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Emerging role of extracellular vesicles in communication of preimplantation embryos *in vitro*

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19 **Abstract**

20

21 *In vitro*, efficient communication between mammalian embryos and between embryos and
22 their environment, e.g. maternal somatic cells, implies that there is a sender, a message and a
23 receiver which is able to decode the message. Embryos are secreting a variety of autocrine and
24 paracrine factors, and among those, extracellular vesicles have recently been implicated as
25 putative messengers in embryo-embryo communication and in communication of the embryo
26 with the maternal tract. Extracellular vesicles (EVs) are membrane-bound vesicles, found in
27 biofluids and in culture media conditioned by the presence of embryos or cells, that carry and
28 transfer regulatory molecules, such as microRNAs (miRNAs), messenger RNAs (mRNA), lipids
29 and proteins.
30 Here, we conducted a systematic search of the literature to review and present the currently
31 available evidence on the possible roles of EVs in embryo communication and embryo
32 development. It is important to note that many of the biologically plausible functions of EVs in
33 embryo communication have not yet been substantiated by conclusive experimental evidence.
34 However, indirect evidence, such as the use of media conditioned by embryos or by somatic
35 cells with improved embryo development as a result, may indicate that EVs can be an
36 important asset for the development of tailor-made media allowing better embryo
37 development *in vitro*, even for single embryo culture.

38

39 **Additional keywords :** Extracellular vesicles, embryo communication, embryo-maternal
40 communication, embryo culture

Introduction

Efficient communication between cells and tissues is paramount in many physiological processes, including embryo development. Typically inside the body, mammalian cells communicate with each other either through direct interaction (juxtacrine signalling) or by secreting molecules such as growth factors, hormones and cytokines. These messengers can turn on the cell or embryo itself (autocrine signalling[1]), or have an effect on both neighboring (paracrine signalling) and distant cells (endocrine signalling). Cell-cell communication is however changing completely when cells are being cultured outside the body, *in vitro*.

Mammalian preimplantation embryos develop *in vivo* inside the female genital tract, i.e. the oviduct and the uterus, and communicate with these dynamic and elastic surroundings on which the embryo depends for its development and survival (Fazeli 2011). In the absence of a genital tract, when embryos are being cultured *in vitro*, the embryo resides in a semi-defined culture medium in which no endocrine or paracrine factors are present, since all communication with the maternal genital tract is cut off. This communication can be restored by embryo co-culture with somatic cells such as cumulus cells (Goto *et al.* 1988; Goovaerts *et al.* 2009), oviduct cells (Eyestone *et al.* 1989; Gandolfi and Moor 1987; Van Soom *et al*. 1996, 1997; Liu *et al*. 2001; Lee *et al.* 2001; Xu *et al.* 2001; Lee *et al.* 2004), and medium conditioned by somatic cells (Mermillod *et al.* 1993; Van Langendonckt *et al.* 1996; Li *et al.* 2004a; Li *et al.* 2004b). This approach was very popular in the late 20th century to mimic the microenvironment conditions associated with the maternal tract. Nevertheless, even without communication with cells from

[1] The term autocrine is here also used to refer to signaling between similar cells, like embryos

62 the genital tract, preimplantation embryos are able to promote their own development *in vitro*
63 by the production of autocrine factors (Paria and Dey 1990), and in this way they are able to
64 communicate to each other. Mostly this accumulation of autocrine factors is typically achieved
65 by culturing bovine embryos in large groups, some 10-25 embryos in a 50 µl droplet of medium
66 covered by oil to avoid evaporation (Sagirkaya *et al*. 2007; Goovaerts *et al.* 2009; Wydooghe *et*
67 *al*. 2013) (Fig. 1a).

68 The presence of these autocrine factors in the medium when embryos are cultured in
69 group lies at the basis of the embryos' superior development in group compared to solitary
70 culture (Paria and Dey 1990; O'Neill 2008). Group culture has been adopted by many research
71 groups as a routine procedure for animal embryo culture, leading to superior embryo
72 development (Vajta *et al.* 2000; Hoelker *et al.* 2010). By playing with embryo density, expressed
73 as the number of embryos per volume of medium, it has been shown that embryos develop best
74 in groups cultured at an embryo-volume ratio ranging from 1:1 (Ferry *et al.* 1994) to 1:5 (Fukui *et*
75 *al. 2000)* (Table 1). When embryo-volume ratio is being kept at 1:10 or 1:20, and embryos are
76 cultured individually in a droplet of medium (Fig. 1b), development to the blastocyst stage is
77 much lower to even non-existing in a suboptimal medium such as medium containing fetal calf
78 serum (FCS) (Table 1). Both group culture of embryos, and co-culture of embryos with somatic
79 cells can reduce the negative effects of serum during embryo culture (Donnay *et al.* 1997;
80 O'Doherty *et al*. 1997; Goovaerts *et al.* 2009; Goovaerts *et al.* 2012). Therefore it appears that
81 some factors released by the adjacent embryos or by the co-cultured somatic cells are either
82 affecting the development of the neighboring embryos in a positive way or are removing a
83 detrimental factor associated with the serum. Interestingly, in serum-free medium, the positive

84 effects of group culture remain present but to a lesser extent, and these so-far unidentified
85 embryotropins have been demonstrated to promote development, with higher blastocyst cell
86 numbers and less apoptosis (Wydooghe *et al.* 2014b). This inter-embryo communication has only
87 been identified after *in vitro* culture of embryos became commonplace. What the exact nature
88 of this communication is, is at present not entirely clear, and a vast range of possible autocrine
89 factors have already been implicated to be important in how embryos 'talk' to each other (for
90 review see: Wydooghe *et al.* 2015). Embryos also 'talk' to the somatic cells used in various co-
91 culture models (for review see : Lee and Yeung 2006; Ulbrich *et al.* 2010).

92 While many studies have been trying to identify the nature of these autocrine factors,
93 or to relate these factors with markers of embryo quality, the main approach so far was to analyze
94 conditioned medium for the presence of proteins, growth factors, or metabolites (Mermillod *et*
95 *al.* 1993; Beardsley *et al.* 2010; Kropp and Khatib 2015; Foresta *et al*. 2016). This may be useful,
96 but in this way an important means of cell-cell communication is being overlooked. Shedding of
97 extracellular vesicles (EVs) is now a well-recognized, important method of cell-cell
98 communication in a number of different cell types: EVs have been purified from every prokaryotic
99 (Kim *et al.* 2015) and eukaryotic (Regente *et al*. 2009; Oliveira *et al.* 2010a; Mantel and Marti
100 2014; Cocucci and Meldolesi 2015) cell type that has been studied to date, including stem cells
101 (Ratajczak *et al.* 2006; Camussi *et al*. 2011; Lai *et al.* 2011; Timmers *et al.* 2011; Chavez-Munoz *et*
102 *al.* 2010), primary cells of immune and nervous systems (Chavez-Munoz *et al*. 2010; Faure *et al.*
103 2006; Guescini *et al.* 2010; Kesimer *et al.* 2009; Potolicchio *et al*. 2005) and various cancer cell
104 lines (Ai- Nedawi *et al.* 2008; Skog *et al.* 2008; Ai-Nedawi *et al*. 2009). Extracellular vesicles are
105 vesicles that are being shed by healthy cells, and are often referred to as microvesicles,

106 exosomes, or microparticles (Raposo and Stoorvogel 2013). They contain as a cargo, amongst
107 other molecules, proteins, lipids, RNAs and miRNAs, that may serve as messengers between cells.
108 However, due to lack of knowledge on the molecular mechanisms for EV formation and lack of
109 methods to interfere with the packaging of cargo or with vesicle release and addressing to
110 receiving cells, it is still difficult to assess the physiological relevance of EVs *in vivo* (Raposo and
111 Stoorvogel 2013; Yañez-Mó *et al.* 2016). *In vitro* model systems such as embryo group culture,
112 and embryo-oviduct co-culture, may become important tools to study these fascinating
113 structures. Here we review the current literature as to release of EVs by preimplantation embryos
114 and we will provide evidence that they may be much more important in embryo-to-embryo or
115 embryo-maternal communication as previously thought. We will also focus on technical aspects
116 of EVs isolation, in order to instigate more research into this fascinating topic. A better
117 understanding of the role of EVs in embryo culture and development may lead to improved
118 knowledge on how embryos communicate with their environment and to the development of
119 new *in vitro* culture systems for both animal and human embryos.
120
121 **Classification and biogenesis of extracellular vesicles**
122 As reviewed by Machtinger *et al.* (2015), EVs have been pointed out to be essential players in
123 gamete maturation, fertilization and embryo implantation. The term 'extracellular vesicle' is
124 generally applied to describe different vesicle types, including exosomes, microvesicles,
125 apoptotic bodies and in pathological situations, necrotic debris.
126
127 a) Exosomes

