



**HAL**  
open science

## Myelinosome-like vesicles in human seminal plasma A cryo-electron microscopy study

M Yefimova, E Bere, A. S. Neyroud, Bernard Jégou, Nicolas Bourmeyster, C  
Ravel

► **To cite this version:**

M Yefimova, E Bere, A. S. Neyroud, Bernard Jégou, Nicolas Bourmeyster, et al.. Myelinosome-like vesicles in human seminal plasma A cryo-electron microscopy study. *Cryobiology*, 2020, 92, pp.15-20. 10.1016/j.cryobiol.2019.09.009 . hal-02304994

**HAL Id: hal-02304994**

**<https://univ-rennes.hal.science/hal-02304994>**

Submitted on 27 May 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Title: Myelinosome-like vesicles in human seminal plasma: a cryo-electron microscopy**  
2 **study**

3

4

5 Yefimova M<sup>1,2,3,4</sup>, Bere E<sup>2</sup>, Neyroud A.S<sup>3</sup>, Jegou B<sup>4</sup>, Bourmeyster N<sup>2</sup>, Ravel C<sup>3,4</sup>.

6

7 *1)Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of*  
8 *Sciences, 44 pr. M Thorez, 194223, St-Petersburg, Russia*

9

10 *2)Laboratoire STIM, Equipe CMCS, ERL CNRS 7368. 1, rue Georges Bonnet 86022 Poitiers*  
11 *Cedex France*

12

13 *3)CHU de Rennes, Département de Gynécologie Obstétrique et Reproduction Humaine-*  
14 *CECOS, Hôpital Sud, 16 boulevard de Bulgarie, 35000 Rennes, France*

15

16 *4)Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et*  
17 *travail) -UMR\_S 1085, F-35000 Rennes, France*

18

19

20 **Corresponding author :**

21 [yefimova3@gmail.com](mailto:yefimova3@gmail.com)

22

23 **Key words :** Extra cellular vesicles; seminal plasma; human; myelinosome; Sertoli cells

24

25 **Abbreviations**

26 **EV:** extra cellular vesicles; **cryo-EM:** cryo electron microscopy; **TEM:** transmission electron  
27 microscopy, **MVB :** multivesicular bodies.

28

29

30

31 **Abstract**

32 Seminal plasma is particularly rich in extracellular vesicles. Myelinosomes are membranous  
33 organelles described throughout the seminiferous epithelium of the testis but never reported in  
34 semen. Our aim was to determine the presence of myelinosomes in human seminal plasma.

35 Transmission electron microscopy and cryo electron microscopy analysis of standard  
36 myelinosome preparation from TM4 Sertoli cells and human seminal plasma samples.

37 We have specified by cryo-EM the morphological aspect of “standard” myelinosomes isolated  
38 from the culture media of TM4 Sertoli cells. Vesicles with the same morphological  
39 appearance were revealed in human seminal plasma samples.

40 Human seminal plasma contains a population of large EV (average diameter 200 nm) whose  
41 morphological appearance resemble those of myelinosomes. Defining the specific biomarkers  
42 and functionalities of myelinosome in human seminal plasma are the concerns to be addressed  
43 in our further research.

44

45

46

47

48

49

50 **INTRODUCTION**

51 Seminal plasma is a complex fluid produced by the secretions from all organs of  
52 seminal tract [15]. Being an environment for the male gametes, seminal plasma is enriched in  
53 extracellular vesicles (EVs). They are produced in the male reproductive tract, by the  
54 accessory sex glands, including seminal vesicles and prostate, as well as by the epididymis,  
55 therefore EVs from seminal plasma are also called prostasomes or epididymosomes. The term  
56 “prostasome” concerns a heterogenous population of bilayered EVs found in seminal plasma  
57 of various species [1]. Structural heterogeneity and protein composition of these exosome-like  
58 vesicles has been shown in human semen [9]. EVs from seminal plasma are known to  
59 participate in various aspects of male fertility, improving spermatozoa function through  
60 regulation of the timing of sperm cell capacitation, induction of the acrosome reaction,  
61 stimulating sperm motility to attain fertilizing capacity. Other proposed functions of  
62 prostasomes include interfering with the destruction of spermatozoa by immune cells within  
63 the female reproductive tract [1]. Indeed, it has been observed that there are significantly  
64 improved outcomes when women are exposed to seminal plasma [4].

65 EVs fraction consists of heterogeneous population of extracellular vesicles. The  
66 particles of human seminal plasma were first classified according morphological criteria,  
67 including multiplicity of vesicles, their shape, their external protrusions and their overall  
68 density [9]. According these features, three types of EV in human seminal plasma were  
69 defined. In recent years, the development of new technologies in Electron Microscopy have  
70 led to an increase of knowledge with a finer and more specific analysis for all types of EVs. A  
71 recent study extended the data revealing 5 major subcategories of EVs and 6 subcategories of  
72 extracellular membrane compartments, including lamellar bodies [7].

73 Myelinosomes are membranous organelles found in the seminiferous epithelium of the  
74 testis [6;16] and seemingly secreted by the somatic Sertoli cells in the lumen of the  
75 seminiferous tubules [10]. It has been suggested that Sertoli cells get rid of misfolded proteins  
76 through myelinosomes-mediating secretion, maintaining thereby cell proteostasis [16]. In  
77 transmission EM micrographs, myelinosomes are recognized as markedly electron-dense  
78 osmiophile membraneous vesicles, showing a stacked, reticulated or whorled arrangement.



