Mapping Mouse Gamete Interaction Forces Reveal Several Oocyte Membrane Regions with Different Mechanical and Adhesive Properties

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Received July 25, 2007. In Final Form: September 12, 2007

This study focuses on the interaction involved in the adhesion of mouse gametes and on the mechanical properties of the oocyte membrane. The oocyte plasma membrane has two distinct regions: the first one is covered with microvilli, and the second one is smaller, without microvilli. With a biomembrane force probe (BFP) adapted to cell−cell measurements, we have quantified the separation forces between a spermatozoon and an oocyte. Microvillar and amicrovillar areas of the oocyte surface have been systematically probed and compared. In addition to a substantial difference in the elastic stiffness of these two regions, the experiments have revealed the presence of two types of membrane domains with different mechanical and adhesive properties, both distributed over the entire oocyte surface (i.e., in both microvillar and amicrovillar regions). If gamete contact occurs in the first type of domain, then the oocyte membrane deforms only elastically under traction. The pull-off forces in these domains are higher in the amicrovillar region. For a spermatozoon contact with the other type of domain, there can be a transition from the elastic to viscoelastic regime, and then tethers are extruded from the oocyte membrane.

Introdução

Mammalian fertilization consists of a series of events leading to the creation of a new being through the merging of a spermatozoon and an oocyte. The oocyte plasma membrane has two distinct regions: the first one is covered with microvilli, and the second one is smaller, without microvilli. In the latter region, fusion rarely, if ever, occurs. Why fusion preferentially takes place in the microvillar area is still unclear. One possible explanation is that binding sites on oocytes for spermatozoon ligands are located in specific domains on the egg surface. A direct measurement of the interaction forces involved when an oocyte and a spermatozoon adhere in both areas may validate such a hypothesis. Another explanation of the differences between microvillar and amicrovillar areas may come from the mechanical properties of the oocyte membrane. These have not yet been characterized and remain to be explored. To detect and quantify local changes in membrane deformability and adhesion behavior on a micrometer length scale, a nanoforce technique is required.

A number of advanced techniques have been developed to investigate cell−substrate and cell−cell interactions. The parallel plate flow chamber,10,11 the atomic force microscopy technique (AFM),1,2,13 the surface force apparatus (SFA),14 and the biomembrane force probe (BFP)15 were used to characterize single ligand receptor systems through their kinetic parameters1,10,11 and energy landscape.16−18 Dual micropipette techniques were used to test the adhesion energies of biological model systems such as giant vesicles19−23 or rupture forces involved between cells overexpressing adhesion proteins.24−26 AFM27−32 and

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10.1021/la072258x CCC: $40.75 © 2008 American Chemical Society
Published on Web 11/21/2007
micropipette techniques were used to study the adhesive properties of single cells on substrates and cell–cell adhesion. The mechanical properties and microstructure of cells or giant vesicles were probed with optical tweezers. Among these studies, very few involved direct cell–cell interactions.

In this study, we aim to investigate the interaction and mechanical properties of two primary cells, an oocyte and a spermatozoon, which in nature are destined to meet and adhere in order to fuse and produce a new being. Our first approach consisted in a qualitative observation of the adhesive and mechanical response of the two gamete membranes when, after a contact time of a few seconds to a few minutes, the spermatozoon and the oocyte were pulled apart with micropipettes. Observations were made by direct and fluorescence light microscopy and have revealed the presence of tethers between the two cells. Tether formation is not unheard of and has been observed in various systems, but this is the first evidence that the interaction between two primary cells is capable of giving rise to tethers. To investigate in a more quantitative way the adhesion behavior and mechanical properties of the gamete membranes and their potential local variations, the BFP technique has been adapted for direct cell–cell measurement. This was made possible by the small size and suitable morphology of the spermatozoon head that allowed its fixation on the head of the BFP transducer. The spermatozoon head can be seen as flat with dimensions of approximately $5 \times 3 \times 1 \, \mu m^3$. The oocyte is around $80 \, \mu m$ in diameter. This difference in size leads to a small contact area (about $10 \, \mu m^2$) between the two gametes and offers the opportunity to select the contact location on the oocyte membrane carefully. Moreover, by controlling the contact time, the cell–cell binding can be reduced to a single attachment point. We cannot know whether this single attachment point corresponds to single molecules or to single molecular complexes.

