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Yang and Lu, Supplemental Table S1

Spacing between two adjacent sperm heads during PSR entrance. Movement of sperm on the GFP trail in the uterus or within the PSR lumen was captured by continuous video imaging. The Nikon NIS-Elements tracking software was used to determine the movement speed and spacing between two adjacent heads.

Sperm Array-1: This group of 14-20 sperm was moving on the GFP trail in the uterus before entering the PSR opening which was captured immediately after the completion of mating. This is considered to be the early phase of sperm storage. The head cluster contained many sperm (estimated to be 5-10). The average speed of this array was 96 μm/second.

Movies	Sperm Name	Starting Time (sec)	Ending Time (sec)	Distances Traveled (μm)	Calculated moving speed (μm/sec)	Distances between this head and the head of the preceding sperm (μm)
9	1					NA
9	2					120
9	Head Cluster (5-10)	1.375	2.75	250	182	120
9	4	4.125	5.375	144	115	270
9	5	7.5	9.5	175	87	290
9	6	7.5	9.5	175	87	70
9	7	15	16.75	180	103	320
9	8	19	21	150	75	230
9	9	22	26	110	28	100
9	10				NA	120

Sperm Array-2: This group of 8 sperm was moving within the PSR lumen at 45 min after the completion of mating which is considered to be the late phase of sperm storage. The three continuous movies, 7-1, 7-2 and 7-3, lasted 60 second each. No sperm were seen in 7-2 and 7-3, resulting in a 4 mm gap between Sperm Array-2 and the Array-3 below. The average speed of this array was 37 μm/second.

Movies	Sperm Name	Starting Time (sec)	Ending Time (sec)	Distances Traveled (μm)	Calculated moving speed (μm/sec)	Distances between this head and the head of the preceding sperm (μm)
7-1	1	7.380	13.940	240	37	NA
7-1	2	7.380	13.940	240	37	0
7-1	3	33.620	38.950	240	45	1,050
7-1	4	37.310	43.460	240	39	148
7-1	5	42.640	48.380	240	42	213
7-1	6	48.790	56.170	240	33	246
7-1	7	48.790	56.170	240	33	0
7-1	8	48.790	56.170	240	33	0
7-2	no					
7-3	no					

Sperm Array-3: Movie 7-4, 7-5 and 7-6 were continuous from Movie 7-3 above. Each movie lasted 60 seconds. This group of 5 sperm appeared to enter the PSR after the Array-2 described above with a 4 mm gap. No sperm entered the PSR after this. The speed was slightly slower than that of the Array-2. Usually, sperm movement slowed after 4 min of imaging-associated UV exposure. The average speed of this array was 30 μm/second.

Movies	Sperm Name	Starting Time (sec)	Ending Time (sec)	Distances Traveled (μm)	Calculated moving speed (μm/sec)	Distances between this head and the head of the preceding sperm (μm)
7-4	9	168.790	213.070	610	14	4,068 (to Array-2)
7-4	10	190.110	209.380	290	15	533
7-5	11	228.790	232.890	160	39	967
7-5	12	230.020	234.530	210	47	31
7-6	13	288.790	307.650	650	34	1,469
7-6	no					

Sperm Array-4: This group of 11 sperm was passing through the PSR at 45 min after mating completion. Each video (8-0; 8-1, 8-2, 8-3) lasted 60 seconds. No sperm entered after 8-3. The average speed of this array was 31 μm/second.

Movies	Sperm Name	Starting Time (sec)	Ending Time (sec)	Distances Traveled (μm)	Calculated moving speed (μm/sec)	Distances between this head and the head of the preceding sperm (μm)
8-0	1	8.546	18.234	400	41	NA
8-0	2	25.645	36.564	357	33	559
8-0	3	31.768	43.435	339	29	178
8-0	4	38.309	50.235	378	25	166
8-0	5	47.923	58.872	389	35	342
8-1	6	67.093	80.345	380	29	550
8-1	7	89.234	101.923	359	28	626
8-2	8	123.342	138.583	370	24	828
8-2	9	140.345	151.342	369	33	571
8-2	10	151.354	163.784	390	31	345
8-2	11	170.675	178.456	348	31	673
8-3	no					