79           The aim of this study was to look for the presence of myelinosome vesicles in human  
80 seminal plasma. Because of the viscosity of seminal gel and its water-holding capacity,  
81 classical transmission electron microscopy does not seem to be an optimal technique to reveal  
82 the presence of myelinosomes in this fluid. Cryo-electron microscopy is a technique that  
83 allows visualization of nanosized structures without prior fixation or addition of heavy metals  
84 for contrast. The sample is therefore visualized as close to its native state as possible [8].  
85 Therefore, using standard myelinosome preparation from TM4 Sertoli cells, we first analyzed  
86 the appearance of “standard” native myelinosomes by cryo EM and then compared it with the  
87 vesicles from human seminal plasma samples.

88

## 89 **MATERIALS AND METHODS**

### 90 *Cells and cell transfection protocol*

91           Murine TM4 Sertoli cells were from IGBMC (Strasbourg, France) cell culture service  
92 collection. TM4 Sertoli cells were cultured in DMEM/F12 medium (Gibco, number 31330),  
93 containing 2.5% FBS, 5% HS, and supplemented with 1% antibiotic mix (10 000 U/ml  
94 penicillin and 50 mg/ml streptomycin, GIBCO 15140).  $0.5 \times 10^6$  cells were plated into 6-well  
95 dishes and cultured at 37°C with 5% CO<sub>2</sub>. Cells were tested for mycoplasma contamination  
96 using mycoplasma detection kit (MycoAlert, LONZA LT07-418).

97           Transient transfection of TM4 cells with expression vectors pEGFP-C2-trHtt-142Q  
98 generated at IGBMC (Strasbourg, France) were performed using Lipofectamine 2000  
99 (Invitrogen) reagent in 6-well plates cells following the manufacturer’s instructions. The ratio  
100 of plasmid/Lipofectamine in 2.0 ml of serum-free incubation mix was 4.4µg/16µl.

101

### 102 *Standard myelinosomes from TM4 Sertoli cells.*

103           Myelinosomes were isolated from culture media of TM4 Sertoli cells transiently  
104 transfected with plasmid vector coding for mutant Huntingtin-exon1-EGFP protein as  
105 previously described [16]. Briefly, 48 h post-transfection the myelinosomes were purified  
106 from the collected culture medium as follows: the cultured medium was centrifuged for 10  
107 min at 600g. The pellet was discarded and the supernatant centrifuged for 90 min at 20 000g.  
108 The supernatant was discarded and the pellet obtained contains the fraction enriched in

109 myelinosomes. 20 000g myelinosome-enriched fraction also contained 100-120 nm  
110 microvesicles. The pellet was suspended in a culture media for immediate use.

111

### 112 *Human samples*

113 Human samples were provided by the GERMETHEQUE BIOBANK. Semen from 4  
114 healthy donors was collected and left to liquefy at room temperature for 30 min. Cells and  
115 large pieces of undissolved seminal gel were removed by centrifugation at 800g for 20 min.  
116 The supernatant was centrifuged at 100 000g for 1 hour, and the pellet was used for cryo-  
117 electron microscopy.

### 118 *Cryo-electron microscopy*

119 Vitrification of the samples was performed using an automatic plunge freezer (EM  
120 GP, Leica) under controlled humidity and temperature [5]. The samples were deposited to  
121 glow-discharged electron microscope grids followed by blotting and vitrification by rapid  
122 freezing into liquid ethane. Grids were transferred to a single-axis cryo-holder (model 626,  
123 Gatan) and were observed using a 200 kV electron microscope (Tecnai G2 T20 Sphera, FEI)  
124 equipped with a 4k × 4k CCD camera (model USC 4000, Gatan). Micrographs were acquired  
125 under low electron doses using the camera in binning mode 1 and at a nominal magnification  
126 of 25,000x.

127

## 128 **RESULTS:**

129 In total, images of 289 slices were analyzed: 100 from TM4 cells as references and 189  
130 from human seminal plasma.

131

### 132 **1) Analysis of TM4 Sertoli cell secretion**

133 Using standard myelinosome preparation from culture media of TM4 Sertoli cells expressing  
134 mutant Huntingtin-exon1-EGFP protein, we analyzed the morphological aspect of secretory  
135 myelinosome vesicles by TEM and Cryo electron microscopy. Figure 1a & b (left panel)  
136 shows the electron micrograph of myelinosome vesicles by TEM technique. Myelinosome

137 vesicles are electron-dense osmiophile particles, which consist of numerous whorled  
138 membranes forming the regular stacks. Because of sectioning of myelinosome preparation  
139 during TEM processing, some of myelinosomes exhibit an interior cavity, filled with  
140 amorphous material, the others seem to be “intact”. Higher magnification of such “intact”  
141 myelinosome allows discerning the stacks of electron-dense membranes, which seem to be  
142 whorled around a central small vesicle, which is also formed of few membrane layers.  
143 Standard preparations of myelinosome vesicles also contain several EVs, which are clearly  
144 discerned in TEM micrographs. Figure 1 (right panel) shows the preparation of myelinosomes  
145 vesicles analyzed by Cryo-Tem technique. Because Cryo-TEM technique does not require  
146 sample sectioning, only “intact” myelinosomes can be seen in Cryo-TEM micrographs. High  
147 magnification allows discerning the regular stacks in myelinosome vesicle, as well as a  
148 central small vesicles, likewise as those seen in TEM preparation (Figure 1a & 1b). Both  
149 TEM and Cryo techniques revealed a structural identity of myelinosome vesicles (electron  
150 density, whorled organisation of membrane layers, distance between membrane layers).

