Supporting Information

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SI Text

CD9 May Induce Small Clusters Causing S Adhesion. We hypothesize that the same number N of receptors are present on WT and *Cd9* null egg membranes. On *Cd9* null eggs, the N receptors are not connected together and they correspond to N isolated potential binding sites for sperm (Fig. 5*A*). On WT membranes, CD9 is able to assemble part of these N receptors into multiprotein patches connecting together i of these receptors on average. This leads to a reshaping of the membrane organization and to a reduction of the effective number of binding sites since one cluster made of i receptors is one binding site only.

If n is the number of clusters formed, the number N' of binding sites on WT membrane verifies:

$$N' = n + (N - ni) \quad \textbf{[S1]}$$

where the second term corresponds to the remaining isolated receptors. The interactions coming from the isolated binding sites have no reason to differ from those observed with *Cd9* null eggs. By contrast, adhesions originating from the clusters cannot be found on *Cd9* null eggs since their existence requires the presence of CD9. We have shown in the Results section and Figs. 3 and 4 that all the adhesive interactions except S-type adhesions are shared between WT and *Cd9* null eggs. The S-type adhesion would therefore correspond to sperm interaction with the clusters due to CD9 tetraspanin. The ratio, r, of receptors involved in the clusters is given by Eq. **S2**.

$$r = \frac{ni}{N} \quad \textbf{[S2]}$$

If we assume that the probability p of adhesion associated to a binding site is the same on WT and *Cd9* null egg, the probability to have no adhesion is $(1-p)^N$ on *Cd9* null eggs and $(1-p)^{N'}$ on WT eggs. Because these probabilities were obtained experimentally and equal to 0.25 for *Cd9* null eggs and 0.54 for WT eggs, the ratio $\frac{N}{N'}$ is equal to 2.25. Eqs. **S1** and **S2** can be rewritten in order to evaluate i and r.

$$i = 1 + \frac{(\frac{N}{N'} - 1)}{\frac{n}{N'}} \quad \textbf{[S3]}$$

$$r = \frac{n}{N'} \times \frac{N'}{N} + 1 - \frac{N'}{N} \quad \textbf{[S4]}$$

Making the simple assumption that single attachments are distributed in the same manner as the sites whether they are free receptors or patches, $\frac{n}{N'}$ corresponds to the fraction of interactions with patches in single attachment events. It can be estimated from the force experimental data. Indeed we have seen that simple attachment events are given by all **B** curves and part of **C** curves (Fig. 2). If one considers that none of the **C** curves correspond to single attachment, then $\frac{n}{N'}$ is equal to the fraction of interaction with patches (those corresponding to the second peak of the **B** distribution) in **B** curves. The S interactions that correspond to the second peak of the **B** distribution represent 40% of the **B**-shape curves (Histogram F_{rupt}, Fig. 4). Hence, 0.4 is an upper bound of $\frac{n}{N'}$. At the opposite, if one considers that all the **C** curves also correspond to single attachment then $\frac{n}{N'}$ is equal to the fraction of interaction with patches (those corresponding to the second peak of the **B** distribution) in **B**+**C** curves. This fraction is equal to $0.27 = 20/75$ because S interactions represent 20% of the total number of events, and **B** and **C** curves represent 75% of the total number of events. Hence $0.27 < \frac{n}{N'} < 0.4$. Injecting these values in Eqs. **S3** and **S4**, one obtains for i and r: $4.1 < i < 5.6$ and $0.68 < r < 0.73$. The result is that around 70% of the N receptors are involved in clusters composed of five receptors on average. Like CD9, these clusters would be localized on WT egg microvilli and accessible to sperm to generate the S-type adhesion.

Acrosome Status of the Sperm in BFP Experiments. In order to check the acrosome status of the sperm in conditions as close as possible to those of the force experiments, we tested live sperm expressing EGFP acrosine prepared with the same protocol as the WT used in the study (incubation of 90 min in medium supplemented with 3% BSA followed by 30 min of incubation in the same medium supplemented with 10 μM ionophore). Fig. S1 shows epifluorescent picture of EGFP acrosine sperm, acrosome reacted or not. We have successively grabbed with a micropipette a high number (64) of EGFP acrosine sperm that were extremely mobile, like the ones used in the BFP experiments. Among them, only one was acrosome intact (less than 2%). During the course of an experiment, sperm is micromanipulated and transferred to the oocyte droplet. This process can only increase the yield of acrosomal reaction. The conclusion is that the near totality of the WT sperm used in the force experiments are acrosome reacted just like in physiological context.