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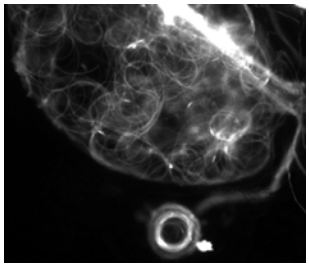
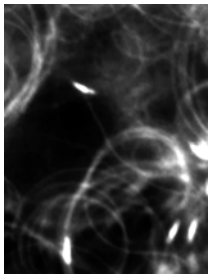
YANG AND LU, SUPPLEMENTAL MOVIE LEGENDS

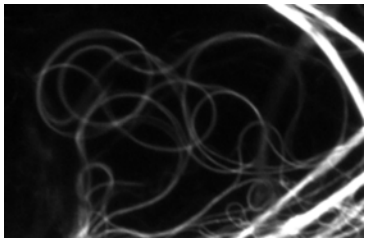
Supplemental Movie 1, Movie1-1, and Movie 2: Circular foci of sperm movement in the uterus. This uterus happened to contain a low number of inseminated sperm. The sperm are propagating circular flagella bends and the head is generally dragged backwards by its flagellum (a close-up view in Movie1-1). Movie2 shows the protrusion of arc-like bends that subsequently close to form circular loops.



Movie1: Circular foci of sperm movement in the uterus.

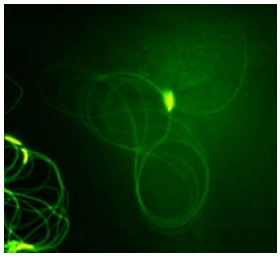


Movie1-1: A close-up view of Movie1 showing that the head is being dragged by its flagellum.



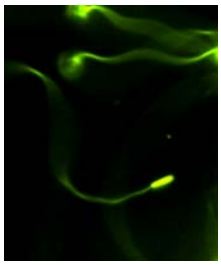
Movie 2: The protrusion of arc-like bends that subsequently close to form circular loops.

Supplemental Movie 3: The arc-line waveform of a figure-8-shaped sperm flagellum. The sperm was released from a uterus that contained freshly inseminated sperm. A single fly flagellum forms up to 10 arc-line bends that stack into a figure-8 configuration. All new bend originate from the tail end. The new bend is placed in alternating bipolar directions, thus producing a figure-8-shaped flagellum. The bend forming speed is approximately one bend per 2.15 seconds. While new bends form at the tail end, the head was being dragged through the figure-8-path, indicating that the bends are propagating from the tail to the head end.



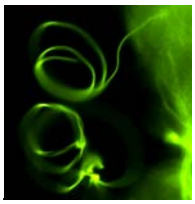
Movie 3: The arc-line waveform of a figure-8-shaped sperm flagellum.

Supplemental Movie 4: The tip-to-base wave of a sperm from the uterus. The sperm was released from a uterus that contained freshly inseminated sperm. Tip-to-base waves were observed when the distal flagellum was anchored. The wave direction generated under this condition was stable and did not reverse. The tip-to-base wave in this particular movie is slower than others to better show the wave direction.



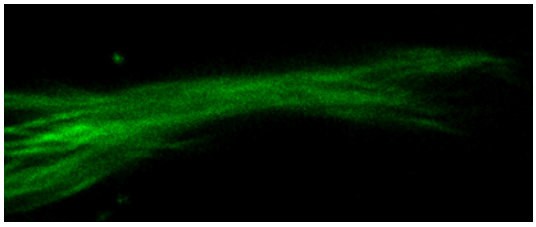
Movie-4: Tip-to-base waves.

Supplemental Movie 5: The base-to-tip wave of a sperm from the uterus. The sperm was released from a uterus that contained freshly inseminated sperm. Base-to-tip waves were observed when the proximal flagellum was anchored. The wave direction generated under this condition was stable and did not reverse.