151 Comparing to TEM samples, more heterogeneity in the morphology of small EVs  
152 were observed in the samples processed by Cryo-TEM technique. Indeed, in Cryo-TEM  
153 micrographs myelinosome vesicles were surrounded by EVs, which were often unequal in  
154 regard of their sizes and shapes (round, elliptic).

155

156

## 157 **2) Analysis of human seminal plasma samples by Cryo-TEM**

158 The viscosity of seminal gel and its water-holding capacity are limiting factors impeding a  
159 good resolution by TEM technique, but not by Cryo-TEM. Careful examination of samples  
160 from human seminal plasma by Cryo-TEM revealed the presence of unusual vesicles, whose  
161 size and morphology resembled those of myelinosomes from TM4 secretions (Figure 2a  
162 & 2b). Indeed, comparing with neighboring EVs, these unusual vesicles were electron dense  
163 and contained multiple membrane layers. In some cases the small central vesicles were  
164 discerned in central part of these organelles, likewise as in secretory myelinosomes from TM4  
165 Sertoli cells (Figure 1). Nevertheless, comparing to “standard” myelinosomes, the unusual  
166 vesicles from human seminal plasma contained fewer membrane layers and exhibited lesser  
167 density of membrane packaging. Remarkably, such vesicles have not been described in  
168 previous studies [7;9]. Intracellular myelinosomes from different cells and/or species are

169 known to exhibit a huge variability of forms by TEM analysis. No such information was  
170 available for the secretory myelinosomes, organelles recently described [16]. Thus, it seemed  
171 likely that the secretory myelinosomes could also present the variability of morphological  
172 organization, including the density of membrane packaging.

173

174

### 175 **3) Comparative analysis of EVs from human seminal plasma and secretion products** 176 **from TM4 Sertoli cells**

177 In preparations from both human seminal plasma and culture media of TM4 Sertoli cells,  
178 myelinosomes vesicles were present together with other EVs. Remarkably, the vesicles from  
179 both species shared striking similarities. Indeed, in both preparations we observed  
180 heterogenous membrane-bound vesicles some of them containing amorphous material (Figure  
181 3). The particles generally varied in size from 50 to 500 nm. Morphologically, some structures  
182 resemble multivesicular bodies, specialized forms of endosomes involved in exocytosis.  
183 Exocytosis is believed to be one of the mechanisms that promote EV release from prostatic  
184 epithelial cells. Light EV appear to be analogous to intraluminal vesicles released during  
185 exocytosis (Figure 3). Some vesicles and membrane compartments showed a more electron  
186 dense interior, indicative of a lumen filled with cargo. We have seen electron density inside  
187 double vesicles, triple or more vesicles. Sporadic multilayered membrane complexes with  
188 large concentric membrane fragments were also visualized (Figure 4). Sporadic small tubules  
189 were also observed in the preparations from both human seminal plasma and from the  
190 secretory products of TM4 Sertoli cells (Figure 5). Main types of EVs from both sources  
191 (human seminal plasma and secretory products from TM4 Sertoli cells) are summarized in  
192 Table 1 based on the classification of Polyakov *et al.* [9]. According this classification, 3 main  
193 class of EVs are present in human seminal plasma. Because the secretory myelinosomes and  
194 myelinosome-like vesicles have not been reported earlier, we added them in the table as the  
195 fourth class of EVs.

196

## 197 **DISCUSSION**

198 Here we show for the first time the presence of myelinosome-like organelle in human  
199 seminal plasma. Secretory myelinosome is a new type of extracellular vesicle, which has been  
200 found *in vitro* in culture media from murine TM4 Sertoli cells, expressing aggregate-prone  
201 proteins mutant Huntingtin and F508delCFTR (cystic fibrosis transmembrane modulator).  
202 While *in situ* secretory myelinosomes were detected in the lumen of seminiferous tubules  
203 from R6/2 HD model mice [16], the presence of myelinosome vesicles in human biological  
204 fluids have never been reported.

205 In TEM micrographs intracellular myelinosomes are discerned as 200-500 nm  
206 osmiophile vesicles, consisting of whorled membrane layers, which can be easily  
207 distinguished on the cross-sections. Intracellular myelinosomes exhibit a huge variability of  
208 forms in TEM. According our data “standard” secretory myelinosomes from TM4 cells-also  
209 revealed a morphological variability in their form, as well as in the number and density of  
210 membrane layers.

211 To our knowledge, no cryo-TEM examination of conventional and secretory  
212 myelinosomes have been undertaken up to now. We specified by cryo-TEM the  
213 morphological aspect of “standard” secretory myelinosomes emitted by TM4 Sertoli cells. By  
214 cryo-TEM technique secretory myelinosome is as round-shaped or elliptic electron-dense  
215 vesicle, consisting of stacked membranes. Thus, both TEM and cryo-TEM techniques seem to  
216 be quasi equal for revealing of myelinosomes in biological samples.

217 Myelinosome-like vesicles we found in human seminal plasma by Cryo-TEM  
218 technique fitted the morphological criteria determining “standard” myelinosome. Indeed,  
219 round-shaped vesicles, formed with whorled membranes, exhibited strong electron density.  
220 Comparing to myelinosome from TM4 Sertoli cells, myelinosome-like vesicle from seminal  
221 plasma looked more friable and seemed to contain fewer membrane layers. The latter is  
222 difficult to assess because no sectioning can be provided by Cryo-TEM technique.

