

Supplementary Information

Self-Assembly of Glycoprotein Nanostructured Filaments for Modulating Extracellular Network at Long Range

Roberto Matassa,^{a*} Marta Gatti,^a Martina Crociati,^{b,c} Roberto Brunelli,^d Ezio Battaglione,^a Massimiliano Papi,^{e,f} Marco De Spirito,^{e,f} Stefania Annarita Nottola,^a and Giuseppe Familiari^a

^a Department of Anatomical, Histological, Forensic and Orthopaedic Sciences, Section of Human Anatomy, Sapienza University of Rome, Via A. Borelli 50, 00161, Rome, Italy.

^b Department of Veterinary Medicine, University of Perugia, Via San Costanzo, 4, Perugia, 06126, Italy.

^c Centre for Perinatal and Reproductive Medicine, University of Perugia, 06129 Perugia, Italy.

^d Department of Gynecological-Obstetric and Urologic Sciences, Sapienza University of Rome, Rome, Italy.

^e Dipartimento di Neuroscienze, Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, 00168 Rome, Italy.

^f Fondazione Policlinico Universitario A. Gemelli IRCSS, 00168 Rome, Italy.

*Correspondence: roberto.matassa@uniroma1.it

Cross-sectioned of Zona Pellucida from Immature to Fertilized stages

Zona Pellucida

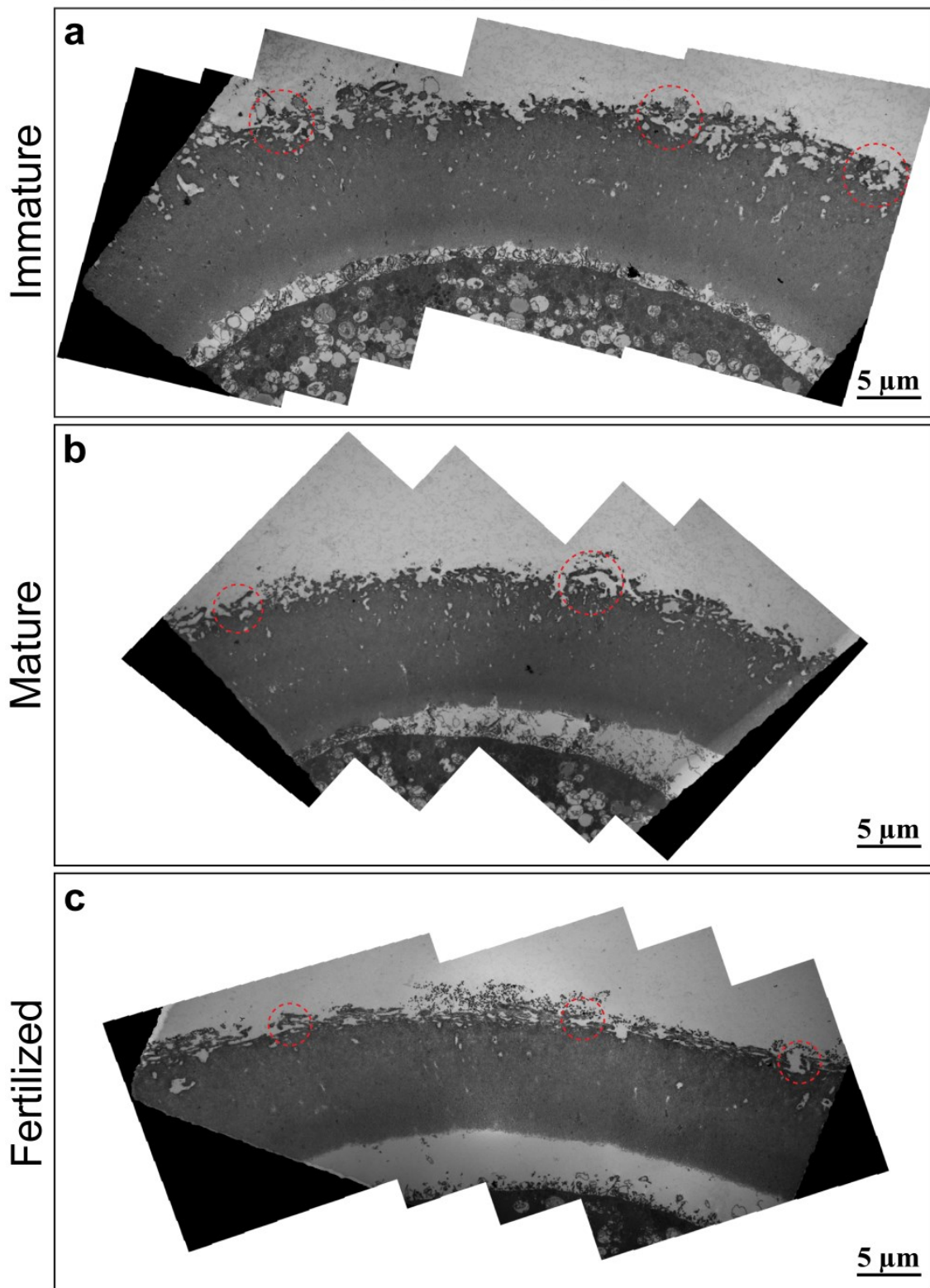


Figure S1. Whole mount view of the transmission electron microscopy images of cross-sectioned Zona Pellucida. **a)** Immature stage. **b)** Mature stage. **c)** Fertilized stage. TEM images were merged to better image their morpho-structure features. Moreover, the outer surfaces of a biological system evidence shading effect capable of hiding depression onto the surface generated by irregular protrusions along the projection direction. Therefore, red dot line circles evidence the real aspect of the highest complex roughness containing forward or backward bending, voids, or other irregular texture profiles that it would not be possible to observe through surface microscopy techniques (i.e.: SEM, AFM, STM, etc..).