128 Exosomes are rounded phospholipid bilayer vesicles, and are in general smaller than
129 microvesicles, with a size ranging from 40-150 nm (Table 2). Exosomes are formed in the
130 late endosomal compartment by inward budding of the membrane of late multivesicular
131 bodies (MVBs) (Fig. 2). Formation of intraluminal vesicles in multivesicular bodies has
132 been shown to involve the endosomal sorting complex required for transport (ESCRT);
133 apart from this, studies indicate that these vesicles can develop independently of this
134 complex (Trajkovic *et al*. 2008). ESCRT has been shown to be involved in inward budding
135 of intraluminal vesicles of MVBs and cleavage of the necks of these vesicles. When the
136 vesicles are present in MVBs they can be released as exosomes by fusion of MVBs with
137 the plasma membrane or alternatively be degraded via lysosomal fusion (Hurley *et al*.
138 2010). Emission of exosomes from the endosomal compartment of MVBs through fusion
139 with the plasma membrane is also dependent on intracellular calcium (Théry *et al*. 1999;
140 Savina *et al.* 2005). Many cytoplasmic proteins are present in exosomes including
141 cytostructural proteins such as actin, annexins, tubulin and actin-binding proteins as well
142 as signaling proteins such as signal transduction kinases, cytokines, and heterotrimeric G-
143 proteins (for the whole known protein contents of exosomes, see Exocarta:
144 http://www.exocarta.org). β integrins and ICAM-1 are also found on the exosomal surface
145 as are the tetraspanins CD9, CD63, CD81, and CD82, which are considered to be exosomal
146 markers (Heijnen *et al*. 1999; Théry *et al*. 2009; Vlassov *et al*. 2012).
147 Once released from producing cells, EVs will reach their target cells in the vicinity or in a
148 distant tissue through transit by biological fluid (blood flow or local fluid). They may be
149 uptaken by target cells through different pathways. EVs can bind randomly to cell

150 membranes and fuse to deliver their contents in the cytoplasm of recipient cell in a non-
151 specific manner. Alternatively, EVs can bind to a cell surface receptor through their
152 surface proteins (integrins, tetraspanins). This pathway requires a specific receptor at the
153 surface of the recipient cell. This binding can end up with activation of the receptor,
154 inducing a signaling cascade in the cell, and/or internalization of the EVs contents by
155 membrane fusion or by phagocytosis of the whole EV. Although these processes are not
156 yet fully elucidated, they are probably all existing together or in different contexts.
157

158 b) Microvesicles
159 Microvesicles are supposed to be formed by outward budding of the plasma membrane
160 (Fig. 2). They are mostly rounded vesicles with a size of around 100-1000 nm (Table 2).
161 They exhibit similar composition in proteins and lipids to plasma membranes (Wolf 1967;
162 Turiák *et al*. 2011; Dragovic *et al*. 2011. György *et al*. 2011a). Microvesicles are released
163 in response to cellular activation or stress: initiated by rises in intracellular calcium, which
164 eventually lead to the activation of scramblase and calpain resulting in microvesicle
165 formation (Cocucci *et al*. 2009; Yuana *et al*. 2013).
166

167 c) Apoptotic bodies
168 Apoptotic bodies also belong to EVs (Yáñez-Mó *et al.* 2015) and are released as the cell is
169 undergoing apoptosis. Apoptotic bodies are consisting of cytoplasm with tightly packed
170 organelles with or without a nuclear fragment. These bodies are subsequently
171 phagocytosed by macrophages, parenchymal cells, or neoplastic cells and degraded

172 within phagolysosomes (Elmore 2007). They are heterogeneous in size ranging from
173 1000-5000 nm (Table 2). Intracellular calcium is increased during apoptosis serving as an
174 initiating event for apoptotic body formation (Cocucci *et al*. 2009; Baj-Krzyworzeka *et al*.
175 2006).

176

177 In the present review we will focus on exosomes and microvesicles because of their emerging
178 role in inter-embryonic and embryo-maternal communication. For reasons of clarity, we will refer
179 to exosomes and microvesicles as EVs in the further text, and will not discriminate between the
180 different classes, even if this was done in the original papers.

181

182 **Isolation and characterization of extracellular vesicles (EVs)**

183 Different isolation techniques have been described to collect EVs from cells or fluids.
184 Extracellular vesicles can be isolated using three major methods; with variations possible, namely
185 (a) ultracentrifugation; (b) adsorption to micro beads, or (c) size exclusion chromatography.
186 After isolation, they can be identified based on morphological properties by several imaging
187 methods which include Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS)
188 analysis (that allows quantification of EVs by size, differentiating exosomes and microvesicles)
189 and immunostaining of exosomal markers like CD9, CD63 or HSP70. It is still a problem to find
190 good markers to differentiate exosomes from microvesicles and different isolation methods can
191 change the content of the EVs and abundance (Sunkara *et al*. 2016).

192 So far no specific marker can be used to distinguish the subtypes of EVs since protein components
193 of the endosomal sorting complexes required for transport (ESCRT complex), such as Alix and
194 TSG101, and membrane proteins such as CD9, CD81 and CD63 are enriched with either exosomes
195 or microvesicles, depending on size and lower relative abundance (Raposo and Stoorvogel 2013).
196 Moreover EV populations are not yet completely defined by researchers, as the EV subtypes
197 released by cells varies from cell to cells.

198

199 **a) Ultracentrifugation**

200 Differential ultracentrifugation can be used for the isolation of EVs (Théry 2006; Witwer
201 *et al*. 2013). The fluid of interest is subjected to repeated centrifugations, each time
202 removing the pellet and increasing the centrifugal force. Separation of EVs is based on
203 their size and density, with larger and denser particles, which are not wanted, pelleting at
204 lower centrifugal forces. During the initial steps conditioned medium is subjected to
205 centrifugation at 300× g for 10 min; after which the supernatant is centrifuged at 2000×
206 g for 10 min, followed by 10,000× g for 30 min of centrifugation. These first three steps
207 of centrifugation are meant to remove intact cells, cell debris and dead cells or apoptotic
208 bodies. In some strategies, these centrifugation step(s) have been replaced by 0.1 µm (Ji
209 *et al.* 2008) or 0.22 µm (Théry *et al.* 2001) filtration. After the 10,000× g spin, the
210 supernatant is then subjected to final ultracentrifugation at 100,000×g for 70 min. The
211 final outcome of this rather time-consuming centrifugation method is an exosome pellet
212 which can be stored for further analysis. The re-suspended pellets can be used for

213 checking the presence of microvesicles or exosomes through electron microscopy (Fig. 3a
214 and b), immunofluorescence, or RNA extraction.
215 It is important to note that the isolation protocol of EVs varies between different cell
216 types, as well as for the targeted population of the EVs is to be extracted. Exosomes (40-
217 100 nm) are usually isolated by centrifugation at 100,000-200,000×g (Théry *et al.* 2006;
218 Witwer *et al.* 2011), whereas microvesicles (10-1000 nm) are isolated by centrifugation
219 at 10,000-20,000×g (Witwer *et al.* 2011; Baran *et al*. 2010). Apoptotic bodies (50-5000
220 nm) are obtained with a centrifugation of 2000×g (Jeppesen *et al*. 2014). It has also been
221 shown that repeated ultracentrifugation steps can reduce the quality of exosome
222 preparations leading to lower exosome yield (Lobb *et al.* 2015). Using ultrafiltration
223 devices results in increased vesicle isolation when compared to traditional
224 ultracentrifugation protocols (Lobb *et al*. 2015).