223 Preparations from both human seminal plasma and secretions products of TM4 Sertoli  
224 cells also contained a heterogeneous population of EVs with different morphological aspect.  
225 In a recent study, Hoog and Lotvall [7] showed that in human ejaculate, about 59% of EVs are  
226 found to be single vesicles, while the rest are oval vesicles, double vesicles, double special  
227 vesicles, triple vesicles, tubules or lamellar bodies. This morphological variability may reflect

228 different subpopulations exerting different functions in physiological and pathological  
229 processes. As believed, these structurally specific EVs originate from several male sexual  
230 organs (prostate, epididymis, testis, seminal vesicles). Remarkably, our data indicate that  
231 nearly all types of secretory product we observed in human seminal plasma were also present  
232 in the secretions from TM4 Sertoli cells. This suggests an important contribution of testicular  
233 secretion in the formation of EV pool of seminal plasma. The former was traditionally  
234 underestimated, and the idea on the main role of prostate gland in the production of EVs in  
235 seminal plasma was prevalent.

236 Our data indicate that in both types of samples the single exosome-like vesicles  
237 predominated and constituted about 50% of EV population. These exosome-like EVs often  
238 surrounded myelinosomes in the preparations of human seminal plasma and TM4 Sertoli cell  
239 culture media. Of note is that the intracellular traffic and excretion of myelinosomes by TM4  
240 Sertoli cells is supported by multivesicular bodies (MVB), which are implicated in the  
241 transport of various specific organelles in other cell types [2;14]. TM4 Sertoli cells secrete  
242 myelinosomes together with the exosomes following the fusion of MVBs with plasma  
243 membrane [16]. Therefore, the presence of exosome-like EVs in close proximity of  
244 myelinosomes in human seminal plasma specimens suggests the MVB-related mechanism of  
245 their release in seminal fluid. Remarkably, “standard” myelinosome vesicles emitted from  
246 TM4 Sertoli cells are devoid of molecular markers of exosomes/prostasomes as CD63 protein  
247 [16].

248 It seems plausible that not only Sertoli cells, but also several cell types of the male  
249 reproductive tract contribute to the production of myelinosomes. Indeed, the epithelial cells of  
250 prostate gland and epididymis are characterized by high secretory activity, being responsible  
251 for the production of prostasome and epididymosome EVs for seminal fluid through apocrine  
252 and merocrine mechanisms. These EVs containing both DNA [11] and RNA [13] are  
253 important for acquisition of the sperm-fertilizing ability and sperm forward motility, for the  
254 protection of sperm against reactive oxygen species [12]. Prostasomes also have  
255 immunosuppressive activity via protection against complement attack, and have a role in the  
256 in the stabilization of the sperm plasma membrane [11].

257 To date no information on the specific markers of myelinosomes as well as on their  
258 physiological role in the human seminal fluid is available. In aggregation diseases such as HD

259 or cystic fibrosis, myelinosome vesicles seem to be the protective particles providing isolation  
260 of cells from potentially toxic aggregate-prone proteins. On the other hand, being absorbed by  
261 neighboring cells, myelinosomes vesicles, carrying the pathological proteins can contribute to  
262 pathological spreading. We are only in the beginning of knowledge about myelinosome  
263 vesicles. To date we only note their presence in human seminal plasma, and upcoming studies  
264 would bring the information about their role and significance for human fertility.

265

## 266 **CONCLUSION**

267 EVs have the capacity to convey information between cells through the transfer of  
268 functional protein and genetic information to recipient cells associated with reproductive  
269 biology. In the male reproductive tract, epididymosomes and prostasomes participate in  
270 regulating sperm motility activation, capacitation, and acrosome reaction [3]. Here we  
271 evidence a new type of EVs, namely a myelinosome-like vesicle in the human seminal  
272 plasma. It remains to define the specific biomarkers and physiological functionalities of this  
273 organelle still poorly known.

274

## 275 **ACKNOWLEDGMENTS**

276 We thank the MRIC Platform of BIOSIT (Rennes), Image UP Platform (Université de  
277 Poitiers) and the Germetheque Biobank (Rennes).

## 278 **Fundings**

279 This work was supported by the CHU of Rennes.

## 280 **Contributions**

281 M.Y. and C.R. conceived the study and wrote the manuscript, M.Y. and AS.N. realized the  
282 preparation of samples, B.J. and N.B. critically revised the manuscript. All authors have  
283 approved the final manuscript.

