponding distance of the sperm cell from the cantilever (Figure 5c). When the sperm cell swims outside the cantilever plane and the distance from the cantilever is high (at least 160 nm), different oscillation frequencies are detected and the amplitude of the oscillations reaches its maximum (time points 1-3 in the figure); on the contrary, the amplitude approaches to zero when the sperm cell swims on the same plane of the cantilever (time point marked as 4 in the figure). Moreover, as it can be better visualized in Supplementary Movie 1, we can optically detect an increase in the sperm cell motility after about 35-40s and this matches with an increase in the amplitude of the lower frequency oscillation (at 1 Hz, depicted in red in the figure and in the movie). On the contrary the change in the amplitude of the higher frequencies oscillation (3 and 6 Hz, depicted in yellow and blue in the figure and in the movie) is not visually detectable because of the low frame rate of the camera (7 fps). We also notice the presence of some free swimming sperm cells passing in the field of view, but their transition doesn't affect the force signal for the different frequencies considered.

The same behaviour is observed for capacitated sperm cells (Figure S4, a-c and Supplemental Movie 2).

3.3. Capacitated sperm cells motion is characterized by a change in the frequencies and the forces

The difference between the motion of the capacitated and the non-capacitated sperm cells is evaluated by detecting the distribution of the maximum force (Fmax) related to the single frequencies and the output power generated by the sperm motion. Capacitation is a reaction that normally occurs in vivo in the female reproductive tract and is triggered by specific factors inside the follicular fluid. This reaction is here chemically induced by the addition of MBCD inside the medium, a factor that changes the sperm cell plasma membrane permeability by sequestering the cholesterol. This results in a change of the motility of the sperm cells that is normally characterized by an asymmetrical swimming pattern in figure-of-height [4].

In figure 6, the distribution of these parameters is shown for capacitated and non-capacitated spermatozoa.

In particular, the distribution of the Fmax values for non-capacitated sperm cells (Figure 6a) shows a significant increase of the Fmax for frequencies between 3-5 Hz at (4.88 ± 3.26) nN. The same trend can be noticed if we look at the distribution of the output power (Figure 6b) a parameter which gauges the amount of energy per time unit that is converted into sperm cell motion for a specific frequency range [24,25]. This is an important parameter because if combined with the total amount of energy available to the sperm cell (that is given from ATP), it could be used to quantify the sperm cell swimming efficiency [10,26]. Also, in this case, the value for frequencies between 3-5Hz is significantly higher compared to the other frequencies and is around $1.45\pm 2.00 \times 10^{-14}$ W.

On the other hand, if we look at the distribution of the Fmax values for capacitated sperm cells (Figure 6c and Figure S5a), we can observe that the Fmax is generally lower compared to non-capacitated ones. Moreover, the Fmax is significantly higher for frequencies between 0-2 Hz compared to the other frequency ranges and has a value of (3.77 ± 2.11) nN. The same trend can be noticed if we look at the distribution of the output power (Figure 6d and Figure S5b): in this case, the higher value is around ($0.49 \pm 0.63 \times 10^{-14}$) W in the frequency range between 0-2 Hz; moreover, there is not a significant difference in the value of this parameter compared to the other frequency ranges of capacitated sperm cells, while it is evident a general decrease in this value compared to the one found in non-capacitated sperm cells.

4. Discussion

In this study, we provide a detailed analysis of the motility of murine sperm cells before and after the capacitation process. The ability of FluidFM to reversibly capture a cell on a cantilever under

physiological conditions enable us to quantify the forces involved in the sperm cell motility and to evaluate the frequencies of the flagellum beating.

The majority of the sperm cells is characterized by a high number of frequencies between 0-20 Hz, as already been identified through image analysis of video microscopy. The range of frequencies changes according to the animal species for instance, in hamsters it is between 0-25 Hz with a mean value of 15 Hz [8, 27] while in shrews it is between 0-10 Hz with a mean value of 3,4 Hz [28]. In humans the range of frequencies is even higher, reaching 30 Hz [9, 10]. However, the contribution of every single frequency to the overall propulsion and/or penetration force has not been investigated so far.

What we additionally observe here is the change in the amplitude related to the single oscillations during the time of the measurement.

This occurs for several reasons. First, as the sperm cell moves the mechanical coupling with the cantilever can change, causing a variation in the value of the detected force. Second, we also observe that the measured intensity of the force changes according to the swimming direction of the sperm cell. In particular, when it moves away from the cantilever on an orthogonal plane, the maximum force value is detected, while when it swims in the same plane of the cantilever a lower force value is measured because the force component on the cantilever plane, which is predominant in this configuration, does not affect the vertical deflection of the cantilever.