225

226 A similar and quicker method is density gradient centrifugation (Tauro *et al.* 2012; Van
227 Deun *et al.* 2014). For density centrifugation, for instance a sucrose gradient can be used
228 to isolate EVs. The primary function of density gradient centrifugation is to separate
229 particles, either on the basis of their buoyant density or their rate of sedimentation.

230

231 (b) Immuno affinity isolation:
232 Another promising method used for EVs isolation involves microbeads, normally
233 magnetic, that are coated with an antibody that recognizes certain markers present on

234 the EV surface. This technique can be used for EVs from cell culture media, or body fluids.
235 Initially the EVs samples are mixed with the antibody coated microbeads, and a magnetic
236 force is applied to a column of microplate. This retains the EV covered microbeads, while
237 the rest of the sample is discarded (www.systembio.com\exosomes). Further on, the
238 microbeads with attached EVs are eluted using appropriate buffers and used for analysis.
239 Compared to other techniques this method has the advantage to select a specific EV
240 population based on specific marker expression regardless of size of the EV (Vlassov *et al*.
241 2012).
242
243 (c) Size Exclusion Chromatography
244 This method is mostly used for a low speed centrifugation step that allows the removal
245 of larger objects from the samples such as cellular debris, cell organelles etc. This is
246 followed by a filtration step (0.8 and 0.2 μM pore size filter) to concentrate the EVs. The
247 filtered EV samples are then subjected to size exclusion chromatography (normally gel
248 filtration column) where small volume fractions are ultracentrifuged to pellet down the
249 EVs (Müller 2012; Taylor *et al*. 2002; Böing *et al*. 2012). The major principle of this
250 technique is that particles based on their size move towards the filtration column at
251 different rates. Hence larger particles will elute more rapidly, whereas small ones will
252 move slowly, due to their ability to penetrate the stationary phase (gel) of the column.
253 However this method has a few limitations, like forcing EVs passage through filter used
254 to per concentrate the samples may lead to EV deformation and eventual rupture into
255 smaller particles (Witwer *et al*. 2013).

256 **Autocrine communication among embryos *in vitro* : role of embryo-derived extracellular**
257 **vesicles**
258
259 Like somatic cells, preimplantation embryos are able to produce and secrete autocrine factors by
260 several mechanisms including active secretion, passive outflow, binding to a carrier molecule, or
261 transport within extracellular vesicles (Wydooghe *et al.* 2015). However, unlike somatic cells, a
262 glycoprotein layer is surrounding mammalian embryos, which is called the zona pellucida. This
263 zona pellucida is composed of four glycoproteins (bZP1, bZP2, bZP3, and bZP4) and is typically
264 visualized under the scanning electron microscope as a complex fibrous network with many pores
265 (Vanroose *et al*. 2000; Van Soom *et al.* 2010). In bovine embryos, the pores are >50 nm in
266 diameter, with 20–50% >200 nm (Vanroose *et al*. 2000). When the passage of fluorescent
267 microspheres through and their location in the zona pellucida was assessed, the smallest beads
268 (40-50 nm) were detected halfway through the thickness of the zona, whereas beads with a size
269 of 200 nm were found only within the outer-fourth part of the zona pellucida (Vanroose *et al.*
270 2000). Using fluorescently labelled markers, Legge (1995) showed that the zona pellucida of
271 murine oocytes is permeable to markers up to 170 kDa. Microvesicles of 40–150 nm diameter
272 should be able to pass through these pores, since most lipids and lipid-containing molecules pass
273 through the zona pellucida relatively easy (Turner and Horobin 1997). This hypothesis has
274 elegantly been proven by Saadeldin *et al.* (2014): they derived EVs from medium conditioned by
275 parthenogenetically activated pig embryos by differential centrifugation. Next the EV pellets
276 were subjected to fluorescent labeling using PKH67 dye, a green fluorescent dye that labels the
277 lipid membranes. Cloned embryos were exposed to these labelled EVs and it was shown that the

278 EVs can pass through the zona pellucida and are internalized by blastomeres. Moreover, analysis
279 of culture media from porcine embryos cultured individually determined the presence of 30–120
280 nm vesicles differing in size according to the embryo's age (less than 40 nm in cultures from two-
281 cell embryos and less than 120 nm in cultures from blastocysts). An important aspect from the
282 experimental set-up was that the culture medium used for porcine embryos was serum-free
283 chemically defined PZM-5 medium (Saadeldin *et al.* 2014). When using serum-containing
284 medium or medium with BSA, EVs derived from serum or BSA could interfere with the results.
285 Gardiner *et al*. (2013) demonstrated that also human IVF embryos release EVs into the culture
286 medium. Increasing EV size was strongly associated with decreasing embryo quality (202 nm
287 good, 218 nm average, 222 nm poor and 227 nm arrested development).
288 Now how do these EVs impact embryo development? In the study of Saadeldin, cloned
289 embryos cultured with porcine parthenogenetic embryos showed a significant increase in their
290 developmental competency (i.e. increased number of blastomeres and better blastocyst
291 formation) compared with cloned embryos cultured alone. Paradoxically, the addition of
292 medium conditioned by parthenogenetic embryos on different time points, either along with
293 the developmental course or preceded by 2 days, was not able to affect embryo development.
294 Authors suggested that a continuous supply of EVs is necessary in contrast to an acute transfer,
295 confirming the highly dynamic microenvironment created by embryonic secretions (Saadeldin
296 *et al*. 2014). The EVs derived from parthenogenetic embryos and conditioned medium
297 contained mRNA of pluripotency transcription factors (*OCT4, SOX2, KLF4,CMYC* and *NANOG*).
298 These transcription factors were also found in EVs derived from embryonic stem (ES) cells
299 (Ratajczak *et al.* 2006). Recently it has also been reported that bovine and human pre-

300 implantation embryos secrete miRNAs into culture medium (Rosenbluth *et al.* 2014; Kropp *et*
301 *al.* 2014). These miRNAs are secreted within EVs into the extracellular environment where
302 these can be taken up by cells and act in autocrine or paracrine manner to impact gene
303 expression. Human embryos cultured for IVF were found to secrete specific miRNAs which are
304 varying depending on the fertilization method, their chromosomal state and whether or not
305 they successfully implanted (Rosenbluth *et al*. 2014). In conditioned medium of aneuploid
306 human embryos, miRNA-191 was more abundant, while miRNA-191, 372 and 645 were mostly
307 highly concentrated in medium from embryos of failed IVF cycles. In horses, an *in vitro* study
308 (Bemis *et al.* 2012) suggested that EVs can be secreted by Day 8 embryos, which can modulate
309 the functions of the oviduct epithelium through transfer of early pregnancy factor (HSP 10) and
310 miRNAs. Kropp *et al.* (2014) examined miRNA secretion in day 5-8 *in vitro* cultured bovine
311 embryos, and observed a clear differentiation of miRNAs expression between the embryos that
312 successfully developed to the blastocyst stage and degenerate embryos. In total four miRNAs -
313 25,302c, 192a2 and 181 were found to be more prevalent in culture medium of degenerating
314 embryos. It is apparently also possible to detect sex determining mRNAs, such as Xist and Sry,
315 in the conditioned medium of *in vitro*–produced embryos cultured individually, which could be
316 used for sexing (Saadeldin *et al.* 2015).
317
318 **Paracrine communication between embryos and somatic cells in vitro: role of maternally-**
319 **derived extracellular vesicles**
320

321 In mammals, maternal-embryo communication is considered the basis for the success of
322 any reproductive event (Rizos *et al.* 2002). The oviduct, or Fallopian tube, which is connecting the
323 ovary to the uterus, plays a vital role in these interactions. In fact, it holds the first maternal cross-
324 talk with gametes and early embryos and provides an optimal environment for fertilization and
325 early embryo development. The oviductal epithelium is composed of ciliary and secretory cells
326 responsible for the secretion of proteins and other factors that together with constituents
327 derived from plasma, contribute to the formation of the oviduct fluid (OF) (Buhi *et al.* 2000; Leese
328 *et al.* 2008). Later, the embryo will migrate to the uterus, and this dialogue will continue with the
329 endometrium to ensure proper implantation. The role of the oviduct has been underestimated
330 based on the ability to produce competent embryos *in vitro* which after transfer to the uterus
331 establish a pregnancy and live calves, lambs, kids, and babies are born. However, it has been
332 evidenced that embryos cultured in the oviducts of different species are of superior quality to
333 those produced *in vitro*, in terms of morphology, gene expression, cryotolerance and pregnancy
334 rate after transfer (Lazzari *et al.* 2010; Rizos *et al.* 2010; Besenfelder *et al*. 2012), indicating that
335 the oviduct is not merely an organ of transit.