A strong difference in the membrane elastic stiffness has been established between microvillar and amicrovillar areas of the oocyte surface. Less intuitively, two types of domains providing locally different mechanical responses have been observed both in amicrovillar and microvillar areas. No tether could ever be formed in the first domains whereas a tether could be extruded in amicrovillar and microvillar areas. No tether could ever be formed in the first domains whereas a tether could be extruded in amicrovillar and microvillar areas. No tether could ever be formed in the first domains whereas a tether could be extruded in amicrovillar and microvillar areas. No tether could ever be formed in the first domains whereas a tether could be extruded in amicrovillar and microvillar areas.

**Gamete Preparation.** Sperm Preparation. Spermatozoa have been prepared as previously described. B6CBA male mice (8–10 weeks old) were purchased from Charles River Laboratories. For sperm isolation, the two caudae epididymis and vas deferens were removed from an adult mouse. Sperm were gently expelled from the caudae epididymis into a 300 mL drop of ferticult medium (FertPro, Belgium) with 3% bovine serum albumin (BSA, Sigma-Aldrich, Lyon, France) under mineral oil. Spermatozoa were incubated at 37 °C in 5% CO$_2$ for 90 min to induce capacitation and acrosome reaction, giving the spermatozoa the ability to fuse with the oocyte. A mouse spermatozoon is composed of an almost flat head and a 60-μm-long flagellum.

**Materials and Methods**

**Fluorescence Microscopy.** Experiments were performed at room temperature on oocytes and spermatozoa prepared as mentioned above. The oocytes were incubated for 45 min at 37 °C in 5% CO$_2$ in ferticult medium containing an anti-CD9 rat antibody coupled with goat anti-rat Alexa Fluor 594 (gift from C. Bouchex, Villejuif, France) at 10 μg/mL. After gentle rinsing of the oocytes, a spermatozoon was manipulated with a micropipette and brought into contact with the microvillar region of a single oocyte for 5 min. Cells were then separated to a distance of around 20 μm. The observation was made on a Leica DM-IRB inverted microscope in epifluorescence under the excitation of an argon lamp. The fluorescence and direct light images were acquired by a Hamamatsu C9585 camera, with an acquisition time of 300 ms for the fluorescence images.

**Experimental Setup for Spermatozoon/Oocyte Interaction Force Measurements.** Our experimental setup is an adaptation of the biomembrane force probe using a force transducer made of a biotinylated red blood cell maintained by a albumin-coated glass pipet and with a streptavidin-coated glass microbead attached to the microvillar region of the oocyte. PMN cells were rich in microvilli. Here, we show that tethers can also be extruded from amicrovillar surfaces. The distribution of these domains on the oocyte membrane and the forces involved in the oocyte–spermatozoon separation are also discussed.
Figure 1. Experimental setup. (A) The oocyte is held by the left micropipette presenting the microvillar region to the probe. The amicrovillar region is indicated by the star. The scale bar is 10 μm. (B) Scheme of the three glass micropipettes showing a probe facing an oocyte and revealing the dimensions of the micropipettes as well as the size of the oocyte compared to the spermatozoon. (C) The probe is made of a red blood cell used as a spring, a streptavidin-coated bead attached to it via strong streptavidin—biotin bonds, and a spermatozoon adhering through nonspecific interactions to the bead. All of the elements are carefully aligned in the axis of the bent micropipette that holds the probe by the suction of the red blood cell. By controlling this suction, the stiffness \( k \) of the red blood cell is adjusted. The scale bar is 5 μm. (D) Schematic view of the whole experimental setup. The main drop of M2 medium is under mineral oil to prevent both evaporation at 37 °C and bacterial contamination. Real-time images acquired at 360 images/s by the CCD camera are used to compute the force of the interaction and control the piezo-electric device for precise positioning of the oocyte through online feedback control while the probe is fixed.

top through the formation of several streptavidin—biotin bonds. This traditional BFP probe is completed by the attachment of a cell to the bead, here a spermatozoon. The red blood cell is used as a spring with known stiffness that can be tuned by the aspiration pressure applied by the micropipette. The glass bead enables precise video tracking because when observed with a slightly unfocused optical microscope it displays a light spot with a Gaussian intensity profile at its center.\(^{18}\)