Table S1. Selected roughness profile parameters computed over the sampling length with a cut-off wavelength of $1/5\lambda$ and ISO 4287 standard [International Organization for Standardization 1997, ISO 4287:1997, Geometrical Product Specifications (GPS) – Surface texture: Profile Method – Terms, Definitions and Surface Texture Parameters, ISO. Geneva, Switzerland.]. ISO 13565-2, 1996, Geometrical Product Specifications (GPS) – Surface texture: Profile method; Surfaces having stratified functional properties – Part 2: Height characterization using the linear material ratio curve, International Organization for Standardization, Geneva.

ZP Stage	Peak Count Number R_{pc}	Total Height R_t (μm)	Mean Height Profile R_c (μm)	Mean Width Profile R_{sm} (μm)	
Immature	8082±909	3.17±0.66	1.47±0.15	1.15±0.20	
Mature	7012±760	2.43±0.59	1.17±0.29	1.50±0.37	
Fertilized	4972±803	1.94±0.31	0.89±0.08	1.72±0.21	
ZP Stage	Skewness R_{sk}	Kurtosis R_{ku}	Roughness Core Profile R_k (μm)	Reduced Peak Height R_{pk} (μm)	Reduced Valley Height R_{vk} (μm)
Immature	-0.18±0.10	3.01±0.19	1.24±0.30	0.39±0.14 Mr1= 6.27±2.58%	0.72±0.19 Mr2= 84.40±3.32%
Mature	-0.45±0.13	3.50±0.25	0.94±0.30	0.32±0.10 Mr1= 7.13±0.84%	0.59±0.24 Mr2= 84.17±1.62%
Fertilized	-0.21±0.10	3.26±0.21	0.62±0.04	0.22±0.07 Mr1= 5.72±1.12%	0.27±0.17 Mr2= 87.74±3.38%

Note: R_t : average height between maximum peak to minimum valley. R_c : mean height of the neighbouring peak-valley pairs. R_{sm} : mean width of the neighbouring peak-valley pairs. R_{sk} : measure of the asymmetry of the profile about the mean line. R_{ku} : measure of the peakedness of the profile about the mean line. R_{pc} : total peak count numbers. R_k : the roughness core profile is the height difference between those two points where the horizontal intersection lines cross the Material Ratio Curve. R_{pk} : average reduced peak height of the protruding peaks above the core roughness profile. R_{pk} is calculated as the height of the right angle triangle, which has the same area as the peak area A1 and Mr1 as its base. R_{vk} : average reduced valley height of the deep valleys below the core roughness profile. R_{vk} is the height of the right angle triangle, which has the same area as the valley area A2 and 100%-Mr2 as its base. Mr_1 : Material ratio where the upper horizontal intersection line which separates protruding peaks from the core roughness profile crosses the Material Ratio Curve. Mr_2 : Material ratio where the lower horizontal intersection line which separates deep valleys from the core roughness profile crosses the Material Ratio Curve.

Table S2. Estimated center of each Gaussian function with the corresponding amplitudes of the ADF profiles