336 Despite the fact that *in vitro* culture conditions are capable of supporting a "relatively
337 high" percentage of blastocysts (30 to 40%), they provide a suboptimal environment reflected on
338 the quality of the produced embryos with short and long term consequences (Rizos *et al.* 2008).
339 Thus, the goal of *in vitro* embryo production is to simulate as closely as possible the conditions *in*
340 *vivo* to obtain high quality embryos capable of continued development and implantation, and
341 resulting in viable births (Menezo *et al.* 1998). Moreover, studying the oviductal environment is
342 crucial to improve our understanding of the regulatory mechanisms controlling early

343 reproductive events (Avilés e*t al.* 2015). While *in vitro* models provide a simple and defined
344 context to study maternal interactions with gametes and embryos, their advantages are not
345 limited to their simplicity. As Van Soom *et al.* (2010) pointed out, when choosing an *in vitro*
346 model, the aim of the experiment is an important consideration. In studies of gamete and embryo
347 interaction with the reproductive tract, the use of BOEC, OF and their EVs may be considered as
348 the most appropriate *in vitro* models to mimic the physiological conditions pertaining *in vivo*.

349 The *in vitro* culture of BOEC has been considered a suitable model to produce embryos
350 of better quality and also to study oviductal-embryo interaction (Ulbrich *et al.* 2010). These cells
351 can be cultured as monolayers or cell suspension (Fig. 4). The drawback of monolayers is that
352 they dedifferentiate losing important morphological characteristics (Rottmayer *et al.* 2006)
353 including reduction of cell height, loss of cilia, and loss of secretory granules and bulbous
354 protrusions (Thibodeaux *et al.* 1992), whereas short-term (24 h) epithelial cell suspension culture
355 maintained morphological characteristics as well as gene markers present in the cells *in vivo* such
356 as *OVGP1*, oestrogen and progesteron receptors (Rottmayer *et al.* 2006).

357 In the present review we will merely focus on the *in vitro* model consisting of coculture
358 of primary BOEC with *in vitro* produced bovine embryos. Using this *in vitro* system, Schmaltz-
359 Panneau and colleagues demonstrated that BOEC adapted their transcriptomic profile in
360 response to the presence of embryos (Schmaltz-Panneau *et al.* 2014). Most of the genes
361 regulated in BOEC by the presence of embryos are known to be interferon regulated, but other
362 pathways may also be involved and triggered by other embryonic signals. Moreover, when the
363 levels of expression of genes suspected to be involved in embryo development support were
364 evaluated (GPX4, OVGP, C3) in different regions of the oviduct (ampulla and Isthmus), a regional

365 difference was found (Cordova, personal communication).

366 Also the other way around, from BOEC to embryo, communication could be detected:
367 Cordova *et al*. (2014) showed that the use of BOEC in embryo culture *in vitro* at the early stages
368 of embryo development, up to day 4, improves embryo development and embryo quality in
369 terms of specific gene transcripts. This period of culture coincides with the *in vivo* conditions
370 where the embryo is still in the oviduct. Furthermore, BOEC co-culture with embryos for the first
371 4 days accelerated the kinetics of blastocyst development, with a significant increase in the
372 number of blastocysts at days 6 and 7 compared to control and coculture during 8 days. BOEC
373 from the isthmus were more capable of supporting early embryo development than BOEC from
374 the ampulla, demonstrating a regional specialization of the oviduct in supporting embryo
375 development (Cordova, personal communication). In addition, embryo transcriptomic analysis
376 revealed that the level of expression of several genes related to embryo quality were altered as
377 a result of the presence of BOEC, reflecting reduced embryo apoptosis and increased capacity to
378 adapt against oxidative stress after coculture.

379 Taken together, these *in vitro* studies have shown the existence of a real dialogue
380 between the early embryo and the oviduct, as a result of which, the embryo regulates its own
381 environment in the maternal tract but also during *in vitro* culture. Soluble factors are probably
382 involved in this cross-talk, binding to receptors on both embryo and maternal sides. However,
383 recent studies indicate that there is room for other players in this embryo-maternal dialogue.
384 Extracellular vesicles have been proposed as intercellular vehicles in the embryo-maternal
385 dialogue in the uterus (Ng *et al*. 2013; Burns *et al*. 2014, 2016; Ruiz-Gonzalez *et al*., 2015) and
386 might also mediate the maternal-gametes/embryo interactions in the oviduct. To date, little is

387 known on how EVs could be taken up by gametes and early embryos and whether they modulate
388 the maternal interactions to promote successful pregnancy. Recently, we demonstrated that an
389 extended culture BOEC monolayer can be used successfully for embryo co-culture and
390 conditioned media (CM) production, improving embryo development and embryo quality, most
391 likely due to the presence of EVs secreted by the cells (Lopera-Vasquez *et al.* 2016a). This
392 hypothesis was confirmed by the presence of $3x10^5$ EVs/ml of a relatively homogeneous
393 population of 150-200 nm in diameter obtained by ultracentrifugation from BOEC CM and
394 assessed by transmission electron microscopy and nanoparticle tracking analysis (Nanosight) (Fig.
395 5). Also, it was verified by Western blot and bead-assisted flow cytometry analysis that these EVs
396 expressed the classical markers of exosomes like tetraspanins CD9 and CD63, TSG101 and ERM
397 proteins (Fig. 5). Furthermore, embryos cultured with EVs, irrespective of concentration
398 ($3x10^5$=100%; $1.5x10^5$=50%; $7.5x10^4$=25% EVs/ml) or processing (fresh or frozen/thawed) had
399 similar blastocyst yield on Day 7, 8 or 9 (range on Day 8: 37.8-43.4%) when compared with
400 controls. Likewise, the survival rate after vitrification/warming was higher at all points in time
401 compared to controls (range at 72h; 48.7-56.5% vs 22.3% respectively). Blastocysts cultured with
402 EVs displayed a higher number of total cells and expressed several genes related with embryo
403 quality. On the other hand, EVs derived from FCS exerted a deleterious effect on embryo quality.
404 Based on this evidence it can be concluded that EVs from BOEC may have an important function
405 in the communication between the oviduct and the embryo during early stages of development.
406 (Lopera-Vasquez *et al.* 2016a).

407 An important component of the oviductal environment is the OF. The composition of OF
408 is very complex, containing simple and complex carbohydrates, ions, lipids, phospholipids and

409 proteins (Avilés *et al.* 2010). Some of these components are metabolic substrates (lactate,
410 pyruvate, amino acids and glucose) and their concentrations are different from those in the
411 uterine fluid and serum (Leese 1988; Hugentobler *et al.* 2007; Leese *et al.* 2008). It has been
412 shown that specific oviductal secretions have an effect on oocyte and sperm function (Killian
413 2011; Mondejar *et al.* 2013), since oviductins, osteopontin, glycodelins and lactoferrin may play
414 a role in gamete interaction (Ghersevich *et al.* 2015). When porcine oocytes were treated with
415 OF before fertilization, a significant increase in cleavage rate and blastocyst yield was evident,
416 suggesting protection of the embryo by OF against apoptosis and against adverse effects on
417 mitochondrial DNA transcription or replication (Lloyd *et al.* 2009). When bovine oocytes were
418 exposed to OF before fertilization, no effect was visible on embryo development and morphology
419 of the resulting blastocysts; but differences appeared in specific transcripts of the embryos
420 produced from oocytes treated with OF (Cebrian-Serrano *et al.* 2013).