For this experiment, three micropipettes with specific inner diameters were used. They were made from borosilicate glass capillary GC100-15 tubing (Harvard apparatus Ltd., Kent., U.K.) using a pipet puller (Sutter Instruments, model P-2000) and a homemade microforge. The micropipette holding the probe had an inner diameter of 1 to 2 μm, and the one used to manipulate the spermatozoon to assemble the probe and to maintain the flagellum throughout the experiment had a slightly larger inner diameter of 2–4 μm. The pipet holding the oocyte had an initial diameter of ~40 μm and was then forged again to obtain a smaller inner diameter of 20 μm and to smooth rounded edges to prevent lysis during the manipulation of the oocyte. Each pipet was held in its own micromanipulator and connected to a combined hydraulic/pneumatic system that provided the necessary control of the aspiration force applied to the probe and cells. The micropipette holding the oocyte was coupled to a linear piezoelectric translator (Physik Instrumente, Karlsruhe, Germany) that allowed accurate control of the oocyte position. Using the microforge, all three pipettes were bent at a 30° angle in order to have the axisymmetric probe horizontal and aligned in the direction of the piezoelectric translator that moves the oocyte, as shown in Figure 1B.

The experiments were performed on the stage of a Leica inverted microscope (DMIRB type, Solms, Germany) positioned on an antivibration platform and equipped with a CCD camera (purchased form JAI, Yokohama, Japan), with a digitally controlled heating box (from Life Imaging Services GmbH, Reinach, Switzerland) maintaining the whole setup at 37 °C.

The experiments took place in a Petri dish filled with two 20 μL drops of M2 medium with 3% BSA under mineral oil (mouse embryo-tested light oil, density 0.84 g/mL, Sigma-Aldrich, Lyon, France). The first drop contained the sperm (at a sufficient concentration to maintain good viability of the cells), and the second one, some streptavidin-coated glass microbeads, the biotinylated red blood cells, and the oocytes. The first step of the experiment consisted of assembling the probe and began with the selection of a single hyperactive spermatozoon from the drop containing the sperm. A rapid stroke on its flagellum immobilized the spermatozoon. It was then aspirated into the pipet and transferred to the main drop. While maintaining the spermatozoon by its flagellum, the spermatozoon head was gently pushed into contact with a streptavidin-coated glass microbead for a few seconds. This forced contact resulted in robust bead/spermatozoon adhesion. This construct was then carefully manipulated into contact with a red blood cell held by a second albumin-coated micropipette in order to finalize the probe with maximum axisymmetric alignment (Figure 1C). During the force measurements, the flagellum of the spermatozoon was continuously held by the pipet at least 20 μm away from the head to keep the probe properly aligned, modifying the stiffness of the overall probe by less than 5%.

The force measurements consisted of performing a series of approach–contact–retraction cycles of the two gametes and measuring the interaction force felt by the gametes during the whole cycle. By translating the oocyte-holding pipet, the microvillar or amicrovillar area is brought in contact with the spermatozoon head and then pulled down (Figure 1A, B and 2). The speed and position of the oocyte were controlled over the approach and traction courses. The time of contact of the gametes and maximum compression forces at contact were also controlled. The interaction force experienced by the two gametes was continuously recorded by measuring the deformation of the red blood cell through a tracking procedure of the glass microbead at a rate of 360 frames/s. The interaction force \( F(t) \) was equal to the red blood cell stiffness \( k \) multiplied by the elastic elongation of the red blood cell on the traction axis \( \Delta l(t) \). The force is positive when the two cells are in compression and negative when in traction (Figure 2d). The interaction force is plotted as a function of the oocyte extension \( \Delta l(t) \) in the traction axis, defined as the length difference of the oocyte in the traction axis at rest and under traction. When retracted at a constant speed \( \frac{dl}{dt} \), the extension of the oocyte \( \Delta l(t) \) on the traction axis (Figure 3) is specified by the product of the retraction speed and time minus the deflection of the transducer \( \Delta l(t) \) that is directly measured. If the origin of distance is taken when the force reaches zero during the pulling phase, then one can plot the interaction...
microvillar and amicrovillar areas of the membrane of the oocyte and for about 60 times for at least 3 different contact zones including both oocyte and spermatozoon, such an approach was not feasible. After a short time (from a few seconds to 1 or 2 min), the spermatozoon head spontaneously made contact with the oocyte. To characterize the adhesion of the two gamete membranes further, the adherent spermatozoon and oocyte were then pulled with micropipettes in order to take them apart. When the oocyte and the spermatozoon were apparently separated and if the aspiration maintaining the oocyte on the pipet tip was stopped, the oocyte left the pipet in a direction toward the spermatozoon and reattached to it. This behavior reveals the formation of one or several tethers when the gametes are separated.