Gaussian curves of the deconvolved ZP-ADFs					
I-ADF		M-ADF		F-ADF	
Center(μm)	Amplitude(%)	Center(μm)	Amplitude(%)	Center(μm)	Amplitude(%)
-1.05±0.02	0.09±0.01	—	—	—	—
-0.94±0.01	0.14±0.03	—	—	—	—
-0.85±0.00	0.19±0.05	-0.82±0.02	0.11±0.01	-0.80±0.01	0.11±0.01
-0.74±0.01	0.20±0.01	-0.73±0.01	0.12±0.05	—	—
—	—	-0.66±0.01	0.15±0.03	-0.65±0.01	0.26±0.01
-0.58±0.01	0.30±0.02	-0.57±0.01	0.28±0.01	-0.54±0.01	0.25±0.05
-0.48±0.01	0.36±0.07	—	—	-0.47±0.01	0.34±0.16
-0.42±0.01	0.41±0.18	—	—	-0.39±0.01	0.44±0.04
-0.34±0.01	0.40±0.03	-0.34±0.01	0.46±0.01	-0.30±0.01	0.50±0.12
—	—	-0.26±0.01	0.54±0.05	-0.24±0.01	0.66±0.12
-0.21±0.01	0.61±0.04	-0.21±0.01	0.59±0.02	-0.20±0.01	0.67±0.17
—	—	-0.16±0.01	0.57±0.11	-0.16±0.01	0.70±0.12
-0.12±0.01	0.57±0.14	-0.11±0.01	0.70±0.04	-0.11±0.01	0.83±0.04
-0.05±0.01	0.55±0.16	-0.02±0.01	0.67±0.04	-0.05±0.01	0.75±0.09
0.01±0.01	0.62±0.07	0.03±0.01	0.70±0.19	0.01±0.01	0.79±0.06
0.08±0.01	0.64±0.14	0.08±0.01	0.69±0.10	0.07±0.01	1.01±0.04
0.14±0.01	0.61±0.23	0.13±0.01	0.78±0.17	0.14±0.01	0.87±0.10
—	—	0.17±0.01	0.67±0.11	—	—
0.21±0.01	0.62±0.17	0.24±0.01	0.78±0.08	0.21±0.01	0.95±0.07
0.27±0.01	0.69±0.16	—	—	—	—
0.34±0.01	0.57±0.19	0.33±0.01	0.68±0.05	0.32±0.01	0.71±0.05
0.43±0.01	0.50±0.09	0.40±0.01	0.47±0.21	0.39±0.01	0.61±0.08
—	—	0.46±0.01	0.38±0.20	0.45±0.01	0.32±0.01
0.52±0.01	0.41±0.11	0.53±0.01	0.26±0.17	0.55±0.01	0.15±0.01
0.60±0.01	0.34±0.12	0.62±0.05	0.17±0.07	—	—
0.68±0.01	0.34±0.06	—	—	0.68±0.01	0.12±0.02
0.78±0.01	0.25±0.01	0.81±0.01	0.07±0.02	—	—
0.95±0.01	0.14±0.01	—	—	—	—
1.05±0.01	0.08±0.04	—	—	—	—
1.15±0.01	0.10±0.01	—	—	—	—

Note: $p < 0.01$ statistical significant difference between experimental and theoretical data fitting

Zona Pellucida

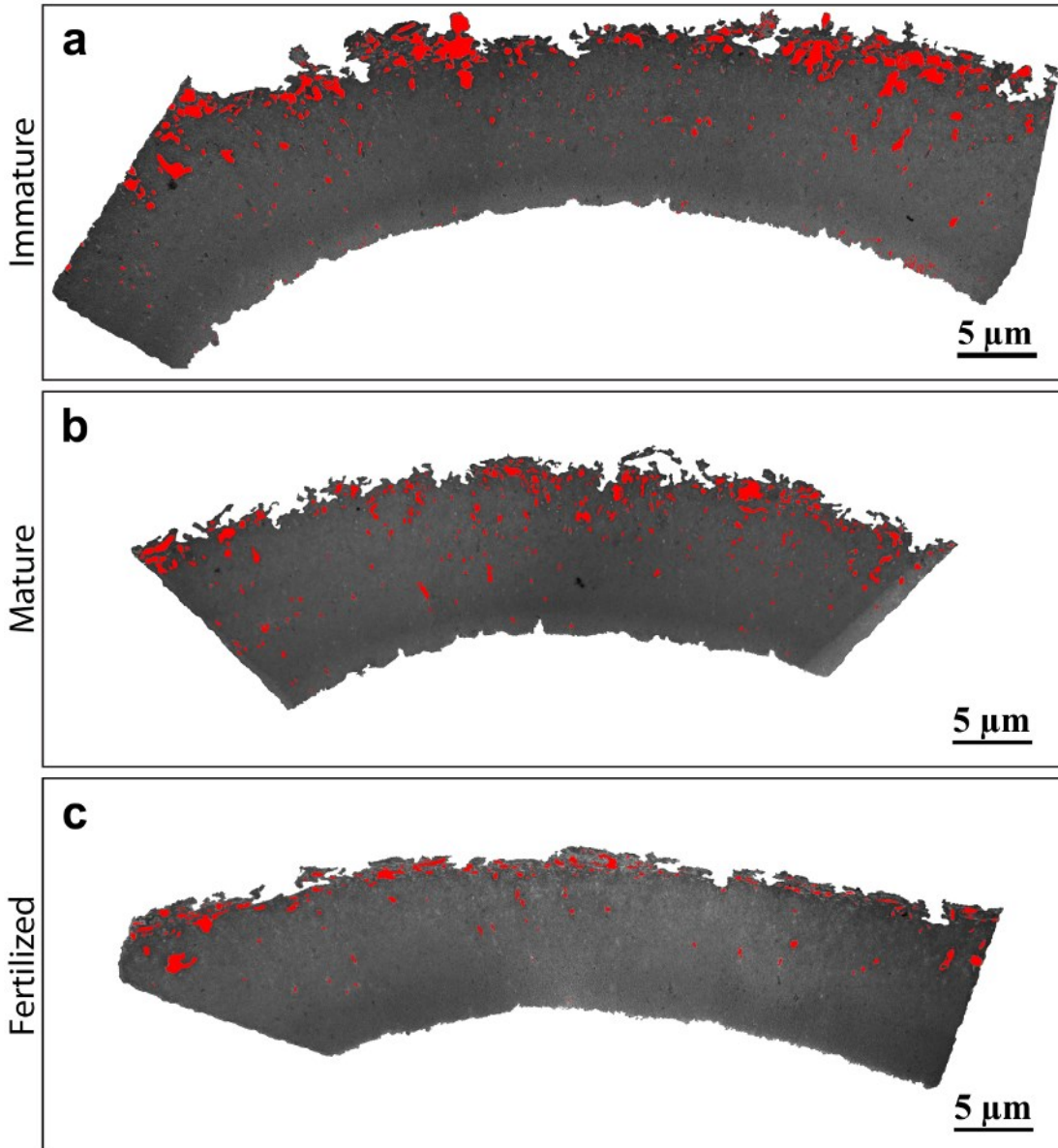


Figure S2. 2D filled pores map of the shell-arc cross-sectional of Zona Pellucida from immature to fertilized stages (Figures S1-a-c).