421 It is worth to mention that, until recently, the OF was only used before fertilization. In a
422 recent study we investigated the developmental competence of bovine zygotes and the quality
423 of blastocysts produced after culture in SOF without FCS, but supplemented with different
424 concentrations of OF. It was clear that >5% OF supplementation was detrimental for embryo
425 development, while low concentrations of OF (1.25%) had a positive effect on development and
426 quality of the produced blastocysts in terms of cryotolerance, cell number and expression of
427 qualitatively related genes (Lopera-Vasquez *et al.* 2015). Thus, enhancing the post fertilization
428 environment *in vitro* with substances present in the oviduct may diminish the limitations of *in*
429 *vitro* embryos and make them comparable to their *in vivo* counterparts. This enhanced
430 development may also be brought about by extracellular vesicles present in the OF. Almiñana

431 and colleagues isolated exosomes from bovine OF and co-cultured them with *in vitro* produced
432 embryos to demonstrate the existence of oviductal-embryo communication via exosomes
433 (Almiñana *et al.* 2015; 2016 published communication IETS). Extracellular vesicles were isolated
434 by serial ultracentrifugation and measured by dynamic light scattering analysis and transmission
435 electron microscopy, detecting exosomes (63-97 nm) and microvesicles (>100nm), both in OF
436 (Fig. 3a) and culture media from BOEC primary culture (Fig. 3b). To demonstrate the existence of
437 the oviductal-embryo communication via exosomes, oviductal exosomes were labelled with
438 green fluorescent dye (PKH67), filtered (0.22µm) to remove microvesicles and co-incubated with
439 *in vitro* produced blastocysts for 20 h, under 5% CO_2 and 5% O_2 conditions. Confocal microscopy
440 observations confirmed that exosomes were internalized by blastocyst cells, demonstrating the
441 existence of an oviductal-embryo communication via exosomes (Fig. 6).

442 Lopera-Vasquez *et al.* (2016b) evaluated the developmental competence and the mRNA
443 abundance of specific genes on bovine blastocysts produced *in vitro* with EVs obtained by
444 ultracentrifugation from ampullary and isthmic OF. EVs from both oviduct regions had a similar
445 size of a mean around 200 nm as quantified with NTA and transmission electron microscopy.
446 Blastocyst rate was not affected by the supplementation of EVs compared to controls (SOF+BSA
447 and SOF+FCS). However, bovine isthmic OF EVs supplementation had a positive effect on gene
448 expression patterns of developmental related genes (*AQP3, LDLR, DNMT3A and SNRPN*)
449 compared with serum supplementation suggesting an association between the oviductal
450 environment and the developing embryo (Lopera-vasquez *et al.* 2016b).

451 In an attempt to decipher the role of oviductal derived EVs, the contents of EVs was
452 analyzed at the proteomic level (Almiñana *et al.* 2015). Knowing that *in vitro* culture could alter

453 the gene expression profile of OEC (Rottmayer *et al*. 2006; Schmaltz-Panneau *et al.* 2015), EVs
454 were analysed from both *in vivo* oviductal fluid and *in vitro* BOEC conditioned medium (Almiñana
455 *et al*., 2015). For this purpose, the same primary BOEC culture system was used as explained
456 previously. EVs secreted by OEC *in vivo* in the oviductal fluid and by OEC *in vitro* in the conditioned
457 media after primary culture were collected by serial ultracentrifugation. Preliminary results by
458 dynamic light scattering analysis revealed different size distribution profiles compatible with
459 exosomes and microvesicle populations from *in vivo* preparations and mostly microvesicle
460 populations from *in vitro* preparations. Protein profile analysis by SDS-PAGE showed quantitative
461 and qualitative differences between both EV samples. In addition, exosomes of *in vivo* and *in vitro*
462 origin exhibited distinct proteomic profiles. Indeed, western blot analysis demonstrated that (i)
463 both types of exosomal protein samples were positive for HSP70, a known exosomal protein; and
464 (ii) *in vivo* exosomes contained OVGP and heat shock protein A8 (HSPA8), oviductal proteins with
465 known roles in fertilization and early development. However, OVGP was not detected in *in vitro*
466 exosomes. This is not surprising since the OVGP gene is known to be downregulated during BOEC
467 culture under these conditions. High throughput analysis of the proteomic content of the *in vivo*
468 vesicles by LC1D-nanoESI-LTQ-Orbitrap revealed 480 proteins in the oviductal EVs. Gene ontology
469 (GO) analysis revealed that a high number of these proteins were involved in metabolism (24.9%),
470 cellular process (19.3%) and 0.8% reproductive processes. Further analysis revealed that more
471 than 56% of EVs proteins involved in cellular processes were associated with cell-to-cell
472 communication (Almiñana *et al.* 2016).

473 In addition to the identification of proteins that may be involved in embryo-embryo
474 communication or embryo-maternal interaction, the analysis of the content of these EVs at

475 mRNA and miRNA levels will bring new insights into the dialogue of the embryos with its
476 environment. Moreover, a better understanding of the molecular mechanisms by which these
477 EVs are recognized and internalized by embryos may contribute to their therapeutic applications
478 in ARTs. Mechanisms involving membrane fusion or endocytosis (Del Conde *et al*. 2005; Parolini
479 *et al*. 2009) have been proposed, but it is still unclear whether these vesicles could use more than
480 one route or whether the vesicular uptake is cell type specific (Feng *et al*. 2010). It becomes more
481 and more apparent that EVs represent ideal natural nanoshuttles for carrying specific *in vivo*
482 molecules that are not present in classical *in vitro* culture media. EVs supplementation could
483 bring a "cocktail" of *in vivo* oviductal proteins, miRNA and lipids to overcome the absence of
484 maternal environment or to complement a deficient coculture system involving partially
485 dedifferentiated BOEC (Fig. 7). Increasing our understanding of the content and function of EVs
486 will highlight the great potential for the use of these vesicles as non-invasive biomarkers in
487 embryo culture or as therapeutic assets in infertility and early pregnancy loss.

488

489 **Conclusion**

490

491 In conclusion, beyond classical ways of cell communication involving ligands binding to
492 membrane receptor to trigger intracellular cascades of phosphorylations, EVs, and especially
493 exosomes, predominate as new players of a complex networking activity of cells and tissues.
494 Indeed, EVs are able to deliver a complex cargo, including proteins, RNA and lipids, to target cells
495 and bypass the classical receptor step to induce deep changes in various cell functions. Number
496 of recent works highlighted the presence and possible functions of such EVs in the reproductive

497 organs and fluids, including oviduct and uterus, as well as in embryonic secretions. Deciphering
498 this newly described communication paradigm will open the way to a better understanding of
499 the regulation of early embryo development and implantation by maternal tissues and by
500 embryos themselves. It will also provide new tools for evaluating the success of these different
501 steps and to improve assisted reproduction biotechnologies.

502

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515 **References**

516 Abumaree, M.H., Chamley, L.W., Badri, M., and El-Muzaini, M.F. (2012). Trophoblast debris
517 modulates the expression of immune proteins in macrophages: a key to maternal tolerance of
518 the fetal allograft?. *J. Reprod. Immunol.* **94**, 131–141.

519

520 Allan, D., and Raval, P. (1983). Some morphological consequences of uncoupling the lipid bilayer
521 from the plasma membrane skeleton in intact erythrocytes. *Biomed. Biochim. Acta.* **42**, 11–16.

522

523 Almiñana, C., Corbin, E., Harichaux, G., Labas, V., Tsikis, G., Soleilhavoup, C., Reynaud, K., Druart,
524 X., and Mermillod, P. (2016). Interception of exosomal messages between the oviduct and the
525 embryo: What are they tweeting about?. *Reprod. Fertil. Dev.* **28**, 168.

526

527 Almiñana, C., Corbin, E., Tsikis, G., Soleilhavoup, C., Galio, L., Sandra, O., and Mermillod, P. (2014).
528 Characterization of Bovine Oviductal Exosomes from in vivo and in vitro origin. *Reprod. Fertil.*
529 *Dev.* **27**, 147.

530

531 Al-Nedawi, K., Meehan, B., and Rak, J. (2009). Microvesicles: messengers and mediators of tumor
532 progression. *Cell Cycle* **8**, 2014–2018.