Most of the time, tethers are too thin to be observed with traditional light microscopy. To determine the origin of the tethers, fluorescence microscopy experiments were performed. It was necessary to use a fluorescent probe that remained at the oocyte surface and was distributed over the whole membrane. Because CD9 proteins were reported to be present in both microvillar and amicrovillar regions of the oocyte, we chose anti-CD9 rat antibody as the fluorescent probe. Tethers that form between oocytes labeled with anti-CD9 rat antibody coupled with a goat anti-rat Alexa Fluor 594 and normal spermatozoon were fluorescent, whereas the spermatozoon did not become fluorescent (Figure 3B). This means that spermatozoon and oocyte membranes had not fused and therefore that tethers are exclusively composed of molecules from the oocyte membrane.

These experiments have provided qualitative information both on sperm/oocyte membrane adhesion and on the mechanical properties of the oocyte. The possibility of extruding tethers from the oocyte plasma membrane assumes that the molecular bonds involved in the adhesion of the gamete membranes are strong enough to resist the traction force applied to form the tethers. Tether formation has been observed in various systems, but to our knowledge, this is the first evidence that some primary cells that interact in a physiological context during fertilization are able to form such tethers.

**Force/Distance Measurements of Spermatozoon/Oocyte Interaction.** To get more quantitative information, we have directly measured the forces involved in the adhesion of the spermatozoon head with the oocyte plasma membrane as well as those involved in the deformation of the oocyte membrane. Because of the morphological and functional differences between the microvillar and amicrovillar regions of the oocyte, both areas have been probed and compared. The experiment consists of bringing into contact the oocyte with the spermatozoon head and measuring the interaction force experienced by the cells during the whole separation phase. The number of bonds created during the contact of two objects varies with the concentration and distribution of the molecules or molecular complexes responsible for the adhesion. All of the quantitative cell force experiments

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**Figure 2.** Schematics of the touch—retraction phase from the probe illustrating the springlike response of the probe and the oocyte response to traction at (a) touch \( F = 0 \) pN, (b) during cell stretching, and (c) during tether elongation. (d) Approach—touch—retraction cycle force—distance curve. The force measured by the probe is expressed as a function of oocyte extension \( \Delta l_o \). During the approach (gray line), the force is zero until the two cells are in contact. During the compression phase, the force is positive. When the predefined maximum force \( F_{\text{max}} \) is reached, the piezotranslator starts moving the oocyte-holding pipet in the other direction (black line with brighter dots), the force decreases, and we set the reference position to \( \Delta l_o = 0 \) when the force comes back to zero or changes sign during the retraction phase. The force becomes negative if an adhesion occurs and jumps back to zero when the two cells are separated. (b) Three distances \( \Delta l_o, v_{\text{pull}}, \) and \( \Delta l_i \) are used to represent the relation \( \Delta l_o = v_{\text{pull}} - \Delta l_i \), where \( v_{\text{pull}} \) is the speed of the oocyte-holding pipet and \( \Delta l_i \) is the elongation of the red blood cell measured by the displacement, relative to the equilibrium position, of the glass bead attached to it.

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**Figure 3.** Tethers obtained after the separation of an oocyte labeled with anti-CD9 rat antibody coupled with goat anti-rat Alexa Fluor 594 in contact for 5 min with a normal spermatozoon in direct light microscopy (A) and under fluorescent excitation revealing only the tethers and the oocyte (B). The scale bar is 5 μm.
The force of force increases linearly with distance, indicating an elastic response. When the cells are separated, the force jumps from a maximum force of $F_{Le}$ to zero. The shapes of the force—distance curves in the separation phase vary considerably with the nature of the interaction. For instance, force—distance curves obtained from multiple attachment points are very complex, and even if the succession of jumps of forces observed in such cases (Figure 4D) may be attributed to the successive rupture of the attachment points, further interpretation is quite hazardous.