Table S3. Morphometric Pores Parameters

ZP Phase	Counts	Total Area (μm ²)	Total Porosity (%)	Total Perimeter (μm)	Average Pore Length (μm)	Average Pore Width (μm)
Immature	1	799.19±0.09	—	317.18±0.04	0.22±0.01	0.16±0.01
	406	45.86±0.01	5.74	592.46±0.09	0.46±0.01 0.75±0.02	
Mature	1	475.22±0.12	—	130.46±0.07	0.27±0.01	0.14±0.01
	285	20.29±0.01	4.23	382.77±0.07	0.44±0.02 0.55±0.04 0.67±0.03	
Fertilized	1	509.73±0.04	—	219.53±0.03	0.31±0.01	0.12±0.01
	159	12.08±0.01	2.34	247.53±0.09	0.55±0.02 0.74±0.01	

Note: $p < 0.001$ statistical significant difference between experimental and theoretical data fitting.

Table S4. Parameters of the deconvoluted Voight curves fitting the radial profile.

Voight Peaks Deconvolution of the ZP-RPs					
I-RP (Std Err - 11.65%, FWHM – 0.40 μm)		M-RP (Std Err – 7.56%, FWHM – 0.43 μm)		F-RP (Std Err - 8.62%, FWHM – 0.40 μm)	
Center (μm)	Amplitude (μm)	Center (μm)	Amplitude (μm)	Center (μm)	Amplitude (μm)
43.34±0.12	0.14±0.10	41.67±0.11	0.18±0.05	52.98±0.11	0.19±0.09
44.00±0.11	0.21±0.07	43.68±0.08	0.23±0.05	53.83±0.10	0.20±0.08
44.70±0.09	0.18±0.10	44.78±0.05	0.19±0.05	54.77±0.09	0.20±0.08
45.31±0.12	0.18±0.11	45.34±0.06	0.25±0.06	55.35±0.10	0.13±0.09
46.15±0.08	0.17±0.08	45.84±0.06	0.18±0.05	55.86±0.08	0.24±0.10
46.60±0.10	0.15±0.09	46.46±0.10	0.20±0.05	56.42±0.08	0.28±0.10
47.07±0.12	0.12±0.08	46.95±0.06	0.20±0.05	57.06±0.07	0.20±0.07
47.69±0.11	0.15±0.07	47.53±0.06	0.13±0.03	57.56±0.08	0.12±0.09
48.31±0.10	0.17±0.08	47.96±0.09	0.16±0.05	58.05±0.04	0.30±0.07
49.00±0.09	0.18±0.08	48.35±0.05	0.15±0.04	58.41±0.07	0.11±0.05
49.64±0.08	0.15±0.06	48.78±0.09	0.19±0.04	58.73±0.09	0.13±0.06
50.41±0.08	0.18±0.06	49.09±0.03	0.16±0.05	59.04±0.08	0.15±0.09
51.14±0.06	0.19±0.06	49.42±0.07	0.12±0.04	59.31±0.08	0.15±0.07
51.64±0.05	0.21±0.06	49.84±0.04	0.23±0.05	59.52±0.04	0.11±0.05
52.14±0.04	0.19±0.06	50.22±0.05	0.13±0.04	59.77±0.09	0.16±0.06
52.69±0.10	0.37±0.08	50.57±0.05	0.21±0.05	60.12±0.07	0.12±0.06
53.25±0.03	0.36±0.07	50.95±0.12	0.13±0.03	60.43±0.08	0.09±0.06
53.95±0.13	0.28±0.13	51.30±0.07	0.15±0.04	60.80±0.08	0.15±0.07
54.51±0.14	0.37±0.11	51.64±0.03	0.20±0.05	61.27±0.10	0.16±0.09
54.93±0.04	0.30±0.05	51.95±0.01	0.24±0.05	61.66±0.11	0.14±0.09
55.27±0.06	0.21±0.06	52.30±0.04	0.17±0.04	—	—
55.66±0.02	0.17±0.06	52.84±0.03	0.31±0.06	—	—
56.03±0.11	0.68±0.09	—	—	—	—
56.64±0.06	0.16±0.06	—	—	—	—
57.15±0.05	0.19±0.07	—	—	—	—
57.56±0.06	0.27±0.08	—	—	—	—
58.06±0.10	0.15±0.09	—	—	—	—
58.61±0.11	0.26±0.10	—	—	—	—
59.30±0.13	0.20±0.11	—	—	—	—
Radial Frequency (μm)	Pore Domain: Number & Rate (, %)	Radial Frequency (μm)	Pore Domain: Number & Rate (, %)	Radial Frequency (μm)	Pore Domain: Number & Rate (, %)
0.57±0.02	20.31±2.02, 5.00 %	0.37±0.01	20.43±1.94, 7.17 %	0.36±0.04	16.64±1.75, 10.47 %

Note: p < 0.01 statistical significant difference between experimental and theoretical data fitting

Table S5. Parameters of the Deconvoluted Voight curves fitting the angular profile.