For these reasons, we choose the maximum force value (Fmax) of each frequency as the relevant parameter for further discussions, and we decide to observe the distribution of this value for each frequency range in two physiological conditions of spermatozoa: capacitated and non-capacitated ones.

In non-capacitated cells, we observe that the measured forces decrease as the frequency increases, indicating that the slow, amplitude motion is the most relevant in the swimming thrust, in particular the range of frequencies between 3-5 Hz, shows the highest propulsion forces as high as (4.88 ± 3.26 nN). After capacitation the exerted forces are generally reduced, however, the force suppression is significant only for the frequencies between 3-5 Hz (figure S5).

As already reported in our previous study [29] and displayed here in the figure S6, we show that a force of 1 nN exerted by a 4,5μm diameter bead (the same order of magnitude as the frontal area size of a sperm cell) is sufficient for the penetration of the zona pellucida up to a depth of 1-2 μm. Here we found that forces as high as 1nN are observed at frequencies as high as 20Hz suggesting that these forces could play a role in the sperm penetration through the ZP. Sperm cell capacitation could have the function to decrease the forces of interaction between the sperm cell and the oocyte, regulating

the access to the oocyte and making other processes (like the acrosome reaction) necessary. This is consistent with the effect of the capacitation only on the 3-5 Hz frequencies.

On the other hand, the higher forces of non-capacitated sperm cells in this frequency range could be linked to the higher swimming ability and progressive motility of these sperm cells [30].

The lower frequency motion of capacitated sperm cells compared to the non-capacitated ones was already detected, together with a more asymmetric motion. The reason for this change in the motility of capacitated sperm cells has been linked to the sperm cells binding to the fallopian tube epithelium and the necessity to overcome this barrier. A change toward a lower beating frequency was also considered advantageous in higher density fluids such as the follicular one [1, 4].

It was also suggested that the higher contribution of the transversal forces at low frequency, characterizing capacitated sperm cells, play a fundamental role in the oocyte penetration by acting on the plastic behaviour that ZP exhibits at higher deformations [31]. Alternatively, we can hypothesize that the lower forces of capacitated sperm cells promote the binding to the ZP surface, but a precise kind of motion is necessary to trigger the acrosome reaction allowing the sperm cell to pass through the ZP.

From the force value, it is also possible to calculate a further parameter, the output power which is the amount of energy per time unit that is converted into sperm movement. The distribution of the values of the output power shows a trend similar to the one of Fmax, highlighting the higher contribution of frequencies between 3-5 Hz in non-capacitated sperm cells to the power generated by the sperm motion; this is not highlighted in capacitated sperm cells that are characterized by three times lower values of the output power compared to non-capacitated ones. Previously, by flagellar waveform tracking and analysis, the modified resistive force theory of Lighthill was applied to calculate the hydrodynamic force exerted by the human sperm flagellum and from this the power dissipation due to the beating of the flagellum. Different values have been found ranging from 0.84 x 10^{-14} W [26], to 3.5 x 10^{-14} W [10], up to 8.9 x 10^{-14} W [9]. However, the values found in the literature deal with an estimation of the dissipated energy rather than with the effective thrust; in our case we directly calculated the power transferred by the tail beating to the sperm forward motion, obtaining values in the same order of magnitude (0.49-1.45 x 10^{-14} W) but smaller, as it was expected from the observation that only a fraction of the dissipated energy is effectively converted in forward displacement.

5. Conclusions

In conclusion, we show that FluidFM allows for the detection of the frequencies and the forces related to the movement of flagellate living systems. While optical tracking methods provide only the calculated value of the viscous power dissipation of the tail, here, for the first time, we directly measure the value of the power transferred to the cantilever and calculate the forces exerted by the sperm tail thrust during the motion. These parameters can be employed to investigate the role of the forces in the process of fertilization and to improve the selection of the semen sample for clinical procedures.

We find that the force values alone involved in the sperm cell motion are in principle, sufficient to penetrate 1-2μm inside the ZP and that the deeper flagellar bending at low frequencies of capacitated sperm cells is characterized by a decrease in the natatory force related to the higher beating frequencies (3-5 Hz). This prompts us to speculate about the functional role of the motility forces in the sperm cell interaction with the egg.

Finally, we identify two quantitative parameters (the maximum force and the output power related to the single oscillation frequencies) that can distinguish two different sperm cell conditions and can be potentially used to detect the fertility potential of sperm samples for in vitro fertilization.