533

534 Al-Nedawi, K., Meehan, B., Kerbel, R. S., Allison, A. C. and Rak, J. (2009). Endothelial expression
535 of autocrine VEGF upon the uptake of tumor-derived microvesicles containing oncogenic EGFR.
536 *Proc. Natl Acad. Sci.* **106**, 3794–3799.

537

538 Al-Nedawi, K., Meehan, B., Micallef, J., Lhotak, V., May, L., Guha, A., and Rak, J. (2008).
539 Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour
540 cells. *Nature Cell Biol.* **10**, 619–624.

541

542 Al-Nedawi, K., Meehan, B., Micallef, J., Lhotak, V., May, L., Guha, A., and Rak, J. (2008).
543 Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour
544 cells. *Nat. Cell Biol.* **10**, 619–24.

545

546 André, F., Chaput, N., Schartz, N.E., Flament, C., Aubert, N., Bernard, J., Lemonnier, F., Raposo,
547 G., Escudier, B., Hsu, D.H., Tursz, T., Amigorena, S., Angevin, E., and Zitvogel, L. (2004). Exosomes

548 as potent cell-free peptide-based vaccine. I. Dendritic cell-derived exosomes transfer functional
549 MHC class I/peptide complexes to dendritic cells. *J. Immunol.* **172**,. 2126–2136.

550 Avilés, M., Coy, P., and Rizos, D. (2015). The oviduct: A key organ for the success of early
551 reproductive events. *Animal Frontiers* **5**, 25–31.
552

553 Avilés, M., Gutiérrez-Adán, A., and Coy, P. (2010). Oviductal secretions: will they be key factors
554 for the future ARTs?. *Mol. Hum. Reprod.* **16**, 896–906.
555

556 Baj-Krzyworzeka, M., Majka, M., Pratico, D., Ratajczak, J., Vilaire, G., Kijowski, J., Reca, R.,
557 Janowska-Wieczorek, A., and Ratajczak, M.Z. (2002). Platelet-derived microparticles stimulate
558 proliferation, survival, adhesion, and chemotaxis of hematopoietic cells. *Exp. Hematol.* **30**, 450–
559 9.

560 Baj-Krzyworzeka, M., Szatanek, R., Weglarczyk, K., Baran, J., Urbanowicz, B., Branski, P.,
561 Ratajczak, M.Z., and Zembala, M. (2006). Tumour-derived microvesicles carry several surface
562 determinants and mRNA of tumour cells and transfer some of these determinants to monocytes.
563 *Cancer Immunol. Immunother.* **55**, 808–18.

564

565 Baran, J., Baj-Krzyworzeka, M., Weglarczyk, K., Szatanek, R., Zembala, M., Barbasz, J., Czupryna,
566 A., Szczepanik, A., and Zembala, M. (2010). Circulating tumour-derived microvesicles in plasma
567 +-of gastric cancer patients. *Cancer Immunol. Immunother*. **59**, 841–850.

568

569 Beardsley AJ, Li Y, and O'Neill C. (2010). Characterization of a diverse secretome generated by
570 the mouse preimplantation embryo in vitro. *Reprod. Biol. Endocrinol.* **23**, 8–71.

571 Bemis, L.T., McCue, P.M., Hatzel, J.N., Bemis, J., Ferris, R.A. (2012) Evidence for production of
572 early pregnancy factor (Hsp10), microRNAs and exosomes by day 8 equine embryos. *J. Equine*
573 *Vet. Sci*. **32,** 398.

574 Besenfelder, U., Havlicek, V., and Brem, G. (2012). Role of the oviduct in early embryo
575 development. *Reprod. Domest. Anim.* **47**, 156–63.
576

577 Beyer, C., and Pisetsky, D.S. (2010). The role of microparticles in the pathogenesis of rheumatic
578 diseases. *Nat. Rev. Rheumatol.* **6**, 21–29.

579

580 Bilyy, R.O., Shkandina, T., Tomin, A., Muñoz, L.E., Franz, S., Antonyuk, V., Kit, Y.Y., Zirngibl, M.,
581 Fürnrohr, B.G., Janko, C., Lauber, K., Schiller, M., Schett, G., Stoika, R.S., and Herrmann, M. (2012).
582 Macrophages discriminate glycosylation patterns of apoptotic cell-derived microparticles. *J Biol*
583 *Chem* **287**:496–503.

584

585 Böing, A.N., van der Pol, E., Grootemaat, A.E., Coumans, F.A., Sturk, A., and Nieuwland, R. (2012).
586 Single-step isolation of extracellular vesicles by size-exclusion chromatography. *J. Extracell.*
587 *Vesicles* **3**, 23430.

588

589 Booth, A.M., Fang, Y., Fallon, J.K., Yang, J.M., Hildreth, J.E., and Gould, S.J. (2006). Exosomes and
590 HIV Gag bud from endosome-like domains of the T cell plasma membrane. *J. Cell Biol.* **172**, 923–
591 935.

592

593 Buhi, W.C., Alvarez, I.M., and Kouba, A.J. (2000). Secreted proteins of the oviduct. *Cells Tissues*
594 *Organs* **166**, 165–79.

595

596 Burns, G., Brooks, K., Wildung, M., Navakanitworakul, R., Christenson, L.K., and Spencer, T.E.
597 (2014). Extracellular vesicles in luminal fluid of the ovine uterus. *PLoS One* **9**, e90913.

598

599 Burns, G.W., Brooks, K.E., and Spencer, T.E. (2016). Extracellular Vesicles Originate from the
600 Conceptus and Uterus During Early Pregnancy in Sheep. *Biol. Reprod.* **94**, 56.

601

602 Camussi, G., Deregibus, M.-C., Bruno, S., Grange, C., Fonsato, V., and Tetta, C. (2011).
603 Exosome/microvesicle-mediated epigenetic reprogramming of cells. Am. J. Cancer. Res. **1**, 98–
604 110.

605 Carolan, C., Lonergan, P., Khatir, H., and Mermillod, P. (1996). In vitro production of bovine
606 embryos using individual oocytes. *Mol. Reprod. Dev.* **45**,145–150.

607

608 Cebrian-Serrano, A., Salvador, I., Garcia-Rosello, E., Pericuesta, E., Perez-Cerezales, S., Gutierrez-
609 Adan, A., Coy, P., and Silvestre, M.A. (2013). Effect of the bovine oviductal fluid on in vitro
610 fertilization, development and gene expression of in vitro-produced bovine blastocysts. *Reprod.*
611 *Domest. Anim.* **48**, 331–8.

612

613 Chamley, L.W., Chen, Q., Ding, J., Stone, P.R., and Abumaree, M. (2011). Trophoblast deportation:
614 just a waste disposal system or antigen sharing?. *J. Reprod. Immunol.* **88**, 99–105.

615

616 Chaput, N., and Théry, C. (2011). Exosomes: immune properties and potential clinical
617 implementations. *Semin. Immunopathol.* **33**, 419–459.

618

619 Chavez-Muñoz, C., Morse, J., Kilani, R., and Ghahary, A. (2008). Primary human keratinocytes
620 externalize stratifin protein via exosomes. *J. Cell. Biochem.* **104**, 2165–2173.

621

622 Chavez-Munozaa, C., Hartwellaa, R., Jaliliaa, R.B., and Ghahary, A. (2010). Immunoprotective role
623 of IDO in engraftment of allogeneic skin substitutes. *Expert Rev. Dermatol.* **5**, 611–616.

624

625 Cocucci, E., and Meldolesi, J. (2015). Ectosomes and exosomes: shedding the confusion between
626 extracellular vesicles. *Trends Cell Biol.* **25**, 364–72.

627

628 Cocucci, E., Racchetti, G., and Meldolesi, J. (2009). microvesicles: artefacts no more. *Trends Cell*
629 *Biol*. **19**, 43–51.

630

631 Cordova, A., Perreau, C., Uzbekova, S., Ponsart, C., Locatelli, Y., and Mermillod, P. (2014).
632 Development rate and gene expression of IVP bovine embryos cocultured with bovine oviduct
633 epithelial cells at early or late stage of preimplantation development. *Theriogenology* **81**, 1163–
634 73.

635

636 Cowan, C.A., Atienza, J., Melton, D.A., and Eggan, K. (2005). Nuclear reprogramming of somatic
637 cells after fusion with human embryonic stem cells. *Science* **309**, 1369–73.