Voight Peaks Deconvolution of the ZP-APs					
I-AP (Std Err - 13.84%, FWHM - 1.41 μm)		M-AP (Std Err – 8.42%, FWHM - 1.41 μm)		F-AP (Std Err – 10.12%, FWHM - 1.03 μm)	
Center (deg.)	Amplitude (μm)	Center (deg.)	Amplitude (μm)	Center (deg.)	Amplitude (μm)
57.25±0.17	0.18±0.16	62.51±0.09	0.19±0.05	64.27±0.12	0.21±0.07
59.46±0.09	0.20±0.06	65.22±0.05	0.31±0.04	65.95±0.13	0.22±0.09
61.32±0.08	0.22±0.06	67.27±0.12	0.19±0.08	68.06±0.10	0.08±0.09
63.80±0.07	0.21±0.06	69.08±0.05	0.13±0.04	70.03±0.09	0.23±0.07
65.91±0.08	0.27±0.06	70.50±0.08	0.10±0.04	72.02±0.10	0.09±0.09
68.07±0.06	0.37±0.07	71.84±0.06	0.23±0.05	74.33±0.09	0.20±0.08
69.76±0.10	0.14±0.09	73.63±0.03	0.13±0.03	76.03±0.08	0.14±0.06
70.94±0.10	0.15±0.09	75.58±0.03	0.36±0.03	79.10±0.08	0.22±0.08
72.72±0.12	0.30±0.09	77.26±0.05	0.15±0.04	83.33±0.09	0.16±0.08
74.70±0.13	0.24±0.09	79.09±0.06	0.19±0.05	85.39±0.08	0.15±0.06
76.84±0.08	0.16±0.05	81.52±0.08	0.18±0.04	87.52±0.06	0.21±0.05
78.84±0.11	0.22±0.08	83.54±0.04	0.17±0.04	89.56±0.09	0.16±0.07
80.64±0.09	0.15±0.06	85.15±0.06	0.29±0.05	90.97±0.08	0.05±0.06
82.55±0.12	0.15±0.08	87.40±0.05	0.23±0.04	92.29±0.10	0.14±0.08
84.49±0.10	0.21±0.09	89.34±0.05	0.21±0.04	94.19±0.08	0.13±0.06
86.64±0.07	0.17±0.06	91.08±0.04	0.15±0.03	95.92±0.09	0.19±0.08
89.61±0.12	0.22±0.10	92.83±0.07	0.20±0.04	98.14±0.07	0.12±0.07
92.64±0.09	0.22±0.08	94.63±0.06	0.18±0.03	100.38±0.05	0.25±0.06
94.52±0.11	0.13±0.09	96.81±0.06	0.19±0.04	102.09±0.08	0.13±0.06
96.89±0.12	0.81±0.09	99.04±0.07	0.16±0.03	104.26±0.11	0.18±0.09
99.00±0.06	0.28±0.08	100.95±0.08	0.25±0.05	106.04±0.10	0.15±0.07
100.60±0.06	0.19±0.07	103.06±0.11	0.26±0.05	108.94±0.11	0.23±0.07
102.42±0.05	0.23±0.05	105.82±0.10	0.26±0.05	111.64±0.08	0.20±0.06
106.00±0.07	0.21±0.06	110.25±0.08	0.27±0.05	112.98±0.09	0.09±0.06
107.95±0.09	0.22±0.08	112.77±0.06	0.29±0.04	114.39±0.10	0.31±0.06
110.02±0.05	0.16±0.07	115.26±0.07	0.17±0.04	115.99±0.11	0.11±0.08
111.85±0.06	0.18±0.07	117.64±0.06	0.26±0.05	—	—
113.55±0.09	0.19±0.08	—	—	—	—
114.75±0.13	0.25±0.11	—	—	—	—
117.09±0.10	0.49±0.08	—	—	—	—
120.32±0.12	0.26±0.10	—	—	—	—
122.49±0.08	0.10±0.10	—	—	—	—
124.45±0.11	0.17±0.10	—	—	—	—
Angular Frequency (deg.)	Pore Domain: Number & Rate (, %)	Angular Frequency (deg.)	Pore Domain: Number & Rate (, %)	Angular Frequency (deg.)	Pore Domain: Number & Rate (, %)
1.90±0.15	13.85±2.40, 3.41 %	1.91±0.03	10.12±1.31, 3.55 %	1.88±0.07	6.65±0.69, 4.18 %

Note: p < 0.01 statistical significant difference between experimental and theoretical data fitting