CRediT authorship contribution statement

A. Battistella: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing-original draft, Visualization. L. Andolfi: Writing-editing and review, Visualization, Project administration. M. Stebel: Investigation, Writing-review and editing. C. Ciubotaru: Software, Data curation. M. Lazzarino: Conceptualization, Resources, Writing-review and editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare no competing financial interests.

Acknowledgements

We acknowledge financial support from the Interreg Italia-Slovenja program through the project Nano-region. We acknowledge Giuseppe Ricci from the “Istituto di Ricovero e Cura a Carattere Scientifico- IRCCS Burlo Garofalo” for the advice and all the people of the animal unit of the University of Trieste.

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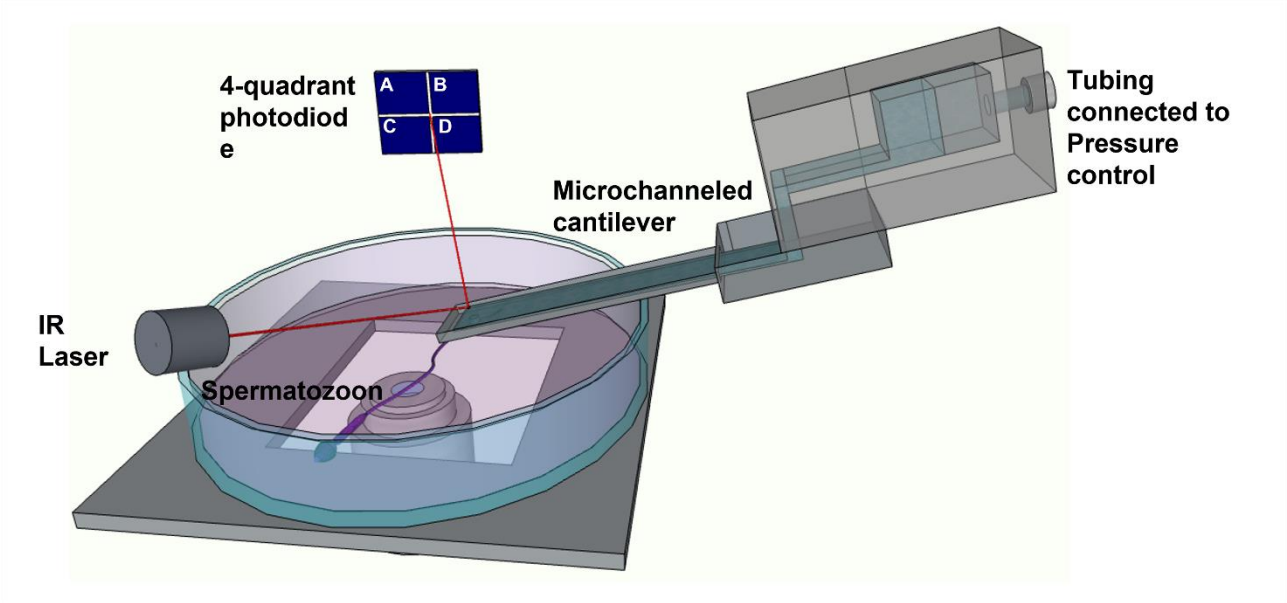

Figure 1: schematic representation of the experimental setup. A spermatozoon is trapped by the tail on the tip of a microchanneled cantilever, by the application of a negative pressure inside the channel by means of a tubing system connected to a pressure controller

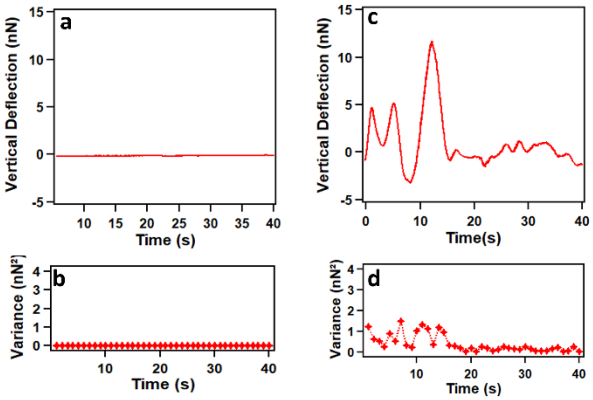

Figure 2: Vertical deflection signal and corresponding variance when no sperm cell is bound to the cantilever. Vertical deflection signal (a) and corresponding variance (b) when no sperm cell is present inside the medium. When the sperm cells are introduced inside the medium the oscillation of the vertical deflection signal increases (c) and also the corresponding variance increases (d).