Protocol and Parameters of Force Measurements. A force distance curve was obtained at each approach-contact-retraction cycle (Fig. S2). By convention, the force was negative when the two cells were in compression and positive when in traction. During the approach phase, the egg was moved at constant speed (10 μm/s) into the direction of the sperm. At the beginning of egg displacement, the interaction force was zero. It started to decrease when the two gametes were touching. When the compression force reached 20 pN, the retraction phase begun, pulling the oocyte away from the sperm at 4 μm/s. Under these conditions, the total time spent by the gametes under compression was approximately 250 ms. If no intercellular bond was formed, approach and retraction force/distance curves were superimposed while when the gametes did adhere, an interaction force was measured until the link between the two cells broke. In order to have direct information on the egg membrane deformation, we plotted (Fig. 2) the interaction forces as a function of egg extension in the traction axis, defined as the difference of the egg at rest and under traction. The origin of distance was taken when the force reached zero during the pulling phase.

Force experiments were performed on a DMIRB Leica inverted microscope positioned in a heating box (Life Imaging Service, GmbH, Switzerland) to ensure stable temperature of 37 °C. Two 20 μl drops of M2 medium supplemented with 3% BSA were deposited on a Petri dish and immersed under mineral oil (mouse embryo tested light oil, density 0.84 g/ml, Sigma-Aldrich) as shown in Fig. S3. The experiment required three home made borosilicate glass micropipettes. Pipette 1 was used for RBC/bead/sperm manipulation. Its inner diameter was approximately 2 μm. Pipette 2 was positioned perpendicularly to pipette 1, to gently maintain sperm flagellum still during the experiment. Its inner diameter was approximately 3 μm. Pipette 3 was positioned in the same axis as pipette 1. Its inner diameter was larger (15 μm), it had a smooth rounded extremity in order to gently

maintain the egg without damaging it. The three pipette extremities were bent with a 30° angle. They were filled with M2 medium, connected to hydraulic systems controlling their aspiration, and placed onto micromanipulators (Narishige and Sutter) allowing their controlled displacements. Pipette 3 was also coupled to a linear 10 μm-range piezoelectric actuator to accurately drive the approach and retraction movements of the egg during the measurement. The use of two M2 drops in the Petri dish was necessary to have separate batches of sperm and eggs so that before the experiment, the eggs had never met any spermatozoon. Sperm were put in the first drop at a concentration of approximately 10^4/ml while eggs were deposited in the second drop as well as streptavidin-coated glass microbeads and the biotinylated RBC. Before each measurement, one hyperactive accrosome reacted sperm was caught by pipette 1, which was introduced into pipette 3 to safely transfer the sperm into the drop containing the eggs. Experiments took place in this latter drop with the transferred spermatozoon.