284

285 **References**

- 286 [1] Aalberts M, Stout TA, Stoorvogel W. Prostatosomes: extracellular vesicles from the prostate. *Reproduction*.  
287 147 (2013) R1-14.
- 288 [2] Berson JF, Harper DC, Tenza D, Raposo G, Marks MS. Pmel17 initiates premelanosome morphogenesis  
289 within multivesicular bodies. *Mol. Biol. Cell*. 12 (2001) 3451–3464.
- 290 [3] Carlos Simon, David W Greening, David Bolumar, Nuria Balaguer, Lois A Salamonsen, Felipe Vilella.  
291 Extracellular Vesicles in Human Reproduction in Health and Disease. *Endocrine Reviews* 39 (2018) 292-332.
- 292 [4] Crawford G, Ray A, Gudi A, Shah A, Homburg R. The role of seminal plasma for improved outcomes during  
293 in vitro fertilization treatment: review of the literature and meta-analysis. *Hum Reprod Update*. 21 (2015) 275-  
294 84.
- 295 [5] Dubochet J, Adrian M, Chang JJ, Homo JC, Lepault J, McDowell AW, Schultz P. Cryo-electron microscopy of  
296 vitrified specimens. *Q Rev Biophys* 21 (1988) 129–228.
- 297 [6] Fouquet JP, Dang DC, Meusy-Dessolle N. Functional differentiation of Leydig cells in the testis of the fetal  
298 monkey (*Macaca fascicularis*). *Ann. Biol. Anim. Biochim. Biophys*. 18 (1978) 1205–1221.
- 299 [7] Höög JL, Lötvall J. Diversity of extracellular vesicles in human ejaculates revealed by cryo-electron  
300 microscopy. *J Extracell Vesicles*. 4 (2015) 28680.
- 301 [8] Mielanczyk L, Matysiak N, Michalski M, Buldak R, Wojnicz R. Closer to the native state. Critical evaluation  
302 of cryo-techniques for transmission electron microscopy: preparation of biological samples. *Folia Histochem*  
303 *Cytobiol*. 52 (2014) 1-17.
- 304 [9] Poliakov A, Spilman M, Dokland T, Amling CL, Mobley JA. Structural heterogeneity and protein  
305 composition of exosome-like vesicles (prostatosomes) in human semen. *Prostate*. 69 (2009) 159-67.
- 306 [10] Ravel C, Jaillard S. [The Sertoli cell]. *Morphologie*. 95 (2011) 151-8.
- 307 [11] Ronquist KG, Ronquist G, Carlsson L, Larsson A. Human prostatosomes contain chromosomal DNA.  
308 *Prostate*. 69 (2009) 737-43.
- 309 [12] Sullivan R. Epididymosomes: a heterogeneous population of microvesicles with multiple functions in sperm  
310 maturation and storage. *Asian J Androl*. 17 (2015) 726-9.
- 311 [13] Vojtech L, Woo S, Hughes S, Levy C, Ballweber L, Sauteraud RP, Strobl J, Westerberg K, Gottardo R,  
312 Tewari M, Hladik F. Exosomes in human semen carry a distinctive repertoire of small non-coding RNAs with  
313 potential regulatory functions. *Nucleic Acids Res*. 42 (2014) 7290-304.



314 [14] Whitsett JA, Weaver TE. Hydrophobic surfactant proteins in lung function and disease. *N. Engl. J. Med.*  
315 347 (2002) 2141–2148.

316 [15] Yefimova M, Bourmeyster N, Becq F, Burel A, Lavault MT, Jouve G, et al. Update on the cellular and  
317 molecular aspects of cystic fibrosis transmembrane conductance regulator (CFTR) and male fertility.  
318 *Morphologie.* 103(2019) 4-10.

319 [16] Yefimova MG, Béré E, Cantereau-Becq A, Harnois T, Meunier AC, Messaddeq N, Becq F, Trottier Y,  
320 Bourmeyster N. Myelinosomes act as natural secretory organelles in Sertoli cells to prevent accumulation of  
321 aggregate-prone mutant Huntingtin and CFTR. *Hum Mol Genet.* 25 (2016) 4170-4185.

322

Journal Pre-proof

323 **Legends of figures:**

324 Fig.1: Preparations of “standard” myelinosome fraction from TM4 Sertoli cells by TEM (a &  
325 b; b is a selection from a) and Cryo-TEM (c & d; d is a selection from c) techniques.

326

327 Fig.2: Preparations human seminal plasma by Cryo-TEM techniques.

328

329 Fig.3: Heterogeneity of EVs in seminal plasma (left) and in the secretion products from TM4  
330 Sertoli cells (right).

331

332 Fig.4: Pleiomorphic aspect of EV from human seminal plasma and from secretion products  
333 released by TM4 Sertoli cells.

334

335

336 Fig.5: Non-vesicular constituents of human seminal plasma (a,b,c) ; tubules observed and  
337 secretion products released by TM4 Sertoli cells (d, e, f,g).

338

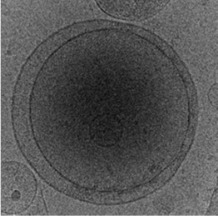
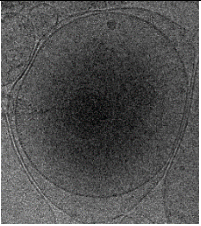
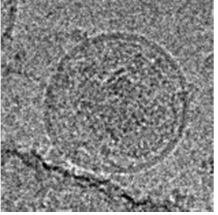
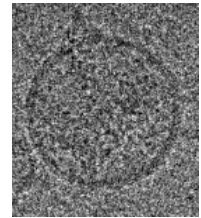
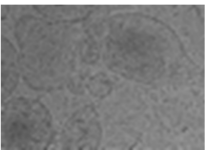
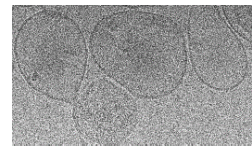
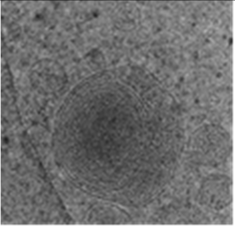
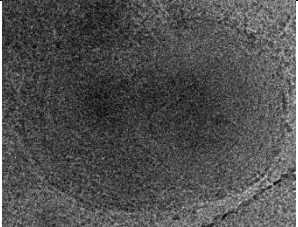
339