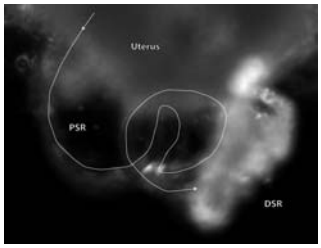
Movie 5: Base-to-tip waves.

Supplemental Movie 6: Stalled sperm tail bundle in the PSR. This movie shows a bundle of moving tails within the PSR lumen at close to the PSR opening, but the forward movement of these tails have stalled. The sperm in this movie was labeled with both the head and tail GFP, indicating that tails were at the moving front. The movie was captured at 7 min after mating onset before the mating pair was separated. This is the time is very soon after the sperm enter the uterus but no sperm has moved into the storage tubules. When the uterus was dissected out of the copulating female at this early time of sperm storage, progressive movement of tails that enter the PSR was found to stall. The stalled movement may be due to insufficient production of the sperm entry signal or sensitivity of early sperm entry to mechanical perturbation from the dissection procedure. When the dissection was done at later times (15 min – 45 min after mating), sperm were found to move from the uterus, through the PSR, into the DSR without a change of moving direction (Movies 7, 8).



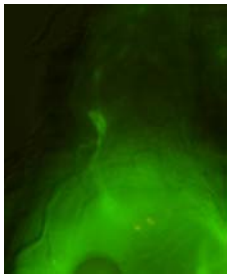
Movie 6: Stalled tail bundle in the PSR.

Supplemental Movies 7 and 8: Sperm passing through the entire PSR without changing direction. The movies were taken at 45 min after mating. Movie7 has a slight bright-field illumination to show the convoluted circular fold of the PSR tubule, which has a slight spatial overlap with the folded DSR on the right that already contained a lot of sperm as indicated by the strong fluorescence. This spatial overlap could give an illusion of some sperm moving out of the DSR and then backing into the DSR, especially when multiple sperm pass through the winding path of PSR. Thus it is important to find out the folding pattern of PSR before accessing the sperm movement. Movie8 is the same sample without bright-field illumination. Multiple sperm heads pass steadily through the entire PSR tubule without 180º flip-flop turns or to-and-fro motions. Only the sperm head was labeled with the GFP marker in the movies. Because sperm form overlapping arrays during their passage through the PSR, the use of tail GFP was found to diminish the visibility of sperm heads. Imaging tracking software and continuous video was used to determine the spacing between two adjacent sperm heads on the PSR path. These analyses found that the PSR-entering sperm formed overlapping arrays consisting of 5, 8, 12 or 19 sperm (see Supplemental Table 1). While sperm from the uterus are still entering the PSR, those sperm that already moved inside the DSR show restricted movement within the DSR. PSR is naturally folded and it normally lies under the folded DSR tubule. Effort is required to distinguish the PSR from DSR by partially unfolding the PSR as shown in the movie.



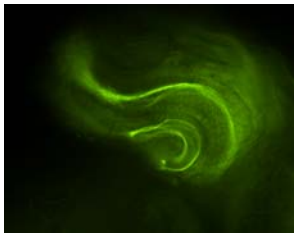
Movies 7 and 8: Sperm move through the PSR without changing direction.

Supplemental Movie 9: Formation of lagging head cluster in the uterus. This uterus was dissected immediately after mating which lasted 18 min. The movie shows that a group of heads is being pulled out of a circularly moving sperm focus. The head cluster moves towards the PSR opening located on the ventral oviduct wall facing away from the image. Several individual sperm heads are also moving towards the PSR opening. These PSR-entering sperm follow the same line of GFP fluorescence (referred to as the GFP trail) that becomes visible due to a combined fluorescence associated with the parallel moving flagella. Individual flagellum is too weak to see distinctively. You also see two atypical sperm heads in the mix of the head cluster by their opposite moving direction away from the head cluster. One of the atypical sperm heads undergoes a rapid U-turn and then moves upwards the GFP trail. The other atypical sperm head moves back into the circular sperm focus and its fate is not known. A working model is proposed to explain the behavior of these atypical sperm (Fig. 8).



