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Collective motility of sperm in confined cells

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1. Introduction

Mammalian fertility analysis is an important industrial issue because of the selection for breeding. This is one reason, beside fundamental interest, for which many studies analyse the individual motion of the spermatozoon (Gaffney et al. 2011). On the other hand, more recent reviews suggest that neither individual motion indicators nor molecular markers can be clearly correlated to fertility (Kastelic and Thundathil 2008; Nathali and Turek 2011). This is why the industry still uses the scoring of sperm motility from the observation of semen sessile drop with a phase-contrast microscope. This observation of pure semen displays wave motion associated with millions of sperm moving together in circular waves and whirlpools. The moving speed, deformation and size of whirlpools were ranked and scored. Similar collective movements have also been observed in different biological suspensions above a certain concentration (Sokolov et al. 2007). But until today, there has been no clear analysis of the origin of the observed whirlpools dynamics in semen. In this article, we provide new insights on the origin of whirlpools. As the sessile drops do not permit a careful control of the micro-hydrodynamic boundary conditions associated with surface tension variations, we investigate collective effects in controlled rectangular cells confined span wise with 20 and 100 μm depth. We mainly analyse the influence of the confinement and the concentration on the appearance of whirlpools.

2. Methods

Samples of ram semen have been collected at CRIOPYC, an insemination center in Toulouse (France). The experimental set-up comprised a fast camera (pco.dimax) coupled with a Nikon DIAPHOT-TMD phase-contrast microscope. The images have been acquired at 50 Hz with a ×4 magnification calibrated at 921 pixels/mm. For the confinement effect observation, the sperm sample has been placed in 20 and 100 μm depth cells. The sample was maintained at 34°C during the experiment. We analyse the robustness of the whirlpool dynamics to dilution by adding NaCl isotonic solution to the semen at various concentrations.

3. Results and discussion

We observed that the whirlpools are both sensitive to the confinement and concentration. As can be observed in Figure 1(a), there is no such structure in a 20-μm depth cell, even in the pure semen sample. On the contrary, when increasing the cell depth to 100 μm, the structures of black whirlpools are clearly visible in pure semen (Figure 1(b)) or even in diluted semen (Figure 1(c)). It is interesting to mention that it takes few seconds before the appearance of the whirlpools after the semen injection in the cell. This can be explained by the fact that the capillary-driven semen in-flow has to settle for a while before the flow due to spermatozoa movement takes on large-scale dynamics.

When increasing the dilution to 5 times, as exemplified in Figure 1(d), the structures of whirlpools disappear. Hence, our experiments show that collective dynamics arises within calibrated cells when two conditions join together: at sufficient concentration and for sufficient in-depth space. The head of ram spermatozoa is roughly ellipsoidal with typical length along the principal axis being 5, 3 and 8 μm. Direct high resolution observations confirm that two of them can hardly span along the 20-μm depth direction. Hence, structures of whirlpools necessitate the interactions of microorganisms in three dimensions. They either result from a concentration or an orientation effect. It is not surprising that the wall confinement plays a significant role in the interactions of micro-swimmers (see e.g. Cosson et al. 2003; Berke et al. 2008; Denissenko et al. 2012). It is, however, the first direct experimental evidence of such an impact on the appearance of whirlpools. Furthermore, note that the lifetime of spermatozoon dynamics is close to 30 min.

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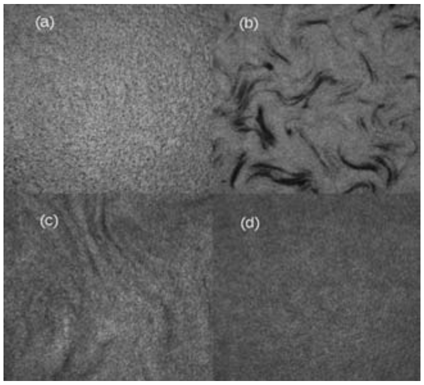

Figure 1. (a) Phase-contrast microscopic observation of ram semen confined in a 20 μm depth cell. (b–d) Same conventions for a 100-μm depth cell with pure (b) and diluted semen in isotonic NaCl (two times diluted in c and five times in d). One can observe the elongated structures of dark whirlpools, in which the orientation looks isotropic and appears only in the 100-μm depth cell (b and c) but not in the 20-μm depth cell (a).

After this short time, the structure of whirlpools remains frozen in the semen even after the spermatozoon death. We then wish to provide some quantitative structural analysis of the statistical spatial properties of whirlpools on the 2048^2 pixels images.

In order to quantify the spatial correlation within the structure of whirlpools, we compute the energy spectral density (ESD) of the image grey level, averaged over several hundred images. To estimate the energy distribution versus the spatial frequency, we have plotted the ESD versus the wave number. Before calculating the ESD, each image has been divided by an average of one to remove stagnant parts. As the 2D spectrum display isotropic wave-vector dependence, we average over the azimuthal direction and represent the ESD versus the wave-vector norm. Figure 2 displays the

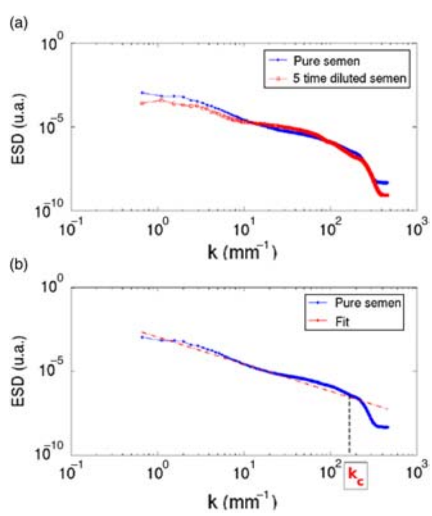

Figure 2. (a) ESD of pure and five times diluted semen. (b) Pure semen's ESD fitted by a power law with a cut-off frequency k_c.

resulting spectrum in bi-logarithmic representation. Figure 2 (a) shows a widely distributed spectrum, which can be associated with long-range power-law correlations. Such an isotropic spatial correlation in the whirlpools elongated structure is the signature of cooperative collective interactions, which built long-range structural organisation within semen, at length scales much larger than the size of sperm cells. In fact, the observed upper cut-off in the spectrum corresponds to 10 μm, which is indeed the size of the sperm head. This result is reminiscent the some recent observations in bacteria dense flow (Wensink et al. 2012), in which the long-range organisational orientation has also been observed. It is interesting to mention that dilution neither affects the length-scale nor the cut-off of the resulting spectrum.

4. Conclusions

In this paper, we have characterised the collective movement in the ram semen. It confirms the existence of collective motion, which is manifested by the appearance of whirlpools. We observed them on sessile pure semen drop, in confined cells of 100 μm depth, but not in more confined cells above a certain dilution rate. Studies are underway to understand the origin of the whirlpools. Furthermore, we characterised their spatial correlation with a power-law with a cut-off frequency.

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