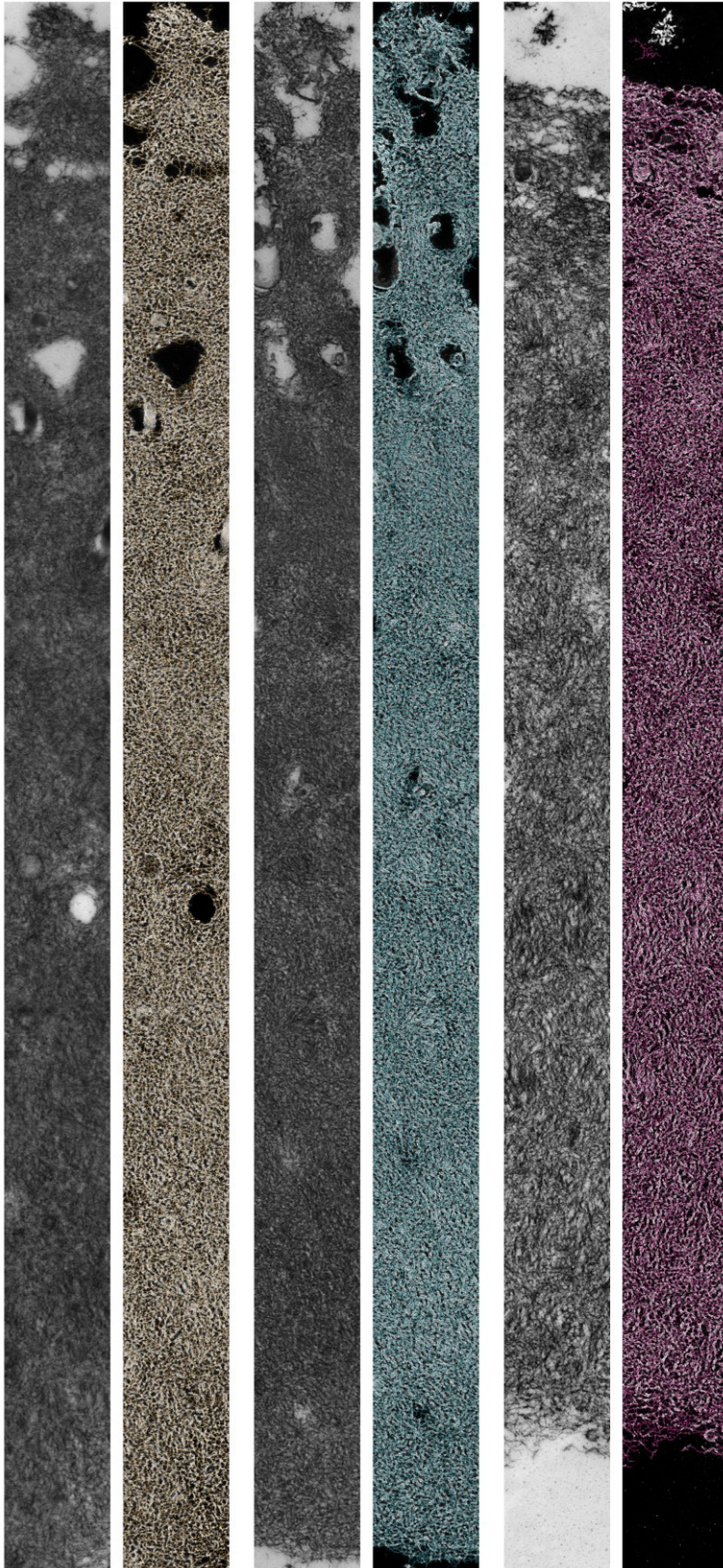
Overlapping Images of Interconnected Glycoprotein Filaments-Skeleton Segments

ZPs Outer Surface

Immature

Mature

Fertilized



ZPs Inner Surface

1000 nm

Figure S3. TEM merged image of cross-sectioned matrix ZPs overlapped by cross-linked segmentation coloured images.

Table S6. Parameters of the deconvoluted Gaussian curves fitting the filament lengths.