638

639 Crawford, N. (1971). The presence of contractile proteins in platelet microparticles isolated from
640 human and animal platelet-free plasma. *Br. J. Haematol.* **21**, 53–69.

641

642 Crescitelli, R., Lässer, C., Szabó, T.G., Kittel, A., Eldh, M., Dianzani, I., Buzás, and E.I., Lötvall, J.
643 (2013). Distinct RNA profiles in subpopulations of extracellular vesicles: apoptotic bodies,
644 microvesicles and exosomes. J. Extracell. Vesicles 2. doi: 10.3402/jev.v2i0.20677.

645

646 Cronqvist, T., Saljé, K., Familari, M., Guller, S., Schneider, H., Gardiner, C., Sargent, I.L., Redman,
647 C.W., Mörgelin, M., Åkerström, B., Gram, M., and Hansson, S.R. (2014). Syncytiotrophoblast
648 vesicles show altered micro-RNA and haemoglobin content after ex-vivo perfusion of placentas
649 with haemoglobin to mimic preeclampsia. *PLoS ONE* **9**, e90020.

650

651 da Silveira, J.C., Veeramachaneni, D.N., Winger, Q.A., Carnevale, E.M., and Bouma, G.J. (2012).
652 Cell-secreted vesicles in equine ovarian follicular fluid contain miRNAs and proteins: a possible
653 new form of cell communication within the ovarian follicle. *Biol. Reprod.* **86**, 71

654

655 Del Conde, I., Shrimpton, C. N., Thiagarajan, P. and López, J. A. (2005). Tissue-factor-bearing
656 microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. *Blood*
657 **106**, 1604–1611.

658

659 Do, J.T., and Schöler, H.R. (2004). Nuclei of embryonic stem cells reprogram somatic cells. *Stem*
660 *Cells* **22**, 941–9.

661

662 Donnay, I., Van Langendonckt, A., Auquier, P., Grisart, B., Vansteenbrugge, A., Massip, A., and
663 Dessy, F. (1997). Effects of coculture and embryo number on the in vitro development of bovine
664 embryos. *Theriogenology* **47**,1549–1561.

665

666 Dragovic, R.A., Gardiner, C., Brooks, A.S., Tannetta, D.S., Ferguson, D.J., Hole, P., Carr, B., Redman,
667 C.W., Harris, A.L., and Dobson, P.J., Harrison, P., and Sargent, I.L. (2011). Sizing and phenotyping
668 of cellular vesicles using Nanoparticle Tracking Analysis. *Nanomedicine* **7**, 780–788.

669

670 EL Andaloussi, S., Mäger, I., Breakefield, X.O., and Wood, M.J. (2013). Extracellular vesicles:
671 biology and emerging therapeutic opportunities. Nat. Rev. Drug Discov. **12**, 347–57.

672

673 Elmore, S. (2007). Apoptosis: a review of programmed cell death *Toxicol. Pathol*. **35**, 495–516.

674

675 Escola, J.M., Kleijmeer, M.J., Stoorvogel, W., Griffith, J.M., Yoshie, O., and Geuze, H.J. (1998).
676 Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes
677 and on exosomes secreted by human B-lymphocytes. *J. Biol. Chem.* **273**, 20121–20127.

678

679 Eyestone, W.H., and First, N.L. (1989). Co-culture of early cattle embryos to the blastocyst stage
680 with oviducal tissue or in conditioned medium. *J. Reprod. Fertil.* **85**, 715–20.

681

682 Fackler OT., Lu X, Frost JA, Geyer M, Jiang B, Luo W, Abo A,. Alberts A S, and Peterlin, B. M. (2000).
683 p21-Activated Kinase 1 Plays a Critical Role in Cellular Activation by Nef. *Mol. Cell. Biol.* **20**, 2619–
684 2627.

685

686 Fang, Y., Wu, N., Gan, X., Yan, W., Morrell, J.C., and Gould, S.J. (2007). Higher-order
687 oligomerization targets plasma membrane proteins and HIV gag to exosomes. *PloS. Biol.* **5**, 158.

688

689 Fauré. J., Lachenal, G., Court, M., Hirrlinger, J., Chatellard-Causse, C., Blot, B., Grange, J., Schoehn,
690 G., Goldberg, Y., Boyer, V., Kirchhoff, F., Raposo, G., Garin, J., and Sadoul, R.(2006). Exosomes are
691 released by cultured cortical neurones. *Mol. Cell. Neurosci.* **31**, 642–648.

692

693 Fazeli, A. Maternal communication with gametes and embryo: a personal opinion. *Reprod.*
694 *Domest. Anim.* **46**, 75–8.

695

696 Feng D., Zhao W.L., Ye Y.Y., Bai X.C., Liu R.Q., Chang L.F., Zhou Q., and Sui S.F. (2010). Cellular
697 internalization of exosomes occurs through phagocytosis. *Traffic* **11**, 675–687.

698

699

700 Ferry, L., Mermillod, P., Massip, A., and Dessy, F. (1994). Bovine embryos cultured in serum-poor
701 oviduct-conditioned medium need cooperation to reach the blastocyst stage. *Theriogenology* **42**,
702 445–453.

703

704 Fevrier, B., Vilette, D., Archer, F., Loew, D., Faigle, W., Vidal, M., Laude, H., and Raposo G. (2004).
705 Cells release prions in association with exosomes. *Proc. Natl. Acad. Sci.* **101**, 9683–8.

706

707 Foresta, C., Ubaldi, F.M., Rienzi, L., Franchin, C., Pivato, M., Romano, S., Guidolin, D., De Caro, R.,
708 Ferlin, A., and De Toni, L. (2016). Early protein profile of human embryonic secretome. *Front.*
709 *Biosci. (Landmark Ed).* **21**, 620–34.

710

711 Fukui, K., Hori, R., Yoshimoto, I., Ochi, H., and Ito, M. (2000). Correlation between progesterone
712 binding sites on human spermatozoa and in vitro fertilization outcome. *Gynecol. Obstet. Invest*.
713 **49**, 1–5.

714

715 Gandolfi, F., and Moor, R. M. (1987). Stimulation of early development in sheep by co-culture
716 with oviduct epithelial cells. *Reprod. Fert.* **81**, 23–28.

717

718 Gardiner, C., Ferreira, Y. J., Dragovic, R. A., Redman, C. W. G., and Sargent, I. L. (2013).
719 Extracellular vesicle sizing and enumeration by nanoparticle tracking analysis. *J. Extracell. Vesicles*
720 **2**, 10.3402/jev.v2i0.19671. http://doi.org/10.3402/jev.v2i0.19671

721

722 Gatti, S., Bruno, S., Deregibus, M.C., Sordi, A., Cantaluppi, V., Tetta, C., Camussi, G. (2011).
723 Microvesicles derived from human adult mesenchymal stem cells protect against ischaemia-
724 reperfusion-induced acute and chronic kidney injury. *Nephrol. Dial. Transplant.* **26**, 1474–1483.

725

726 George, J.N., Potterf, R.D., Lewis, P.C., and Sears, D.A. (1976). Studies on platelet plasma
727 membranes. I. Characterization of surface proteins of human platelets labeled with diazotized
728 (125i)-diiodosulfanilic acid. *J. Lab. Clin. Med.* **88**, 232–246.

729

730 Ghersevich, S., Massa, E., and Zumoffen, C. (2015). Oviductal secretion and gamete interaction.
731 *Reproduction* **149**, R1–R14.
732
733 Goovaerts, I. G., Leroy, J. L., Van Soom, A., De Clercq, J. B., Andries, S., and Bols, P. E. (2009). Effect
734 of cumulus cell coculture and oxygen tension on the in vitro developmental competence of
735 bovine zygotes cultured singly. *Theriogenology*, ***71***, 729–738.

736

737 Goovaerts, I.G., Leroy, J.L., Langbeen, A., Jorssen, E.P., Bosmans, E., and Bols, P.E. (2012).
738 Unravelling the needs of singly in vitro-produced bovine embryos: from cumulus cell co-culture
739 to semi-defined, oil-free culture conditions. *Reprod. Fertil. Dev.* **24**, 1084–92.