Previously performed with AFM,27–34 BFP,16–18 or other dual pipet assay techniques44,26,47 involved the use of functionalized surfaces, vesicles, or cell lines for which the molecular concentration and distribution parameters can be adjusted. In this study, we used primary cells that imply that the molecular concentration and distribution are imposed. A possible way of limiting the number of attachments in the spermatozoon/oocyte system is to optimize the area and time of contact by adjusting both the maximum compression force at contact and the approach speed of the cells. The contact time is the time for which cells are under compression. It starts with the two cells coming into contact, continues with the force of the interaction increasing up to a maximum compression force, and then ends with the force decreasing back to 0 pN. Satisfactory conditions were obtained for a red blood cell stiffness of 125 pN/μm, a 10 μm/s approach speed of the oocyte-holding pipet, and a maximum compression force of 20 pN without pause before the beginning of the retraction phase at 4 μm/s. Under these conditions, the total time spent by the cells under compression was around 250 ms. Indeed, under such experimental conditions, 15% of gamete contacts gave rise to well-defined single attachment (Figure 4A,B). For 60% of touches, no adhesions were detected (Figure 4C). The last 25% of touches gave rise to different kinds of interactions, including very complex profiles for which multiple attachment points are involved (Figure 4D). These proportions were identical (within 10%) for both microvillar and amicrovillar areas.

The part of the force—distance curve corresponding to the approach phase is always the same (i.e. force is zero until the cells come into contact) and then increases up to a value set by the operator (20 pN). When no attachment occurs (Figure 4C), the force decreases back to zero as the two cells are separated from each other. If an adhesion occurs, then the interaction force becomes negative during the traction phase before jumping back to zero. The shapes of the force—distance curves in the separation phase vary considerably with the nature of the interaction. For instance, force—distance curves obtained from multiple attachment points are very complex, and even if the succession of jumps of forces observed in such cases (Figure 4D) may be attributed to the successive rupture of the attachment points, further interpretation is quite hazardous. In this study, we have focused on the 15% of touches giving single attachment events (Figure 4A,B). At the start of separation, the oocyte deformation always increases linearly with force, indicating an elastic response to the oocyte’s stretching. Then two behaviors are observed: either the membranes completely separate (Figure 4A), or there is a transition to a damped regime (Figure 4B). This second regime corresponds to viscoelastic behavior of the oocyte plasma membrane associated with the extrusion of a tether. In the $F = f(Δl_o)$ representation given in Figure 4, the maximal elastic elongation experienced by the oocyte membrane and the local elastic stiffness of the oocyte membrane are directly accessible on the experimental curves. Indeed, the first one is given by the end distance of the elastic regime ($s$ coordinate of $F_{Le}$ in Figure 4A and $F_{Lv}$ in Figure 4B). The local elastic stiffness of the membrane is the slope of the linear decrease in force with distance.

**Stronger Membrane Elastic Stiffness in the Amicrovillar Area.** A systematic study of the local elastic stiffness of the membrane has been performed both in the microvillar and amicrovillar areas of the oocyte. If, for one given couple of spermatozoon and oocyte, we plot the force at the end of the linear regime as a function of the associated membrane deformation ($Δl_o$), then the data can be well fitted linearly, with slopes corresponding to two distinct stiffness regimes. Data obtained for several positions inside the amicrovillar region are associated with the high-stiffness regime, and that obtained for the microvillar region, with the lower-stiffness regime (Figure 5A).

This means that the membrane stiffness values measured from distinct traction positions in each area are quite stable. From one

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oocytes are reported to recover a good level of expression for during oocyte preparation could be responsible for the modification of reticular acid treatments. These chemical treatments performed on oocytes could be invoked because experiments were performed with oocytes probed between 3 and 5 h after hyaluronidase and tyrode acid treatments. These chemical treatments performed during oocyte preparation could be responsible for the modification of the expression level of surface adhesion proteins. However, oocytes are reported to recover a good level of expression for a maximum fertilization rate after the 3 h recovery procedure. The last factor for such a dispersion of the stiffness data is the natural inhomogeneities in a population of cells, inherent to biological material.

Even for a single attachment point between the gamete membranes, the elastic deformation of the oocyte membrane is perceptible on a surface of a few tens of square micrometers. The elastic stiffness that we measure therefore accounts for the elastic properties of the membrane on this scale. The fact that its value is consistent from one traction point to another inside the same area means that on the scale of a few tens of square micrometers the inhomogeneities in molecular composition of the membrane and in the interaction with the cytoskeleton are averaged. The significant differences in the stiffness values for amicrovillar and microvillar areas are probably correlated to the molecular composition of the membrane, which is different in the two regions. The denser cytoskeleton network in the amicrovillar area, responsible for the pear shape of the oocyte, is also likely to be involved in the stronger stiffness value measured in the amicrovillar area. Indeed, the cell membrane is connected to the cytoskeleton through molecular bonds. Before the rupture of these bonds, a deformation of the membrane oocyte also involves the deformation of the cytoskeleton. A denser cytoskeleton network should therefore exhibit a stronger resistance to deformation. Such behavior is perfectly consistent with the higher stiffness measured in the amicrovillar area of the oocyte.