340 **Legends of the table:**

341 Table 1: description of EV in seminal plasma according modified Poliakov’s classification  
342 [9].

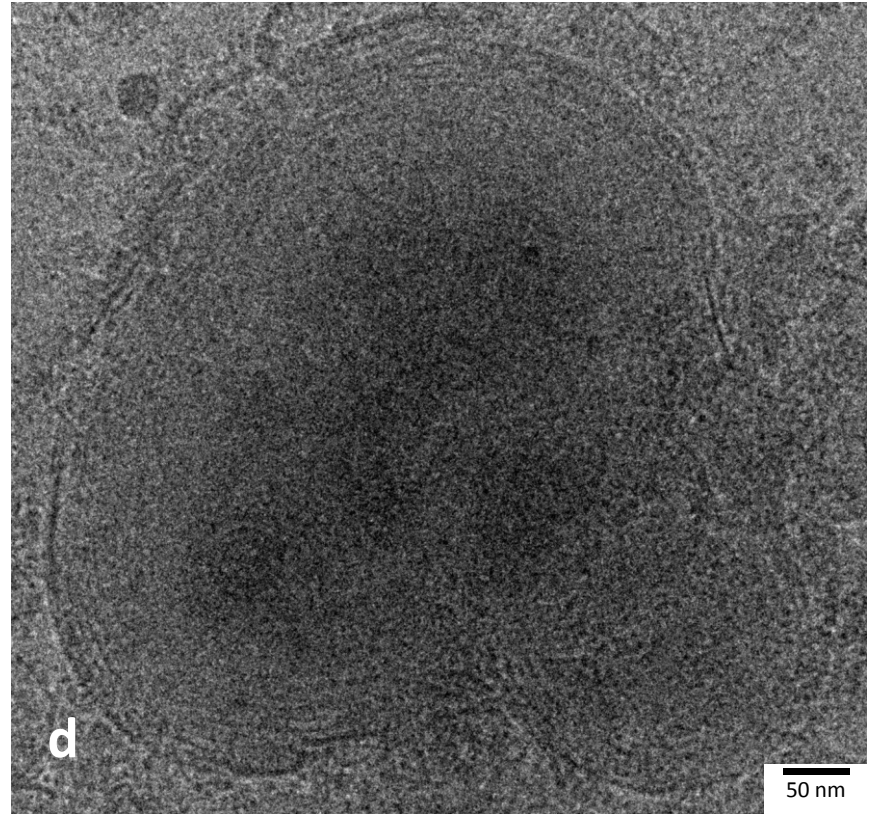
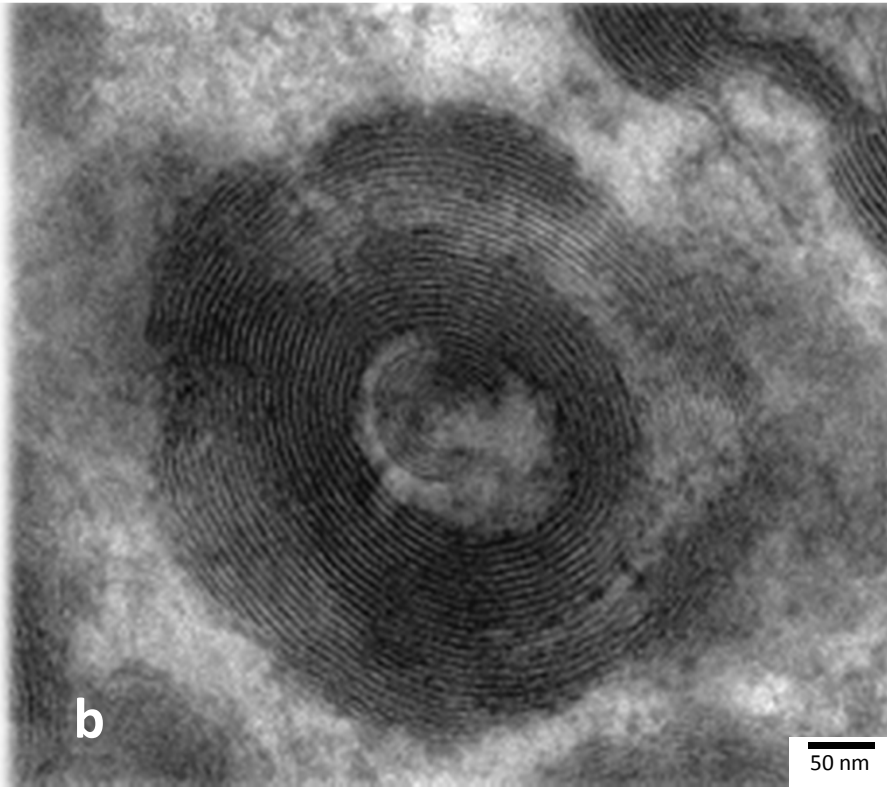
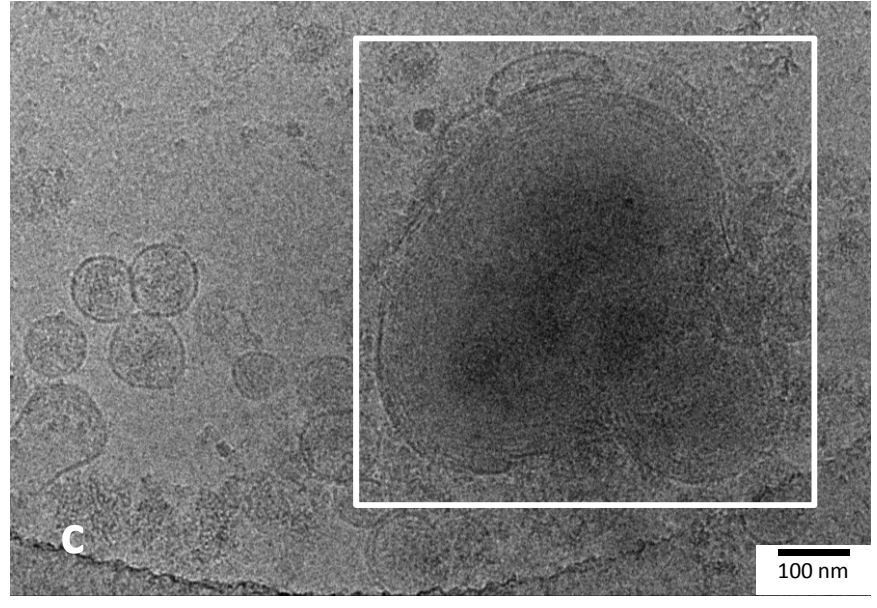
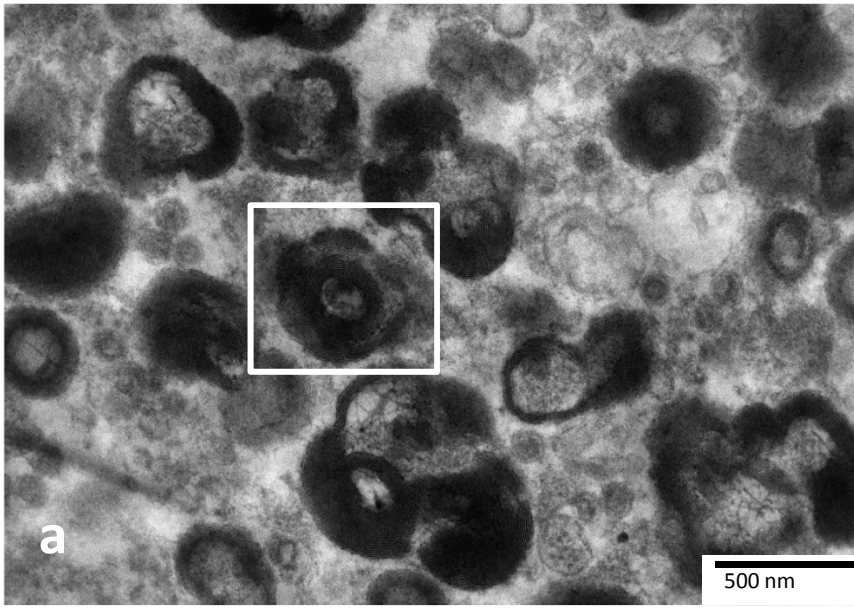
343