Gaussian Peaks Deconvolution of the ZP Stages					
I-ZP (Std Err – 5.80%, FWHM – 3.61 nm)		M-ZP (Std Err – 9.42%, FWHM – 4.22 nm)		F-ZP (Std Err – 4.25%, FWHM – 3.58 nm)	
Center (nm)	Amplitude (%)	Center (nm)	Amplitude (%)	Center (nm)	Amplitude (%)
9.99±0.47	0.40±0.33	9.90±0.37	0.27±0.15	9.89±0.14	0.46±0.10
14.23±0.10	0.64±0.06	14.19±0.11	0.43±0.10	14.64±0.08	0.72±0.05
17.19±0.08	0.83±0.10	17.11±0.10	0.62±0.09	17.21±0.09	0.71±0.05
23.02±0.16	0.57±0.05	23.89±0.23	0.60±0.08	23.43±0.11	0.70±0.05
27.11±0.13	0.67±0.06	27.82±0.17	0.70±0.10	26.87±0.12	0.66±0.06
30.92±0.16	0.61±0.06	31.02±0.18	0.72±0.09	30.64±0.09	0.68±0.07
35.93±0.10	0.82±0.06	36.77±0.18	0.79±0.08	36.47±0.09	0.77±0.06
40.12±0.13	0.71±0.07	41.60±0.13	0.69±0.09	40.34±0.10	0.86±0.06
44.22±0.12	0.76±0.07	45.63±0.18	0.78±0.10	44.58±0.08	0.85±0.08
48.66±0.14	0.79±0.07	49.93±0.25	0.78±0.08	49.31±0.12	0.83±0.08
53.46±0.15	0.75±0.07	55.92±0.19	0.78±0.08	53.40±0.11	0.94±0.09
57.71±0.17	0.70±0.08	60.13±0.20	0.78±0.11	57.72±0.12	0.95±0.09
62.10±0.18	0.82±0.07	63.44±0.20	0.86±0.10	62.57±0.14	0.90±0.11
66.79±0.18	0.79±0.09	68.74±0.20	1.02±0.08	66.97±0.10	1.09±0.09
70.78±0.15	0.86±0.10	—	—	71.41±0.10	1.03±0.10
74.94±0.20	0.90±0.09	74.83±0.21	0.96±0.13	—	—
—	—	76.85±0.19	0.96±0.10	75.70±0.08	1.12±0.10
80.22±0.18	1.02±0.08	82.70±0.16	1.01±0.11	81.11±0.09	1.16±0.10
84.42±0.11	0.87±0.11	—	—	85.45±0.09	1.02±0.12
88.02±0.19	0.95±0.08	87.83±0.20	1.11±0.09	88.79±0.09	1.11±0.10
93.70±0.17	0.96±0.09	—	—	94.00±0.11	1.04±0.08
98.87±0.22	0.85±0.08	98.06±0.21	1.09±0.08	98.90±0.16	0.80±0.09
—	—	104.00±0.17	0.99±0.09	101.91±0.17	0.76±0.10
106.47±0.18	0.89±0.06	—	—	107.69±0.19	0.73±0.08
110.89±0.14	0.66±0.10	111.81±0.22	0.97±0.08	112.96±0.24	0.63±0.07
114.61±0.19	0.75±0.08	—	—	—	—
120.04±0.21	0.76±0.07	118.61±0.26	0.87±0.08	115.71±0.20	0.65±0.06
124.63±0.17	0.72±0.08	124.06±0.29	0.73±0.13	123.53±0.15	0.53±0.07
128.95±0.21	0.68±0.07	127.45±0.29	0.79±0.09	127.83±0.19	0.55±0.05
133.61±0.27	0.57±0.08	133.62±0.19	0.86±0.07	133.97±0.21	0.48±0.04
137.75±0.25	0.53±0.06	—	—	140.88±0.17	0.37±0.04
143.70±0.26	0.45±0.06	—	—	—	—
149.20±0.24	0.46±0.05	—	—	147.74±0.16	0.34±0.04
—	—	—	—	153.44±0.20	0.27±0.04
156.61±0.29	0.38±0.04	157.50±0.22	0.58±0.04	157.33±0.16	0.33±0.04
164.29±0.34	0.36±0.04	—	—	162.13±0.24	0.24±0.04
—	—	—	—	168.48±0.20	0.23±0.03
—	—	172.17±0.25	0.37±0.05	—	—
—	—	—	—	176.49±0.26	0.19±0.02
—	—	187.79±0.31	0.31±0.05	—	—
—	—	201.95±0.36	0.20±0.05	—	—

Note: p < 0.01 statistical significant difference between experimental and theoretical data fitting

Table S7. Parameters of the Deconvoluted Gaussian curves fitting the network filament segments. And theoretical prediction of the filament lengths related to glob-rod angles.