**Elastic and Viscoelastic Response of the Membrane Oocyte to Mechanical Traction.** Two types of force–distance curves are obtained with contacts producing single attachment events. Either the initial elastic regime is followed by the viscoelastic one in the same force–distance curve (Figure 4B) or the attachment point breaks directly during the elastic regime of the plasma membrane and no viscoelastic behavior is detected (Figure 4A). These two types of curves (A and B) were observed both in the microvillar and amicrovillar areas of the oocyte. Transition from an elastic to a viscoelastic regime has recently been reported by Evans and co-workers on human neutrophils (PMN), whose membranes are rich in microvilli. According to these authors, this transition from the elastic to the viscoelastic regime would be the signature of membrane detachment from the inner cytostructures. In this study, the tether formation rates are respectively 30% and 51% in the amicrovillar and microvillar regions. These results show than even if it is less probable, tethers can also be extruded from the amicrovillar region.

Figure 6 gives the histograms of force distributions with the most probable forces centered around 8.5 and 19.5 pN for the microvillar area and around 8 and 25.5 pN for the amicrovillar area. By contrast, the histograms show two distinct force distributions with the most probable forces centered around 8.5 and 19.5 pN for the microvillar area and around 8 and 25.5 pN for the amicrovillar area. Because the second peak of the histogram corresponds to larger forces than in the microvillar and amicrovillar areas, the shape of the histograms and the range of forces involved are similar in the microvillar and amicrovillar regions of the oocyte, but they significantly depend on the type of experimental curves. Indeed, the histograms show two distinct force distributions with the most probable forces centered around 8.5 and 19.5 pN for the microvillar area and around 8 and 25.5 pN for the amicrovillar area. Because the second peak of the histogram corresponds to larger forces than in the microvillar and amicrovillar areas, the shape of the histograms and the range of forces involved are similar in the microvillar and amicrovillar areas.

The location of the attachment point on the oocyte membrane appears to be a key parameter regarding the capability of the membrane to create a tether. This suggests that the oocyte membrane accessible to the spermatozoon is composed of different kinds of zones with different mechanical characteristics, some of them suitable for tether formation and the others not suitable. In the following text, the former zones will be referred to as v domains, and the latter, as e domains (Figure 7). When the attachment point between the spermatozoon and the oocyte membrane takes place on a v domain, the membrane is capable of undergoing a transition from the elastic to the viscoelastic regime. Even if speculative, the involvement of inhomogeneities of the spermatozoon in the possibility of binding to a v or e domain of the oocyte is probable. Indeed, the attachment points of the two gametes are most probably due to the recognition of

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Figure 7. v and e domains on the oocyte membrane probed by the spermatozoon. From v domains, tethers can be extruded after the initial elastic deformation of the oocyte membrane. Two types of curves are therefore obtained depending on whether the elastic regime is followed by the viscoelastic one. From e domains, the membrane can deform only elastically. The curves obtained from v domains give rise to the $F_{Lv}$ histogram and to the first peak of the $F_{Le}$ histogram. The curves obtained from e domains give rise to the second peak of the $F_{Le}$ histogram.

It often happens that force—distance curves with only the elastic regime (type A in Figure 4) alternate with curves showing a tether extrusion (type B in Figure 4) during consecutive approach—retraction cycles. Let us take the example of the three consecutive approach—retraction cycles with the BAB sequence. According to the interpretation of the lower peak of the $F_{Le}$ histogram (Figure 7), two cases have to be considered. In other words, among the forces obtained at the end of the linear regime for gamete attachment in a v domain, a majority composes the $F_{Lv}$ histogram, and the rest composes the first peak of the $F_{Le}$ histogram. The second peak of the $F_{Le}$ histogram comes from the spermatozoon/oocyte contacts situated in e domains of the oocyte.