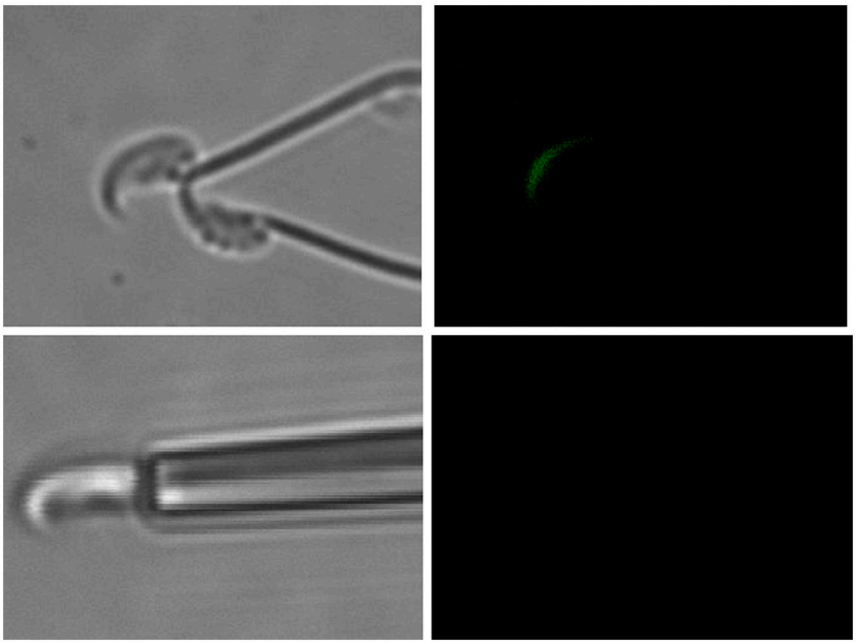

Fig. S1. Transmission and corresponding epifluorescent images of EGFP acrosine sperm. Top: Both acrosomal status can be found before incubation in the ionophore (the sperm showing fluorescence is not acrosome reacted, the other one is acrosome reacted). Bottom: After incubation, the great majority (63 over 64) of the super active sperm grabbed into the pipette was acrosome reacted (no fluorescence is observed).

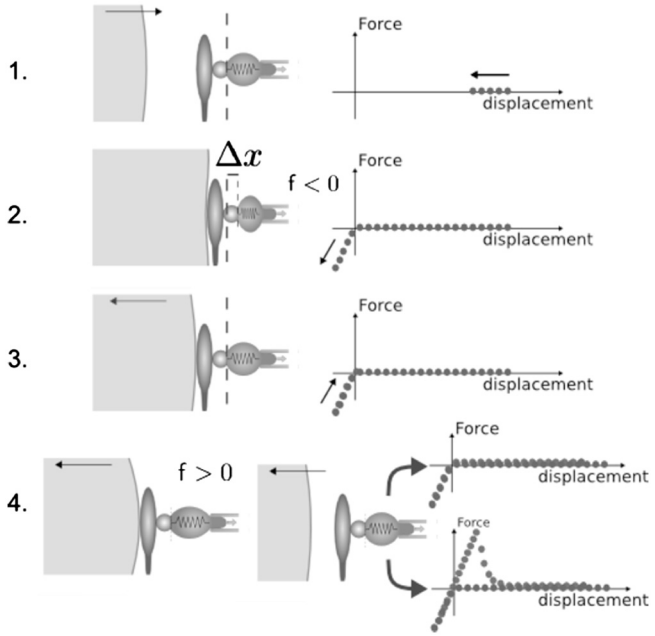

Fig. S2. The approach–contact–retraction cycle (1) starts with the egg moving toward the sperm attached to the spring. (2) The egg and sperm are in contact and the force recorded is negative by convention. Once the maximum compression force is reached, the egg is moved backward, (3) the force decreases back to zero. (4) When separating the two gametes, either no adhesion occurs (top), or gametes adhere (bottom) and the force is recorded until the complete separation of the egg and the sperm.

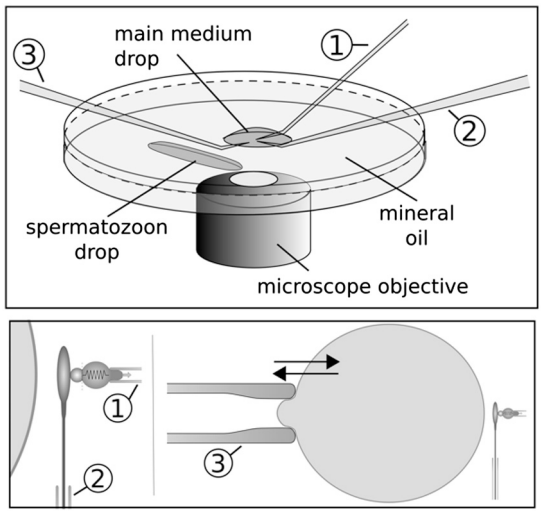

Fig. S3. Two drops of M2 medium are deposited on a Petri dish. Sperm are deposited in the elongated drop. The eggs are in the main drop where the force measurements are done. Pipette 1 holds the red blood cell. Pipette 2 maintains the sperm flagellum still. Pipette 3, facing pipette 1, holds the egg and is translated by a piezoelectric transducer during each approach–contact–retraction cycle.