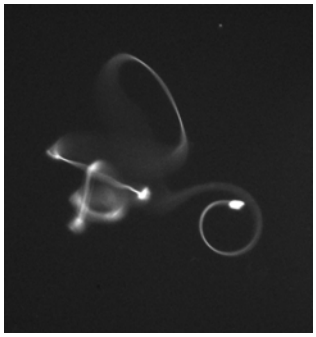
Movie 9: Lagging head cluster on the GFP trail.

Supplemental Movie 10: Sperm movement in the DSR. Consecutive mating of one male with multiple females was carried out in attempts to reduce the number of wild-type sperm inside the DSR. Sperm movement was captured on the same or the next day. Inside the DSR, sperm move rapidly. The movement tends to slow down beyond 4 min of imaging. The movement is associated with the formation of hairpin-shaped flagellar bends that may possibly be similar to those of arc-line waves found in the uterus. Spatial confinement of the bends by the DSR lumen may lead to hairpin-shaped bends. The head is mostly being pulled backwards as the flagellum slithers through its folded form. Occasionally, the head pauses and reverses to head-leading movement briefly before being dragged backwards again by its flagellum.



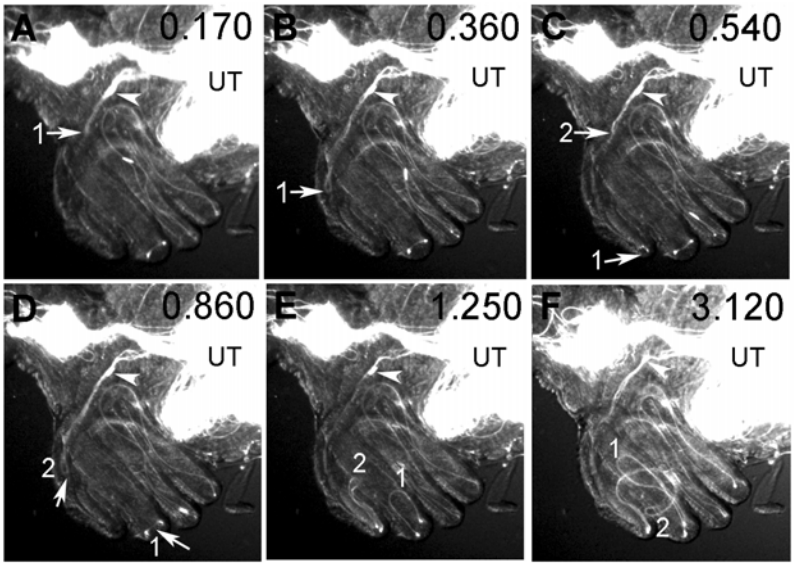
Movie 10: Sperm move in the DSR with hairpin-shaped flagellar bends.

Supplemental Movie 11: Head-leading helical movement of sperm released from the DSR. The sperm in this movie was released from the DSR after 11 days of storage and the seprm is free floating on the slide. All sperm released under this condition make head-leading rotations, consisting of 3.5 to 8 360º-turns, in a clockwise direction on a left-handed helical path. The sperm will then stop and reverse to the counter-clockwise direction with limited angles of 45º - 90º. A single sperm can form a helix with three modules that are separated by link regions. Low amplitude vibrating waves make the flagellum appear thicker than it actually is.



Movie 11: Head-leading helical movement.

Supplemental Movie 12: Formation of stuck-in-motion flagellar hairpins by a *Pkd2*^{KO67} mutant sperm located at the uterus-PSR junction. This movie was made by imaging intact SR complex at 15 min after mating. The coiled DSR is situated against the anterior uterus wall that is facing downwards. The DSR consists of the undulating tubules directly visible to your eyes, whereas the PSR tubule forms the left outer rim of the coiled complex, going underneath the DSR tubule. The sperm (arrowhead points to the head) is located near the uterus-PSR junction and it protrudes two flagellar hairpins (1 and 2) into the PSR lumen and becomes stuck soon after.



Movie 12: abnormal propulsion of the *Pkd2*-mutant sperm.