The consistency between experimental and predicted data is very good in the microvillar area. The difference between the predicted and experimental data in the amicrovillar area may indicate that in this area a non-negligible number of curves just underwent the transition to the viscoelastic regime when the rupture event happened and were counted as elastic curves because of uncertainty while sorting the curves. An alternate explanation could be that approximating $P_{Lv}(f)$ by the second peak of the $F_{Le}$ distribution is not a valid assumption in the amicrovillar region. This would indicate that molecular bonds involved in the amicrovillar e and v domains are different.

From the experimental evaluation of the rate of gamete attachment in a v domain that breaks in the elastic regime, it is possible to give the proportions of the one attachment point occurring in the e and v domains (Table 1). v and e domains are almost equally accessible in amicrovillar and microvillar areas.

$F_{Le}$ is the only experimental data that provides information on the robustness of the link between the gamete membranes. One
can note that $f_{Le}$ is higher in the amicrovillar area than in the microvillar region. This result tends to show that in e zones the type of links involved in the spermatozoon/oocyte adhesion are different in the microvillar and amicrovillar areas. This indicates that proteins and/or molecular arrangement involved in the spermatozoon/oocyte adhesion are different in both areas.

**Conclusions**

This experimental study deals with the interaction between an oocyte and a spermatozoon and with the mechanical properties of the oocyte membrane. The first approach combining micropipette manipulation and fluorescence microscopy has shown that when a spermatozoon is in contact with the oocyte plasma membrane it rarely detaches spontaneously in spite of the large movements of its flagellum. When separation is forced after a long contact time, tethers connecting the cells are extruded from the oocyte membrane. With a BFP adapted to cell−cell measurements, we have measured the interaction forces experienced by the two gametes when they are brought into contact and then pulled apart from each other. Microvillar and amicrovillar areas of the oocyte surface have been systematically probed and compared. We found that the amicrovillar area is characterized by an average elastic stiffness that is substantially stronger (48%) than the amicrovillar area, which is consistent with a denser cytoskeleton network in this area.46 The experiments have revealed the presence of two types of membrane domains of micrometer sizes or smaller, distributed or at least accessible in comparable proportions in both microvillar and amicrovillar areas. The mechanical properties of these domains are different. In an e domain, the membrane deforms only elastically under traction, whereas in a v domain the elastic regime can be followed by a viscoelastic one. In the latter case, tethers can be extruded from the oocyte membrane. As a result, tethers can be extruded in microvillar regions in agreement with what was observed with other types of microvillar membranes41,42 but also in amicrovillar areas. In spite of an equal distribution of e and v domains over the entire oocyte surface, tethers are more often observed in the microvillar area. A possible explanation is that the molecules involved in the spermatozoon−oocyte link in a v domain are different in the microvillar and amicrovillar areas. In such a case, the binding would be stronger in the amicrovillar region. Another explanation may be the stronger binding of the membrane to the cytoskeleton in the amicrovillar area. In the e domains, spermatozoon/oocyte links are significantly stronger in the amicrovillar than in the microvillar area. This tends to show that proteins and/or molecular arrangement involved in the spermatozoon/oocyte adhesion are different in microvillar and amicrovillar areas. Because the attachment points of the two gametes are most probably due to the recognition of specific spermatozoon membrane ligands and molecular receptors present at the oocyte surface, both oocyte and spermatozoon molecular arrangements play a decisive role. To create a bond, such receptors and associated ligands have to meet. The rate of bond formation will therefore depend on the molecular inhomogeneities of both oocyte and spermatozoon membranes.

This study has therefore proved the capability of the modified BFP technique used here in quantitatively measuring local changes in gamete membrane adhesion and in probing the mechanical behavior of the oocyte membrane on the micrometer scale. The nature of the e and v domains and the molecular players involved in the microvillar and amicrovillar areas of the oocyte and at the spermatozoon membrane remain to be further investigated. This can be achieved by combining the approach presented here and strategic antibody molecules, knock-out gametes, or drugs modifying the binding between the cell membrane and its cytoskeleton. This approach could therefore become an efficient way of studying the molecular basis of spermatozoon/oocyte plasma membrane interaction during mammalian fertilization, which is very complementary to the usual biological strategies.5,50,51 With the latter, it is often impossible to discriminate whether the involved proteins play a role in adhesion, fusion, or both steps of fertilization. By contrast, here, gamete adhesion is probed independently from fusion. Moreover, it allows the study of gamete adhesion under conditions close to physiological ones because two isolated gametes are involved the same way as in fertilization.

LA702258X