Gly-filament Arrangements of Immature ZP Filaments							Gly-filament Arrangements of Mature ZP Filaments							Gly-filament Arrangements of Fertilized ZP Filaments						
Exper.	Theoretical Filament Lengths and Glob-Rod Angles						Exper.	Theoretical Filament Lengths and Glob-Rod Angles						Exper.	Theoretical Filament Lengths and Glob-Rod Angles					
Center (nm)	Length (nm)	Angle (°)	Length (nm)	Angle (°)	Length (nm)	Angle (°)	Center (nm)	Length (nm)	Angle (°)	Length (nm)	Angle (°)	Length (nm)	Angle (°)	Center (nm)	Length (nm)	Angle (°)	Length (nm)	Angle (°)	Length (nm)	Angle (°)
9.99	9.99	—	9.99	—	9.99	—	9.90	9.90	—	9.90	—	9.90	—	9.89	9.89	—	9.89	—	9.89	—
14.23	—	—	—	—	14.23	—	14.19	—	—	—	—	—	14.19	—	—	—	—	—	14.64	—
17.19	17.19	—	17.19	—	—	—	17.11	17.11	—	17.11	—	—	—	17.21	17.21	—	17.21	—	—	—
23.02	—	—	—	—	24.15	116.87	23.89	—	—	—	—	24.89	122.57	23.43	—	—	—	—	24.56	112.56
27.11	27.11	104.09	—	—	—	—	27.82	27.82	108.77	—	—	—	—	26.87	26.99	103.28	—	—	—	—
30.92	—	—	29.64	119.11	—	—	31.02	—	—	30.22	124.03	—	—	30.64	—	—	29.44	117.80	—	—
35.93	—	—	—	—	36.63	122.56	36.77	—	—	—	—	37.49	125.23	36.47	—	—	—	—	37.31	119.44
40.12	40.65	103.93	—	—	—	—	41.60	41.60	107.29	—	—	—	—	40.34	40.51	103.55	—	—	—	—
44.22	—	—	44.06	114.03	—	—	45.63	—	—	45.22	122.62	—	—	44.58	—	—	43.93	114.90	—	—
48.56	—	—	—	—	49.14	123.07	49.93	—	—	—	—	50.11	125.58	49.31	—	—	—	—	50.05	119.29
53.46	54.22	104.26	—	—	—	—	55.92	55.52	108.89	—	—	—	—	53.40	54.11	104.41	—	—	—	—
57.71	—	—	57.71	105.13	—	—	60.13	—	—	59.93	118.44	—	—	57.72	—	—	57.98	108.48	—	—
62.10	—	—	—	—	61.61	122.40	63.44	—	—	—	—	62.72	125.40	62.57	—	—	—	—	62.86	120.37
66.79	67.73	103.71	—	—	—	—	68.74	69.39	108.31	—	—	—	—	66.97	67.65	103.76	—	—	—	—
70.78	—	—	71.35	105.13	—	—	74.83	—	—	74.20	113.14	—	—	71.41	—	—	71.74	106.34	—	—
74.94	—	—	—	—	74.03	121.57	76.85	—	—	—	—	75.33	125.40	75.70	—	—	—	—	75.80	122.48
80.22	81.27	103.82	—	—	—	—	82.70	83.31	108.89	—	—	—	—	81.11	81.20	103.98	—	—	—	—
84.42	—	—	85.06	105.79	—	—	87.83	—	—	88.94	118.83	87.34	115.63	85.45	—	—	85.45	105.79	—	—
88.02	—	—	—	—	86.67	125.31	98.06	97.64	113.75	—	—	99.34	115.48	88.79	—	—	—	—	88.79	123.27
93.70	94.77	103.50	—	—	—	—	104.00	—	—	103.67	118.83	—	—	94.00	94.73	103.55	—	—	—	—
98.87	—	—	99.31	111.99	99.26	124.44	111.81	112.21	116.76	—	—	111.71	121.32	98.90	—	—	99.13	105.46	—	—
106.47	108.29	103.71	—	—	—	—	118.61	—	—	118.50	120.16	—	—	101.91	—	—	—	—	101.81	123.66
110.89	—	—	—	—	111.77	123.07	124.06	—	—	—	—	124.06	120.99	107.69	108.21	103.12	—	—	—	—
114.61	—	—	114.01	117.55	—	—	127.45	127.03	120.03	—	—	—	—	112.96	—	—	112.96	107.13	—	—
120.04	121.81	103.71	—	—	—	—	133.62	142.00	122.07	133.44	121.65	—	—	115.71	—	—	—	—	114.82	123.57
124.63	—	—	—	—	124.38	124.78	157.50	157.01	122.62	—	—	—	—	123.53	121.75	103.76	—	—	—	—
128.95	—	—	128.55	115.52	—	—	172.17	171.97	121.93	—	—	—	—	127.83	—	—	127.06	110.21	—	—
133.61	135.39	104.36	—	—	—	—	187.79	186.89	121.38	—	—	—	—	133.97	135.29	103.76	—	—	—	—
137.75	—	—	—	—	136.91	123.41	201.95	201.85	121.93	—	—	—	—	140.88	—	—	140.78	105.90	—	—
143.70	—	—	143.23	117.29	—	—	—	—	—	—	—	—	—	147.74	148.85	103.98	—	—	—	—
149.20	149.20	103.71	—	—	—	—	—	—	—	—	—	—	—	153.44	—	—	154.58	106.79	—	—
156.61	—	—	157.04	106.90	—	—	—	—	—	—	—	—	—	157.33	—	—	—	—	—	—
164.29	163.09	107.81	—	—	—	—	—	—	—	—	—	—	—	162.13	162.43	104.19	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	168.48	—	—	168.48	107.92	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	176.49	176.29	107.28	—	—	—	—

Table S8. Summary results of the structural parameters of gly-filaments.

ZP Stage	Total Filament Segments		Total Interconnections Rate (%)	Unbranched Filaments Rate (%)		Triple Interconnections Rate (%)			
Immature	11056		64.77	4.63		63.70			
Mature	11408		64.38	7.82		63.32			
Fertilized	9654		61.02	16.22		60.27			
ZP Stage	R _{Hamilton} (%) Lengths (type I)	R _{Hamilton} (%) Lengths (type II)	R _{Hamilton} (%) Lengths (type III)	Type I		Type II		Type III	
				Average Glob-Rod Angles	Total Sub-Unit	Average Glob-Rod Angles	Total Sub-Unit	Average Glob-Rod Angles	Total Sub-Unit
Immature	1.12	1.88	1.13	104.53±1.43°	12	111.83±5.64°	11	122.75±2.36°	11
Mature	0.58	0.65	0.56	115.10±6.39°	13	119.72±4.33°	9	121.96±4.85°	10
Fertilized	0.94	1.03	1.40	103.99±1.05°	14	108.90±3.94°	11	120.59±3.72°	9

Note: $p < 0.001$ statistical significant difference between experimental and theoretical data fitting.