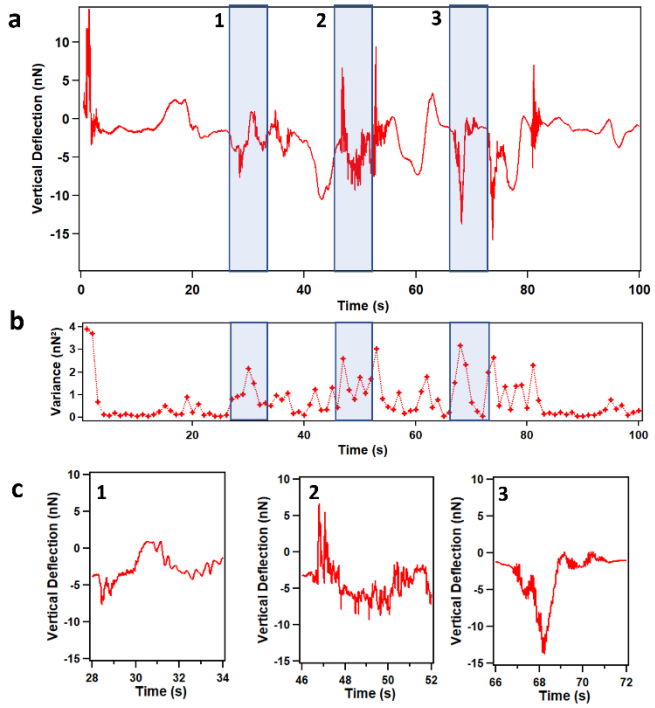

Figure 3: Vertical deflection signal and corresponding variance when a sperm cell is bound to the cantilever. Sharp oscillations of the vertical deflection signal (a) and the corresponding variance (b) appear at specific times (subsections 1-3 marked in blue in the figure) when a sperm cell is linked to the cantilever. The same periodic oscillations are highlighted (c).

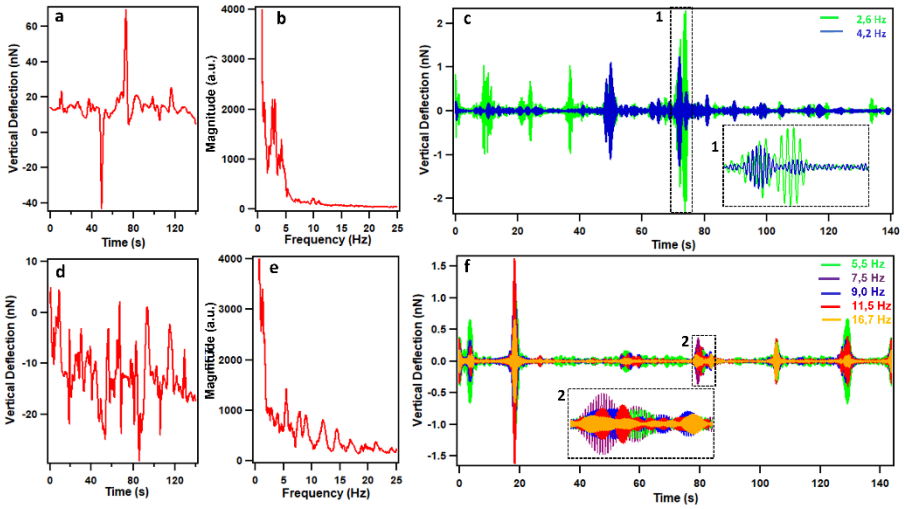

Figure 4: Vertical deflection, fast Fourier transform (FFT) and inverse FFT (iFFT) of the selected frequencies when the sperm cell is linked to the cantilever. In a small fraction of the analysed sperm cells (N=14) we detected the presence of few oscillation frequencies between 0-5 Hz (a, b, c). But most of the sperm cells analysed (85%) are characterized by the presence of multiple frequencies in the range 0-20Hz (d, e, f). Vertical deflection signal (a, d), FFT in which a 51-point Savitzky-Golay filter is applied (b, e). IFFT of the isolated oscillation frequencies (c, f).