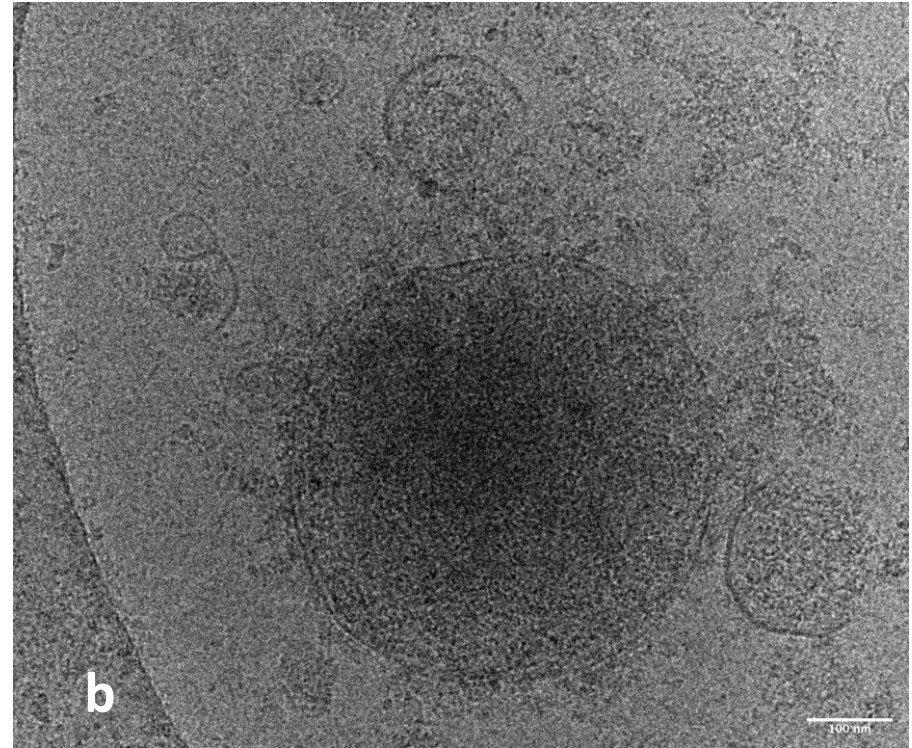
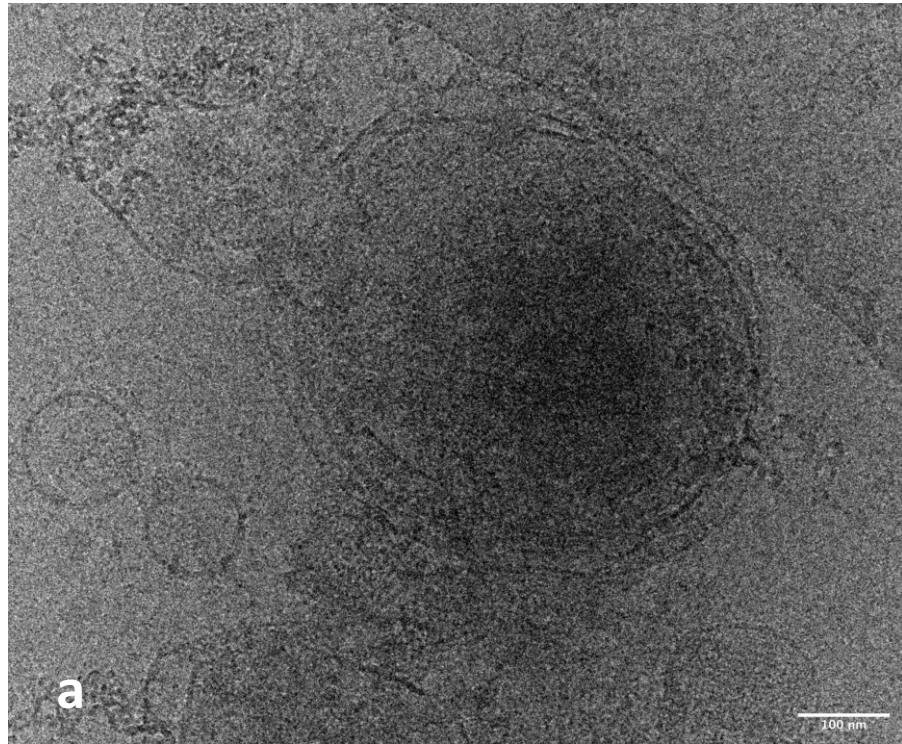
344

Human seminal plasma	EV secreted by murine TM4 Sertoli cells	Type	Description	Size	Fraction in the
		Type 1	<p><b>Dark vesicles</b></p> <p>High electron density vesicles with asymmetric deposition of dark substance surrounding one or more secondary vesicles. These particles are invariably smooth on the outside, lacking protrusions, and are nearly round or slightly elongated.</p>	from 100 to 200 nm.	around 50%.
		Type 2	<p><b>Light vesicles</b></p> <p>Low electron density vesicles with abundant protrusions. These vesicles do not have internal depositions of dark substance, but sometimes contain daughter vesicles, and are nearly round or only slightly elongated.</p>	from 50 to 200 nm.	25–30% of all v
		Type 3	<p><b>Low electron density vesicles</b></p> <p>without protrusion features, presenting as elongated, or pear-shaped, with no dark depositions, and smooth outer surface.</p>	they can be as long as 400 nm and about 50–100 nm wide	20–25% of the
		Type 4	<p><b>Myelinosomes</b></p> <p><b>round-shaped vesicles, formed with whorled membranes, exhibited strong electron density</b></p>	200-700 nm	<1%

**Table 1: description of EV in seminal plasma according modified Poliakov's classification [9].**

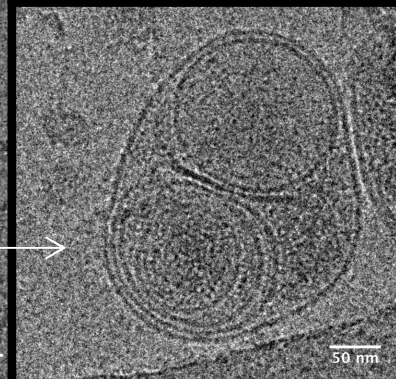
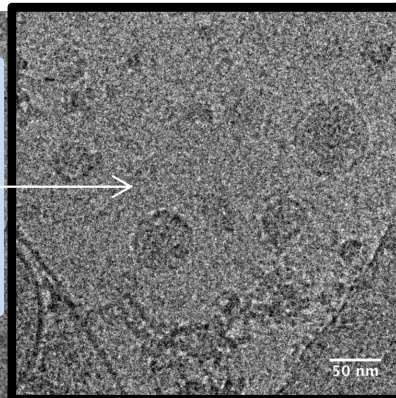
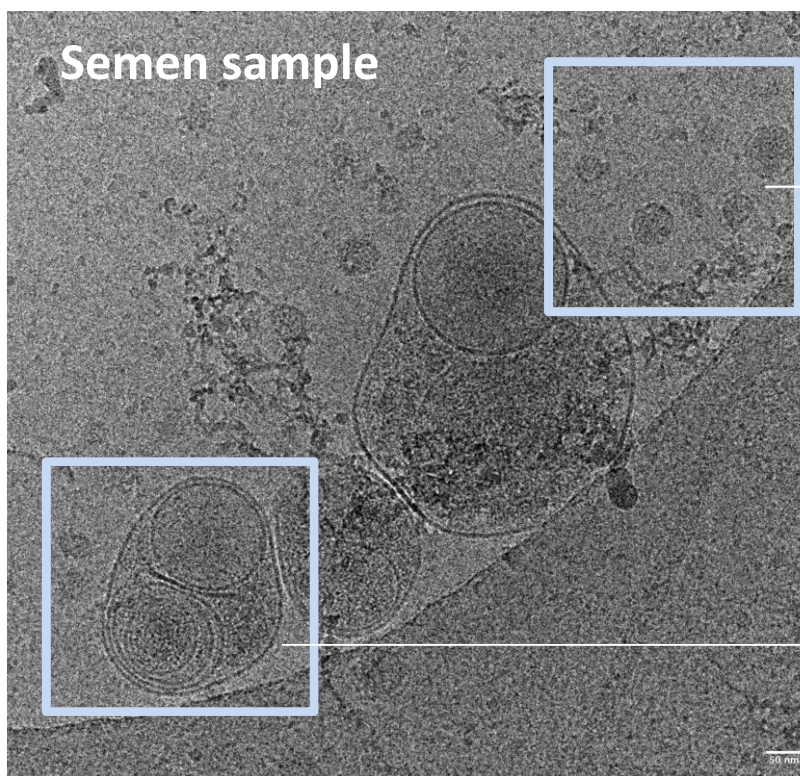
Journal Pre-proof







Semen sample



TM4 cells production