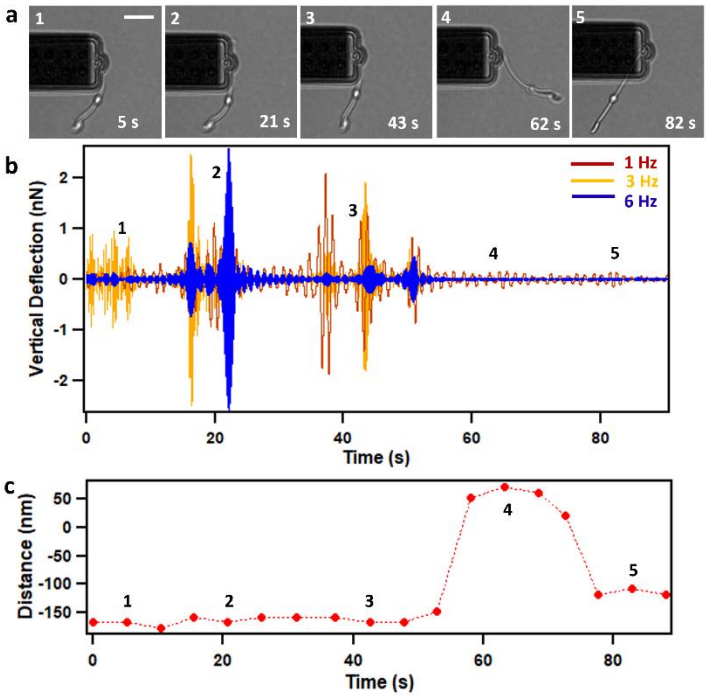

Figure 5: Swimming direction of a non-capacitated sperm cell influence the oscillation amplitude. Different frames from Supplemental Movie 1 at 5s (1), 21s (2), 43s (3), 62s (4), 82s (5) highlighting different orientations of the sperm cell attached to the cantilever (a) Scale bar is set to 10µm. The oscillation trace as a change in the cantilever vertical deflection is depicted (b). Numbers on the plot (1-5) refers to the frames depicted in a. The corresponding distance of the sperm cell from the cantilever is depicted (c).

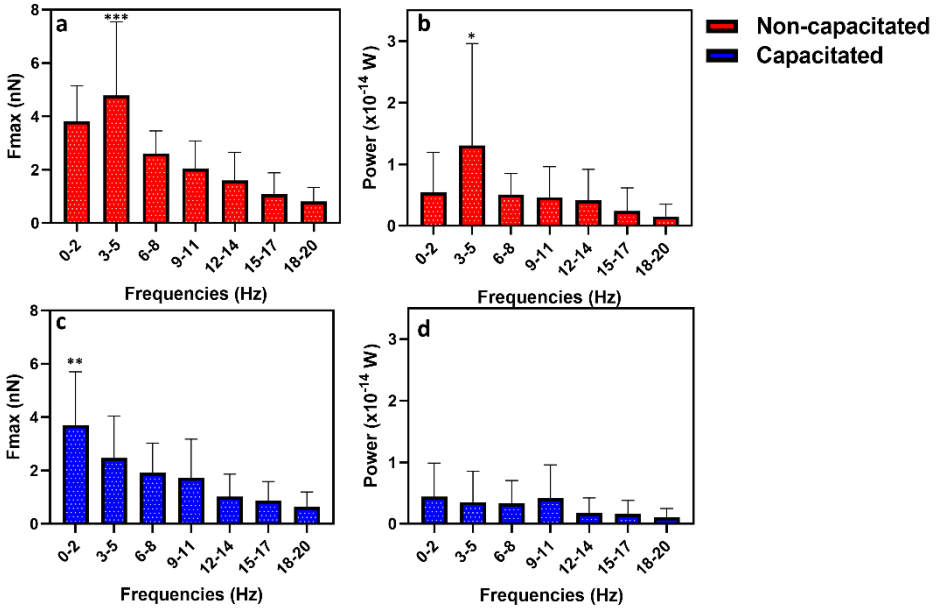

*Figure 6: Change in the maximum force and the output power generated from the sperm cell movement in two sperm cell groups. Mean values and SD for each range of frequencies are depicted. In non-capacitated sperm cells (N=14) the maximum force exerted is about 4.88 ± 3.26 nN in the range of frequencies of 3-5 Hz. This value is significantly higher compared to all the other frequency ranges (a). The output power follows the same trend reaching maximum values around 1.45 ± 2.00 x 10^{-14} W in the same frequency range, also in this case this value is significantly higher compared to all the other frequency ranges (b). Capacitated sperm cells (N=14) are characterized by significantly higher forces about 3.77 ± 2.11 nN in the range of 0-2 Hz compared to the other frequency ranges (c) and very low output power with a maximum value of 0.49 ± 0.63 x 10^{-14} W (d). The significance level was set at *p<0.05, **p<0.001 and ***p<0.0001*