740

741 Goto, K., Kajihara, Y., Kosaka, S., Koba, M., Nakanishi, Y., and Ogawa, K. (1988). Pregnancies after
742 co-culture of cumulus cells with bovine embryos derived from in-vitro fertilization of in-vitro
743 matured follicular oocytes. *J. Reprod. Fertil.**83***, 753–758.

744

745 Greening, D.W., Xu, R., Ji, H., Tauro, B.J., and Simpson, R.J. (2015). A protocol for exosome
746 isolation and characterization: evaluation of ultracentrifugation, density-gradient separation,
747 and immunoaffinity capture methods. *Methods Mol. Biol*. **1295**,179–209.
748
749 Guescini, M., Genedani, S., Stocchi, V. and Agnati, L. F. (2010). Astrocytes and Glioblastoma cells
750 release exosomes carrying mtDNA. *J. Neural Transm.* **117**, 1–4.

751 György ,B., Módos, K., Pállinger, E., Pálóczi, K., Pásztói, M., Misják, P., Deli, M.A., Sipos, A., Szalai,
752 A., Voszka, I., Polgár, A., Tóth, K., Csete, M., Nagy, G., Gay, S., Falus, A., Kittel, A., and Buzás, E.I.
753 (2011a). Detection and isolation of cell-derived microparticles are compromised by protein
754 complexes resulting from shared biophysical parameters. *Blood.* **117**, 39–48.

755

756 Harding, C., Heuser, J. and Stahl, P. (1984). Endocytosis and intracellular processing of transferrin
757 and colloidal gold-transferrin in rat reticulocytes: demonstration of a pathway for receptor
758 shedding. *Eur. J. Cell Biol.* **35**, 256–263.

759

760 Harding, C., Heuser, J., and Stahl, P. (1983). Receptor-mediated endocytosis of transferrin and
761 recycling of the transferrin receptor in rat reticulocytes. *J. Cell. Biol.* **97**, 329–339.

762

763 Hagemann, L.J., Weilert, L.L., Beaumont, S.E., Tervit, H.R. (1998). Development of bovine
764 embryos in single in vitro production (sIVP) systems. *Mol. Reprod. Dev.* **51**,143–147.

765

766 Heijnen, H.F, Schiel, A.E., Fijnheer, R., Geuze, H.J., and Sixma, J.J. (1999). Activated platelets
767 release two types of membrane vesicles: microvesicles by surface shedding and exosomes
768 derived from exocytosis of multivesicular bodies and alpha-granules. *Blood* **94**, 3791–9.

769

770 Hoelker, M., Rings, F., Lund, Q., Phatsara, C., Schellander, K., and Tesfaye, D. (2010). Effect of
771 embryo density on in vitro developmental characteristics of bovine preimplantative embryos
772 with respect to micro and macroenvironments. *Reprod. Domest. Anim.* **45**, 138–45.

773

774 Hristov, M., Erl, W., Linder, S., and Weber, P.C. (2004). Apoptotic bodies from endothelial cells
775 enhance the number and initiate the differentiation of human endothelial progenitor cells in
776 vitro. *Blood* **104**, 2761–2766.

777

778 Hugentobler, S.A., Diskin, M.G., Leese, H.J., Humpherson, P.G., Watson, T., Sreenan, J.M., and
779 Morris, D.G. (2007). Amino acids in oviduct and uterine fluid and blood plasma during the
780 estrous cycle in the bovine. *Mol. Reprod. Dev.* **74**, 445–54.

781

782 Hunter, R. (1989). The fallopian tubes: Their role in fertility and infertility, by R.H.F. Hunter;
783 Springer-Verlag, Berlin, 1988, 191 pp., 55 Figs., 2 in color. $120. In 'Gamete Research. Vol. 23.'
784 (Ed. Springer-Verlag) pp. 475-475. (Wiley Subscription Services, Inc., A Wiley Company: Berlin).

785

786 Hurley, J. H., Boura, E., Carlson, L.A., and Rózycki, B. (2010). Membrane Budding. *Cell* **143**, 875–
787 887.

788

789 Hurley, J.H., and Hanson, P.I. (2010). Membrane budding and scission by the ESCRT machinery:
790 it's all in the neck. *Nat Rev Mol Cell* Biol. **11**, 556–66.

791

792 Janowska-Wieczorek, A., Majka, M., Kijowski, J., Baj-Krzyworzeka, M., Reca, R., Turner, A.R.,
793 Ratajczak, J., Emerson, S.G., Kowalska. M.A., and Ratajczak, M.Z. (2001). Platelet-derived
794 microparticles bind to hematopoietic stem/progenitor cells and enhance their engraftment.
795 *Blood* **98**, 3143–9.

796

797 Janowska-Wieczorek, A., Wysoczynski, M., Kijowski, J., Marquez-Curtis, L., Machalinski, B.,
798 Ratajczak, J., and Ratajczak, M.Z. (2005). Microvesicles derived from activated platelets induce
799 metastasis and angiogenesis in lung cancer. *Int. J. Cancer* **113**, 752–60.

800

801 Jeppesen, D.K., Hvam, M.L., Primdahl-Bengtson, B., Boysen, A.T., Whitehead, B., Dyrskjøt, L.,
802 Orntoft, T.F,, Howard, K.A,, and Ostenfeld, M,S. (2014). Comparative analysis of discrete exosome
803 fractions obtained by differential centrifugation. *J. Extracell. Vesicles* **3**, 25011.

804

805 Ji, H., Erfani, N., Tauro, B. J., Kapp, E. A., Zhu, H. J., Moritz, R. L., Lim, J.W., Simpson, R.J. (2008).
806 Difference gel electrophoresis analysis of Ras-transformed fibroblast cell-derived exosomes.
807 *Electrophoresis* ***29***, 2660–2671.

808

809 Kerr, J.F., Wyllie, A.H., and Currie, A.R. (1972). Apoptosis: a basic biological phenomenon with
810 wide-ranging implications in tissue kinetics. *Br. J. Cancer* **26**, 239–257.

811

812 Kesimer, M., Scull, M., Brighton, B., DeMaria, G., Burns, K., O'Neal, W., Pickles, R.J., and Sheehan,
813 J.K. (2009). Characterization of exosome-like vesicles released from human tracheobronchial
814 ciliated epithelium: a possible role in innate defense. *FASEB J.* **23**, 1858–1868.

815

816 Killian, G. (2011). Physiology and endocrinology symposium: evidence that oviduct secretions
817 influence sperm function: a retrospective view for livestock. *J. Anim. Sci.* **89**, 1315–22.
818
819 Kim, M., Cho, A., Lim, H.S., Hong, S.G., Kim, J.H., Lee, J., Choi, T., Ahn, T.S., and Kim, O.S. (2015).
820 Highly heterogeneous soil bacterial communities around Terra Nova Bay of Northern Victoria
821 Land, Antarctica. *PLoS One* **10**, e0119966.

822

823 Kim, D.K., Kang, B., Kim, O.Y., Choi, D.S., Lee, J., Kim, S.R., Go, G., Yoon, Y.J., Kim, J.H., Jang, S.C.,
824 Park, K.S., Choi, E.J., Kim, K.P., Desiderio, D.M., Kim, Y.K., Lötvall, J., Hwang, D., and Gho, Y.S.

825 (2013). EVpedia: an integrated database of high-throughput data for systemic analyses of
826 extracellular vesicles. *J. Extracell. Vesicles.* **2**, doi: 10.3402/jev.v2i0.20384.

827

828 Kropp, J., and Khatib, H. (2015). Characterization of microRNA in bovine in vitro culture media
829 associated with embryo quality and development. *J. Dairy Sci.* **98**, 6552–63.
830

831 Kropp, J., Salih, S. M., and Khatib, H. (2014). Expression of microRNAs in bovine and human pre-
832 implantation embryo culture media. *Front. Genet.* **5**, 91.

833

834 Lai, R. C., Chen, T. S. and Lim, S. K. (2011). Mesenchymal stem cell exosome: a novel stem cell-
835 based therapy for cardiovascular disease. *Regen. Med.* **6**, 481–492.

836

837 Lazzari, G., Colleoni, S., Lagutina, I., Crotti, G., Turini, P., Tessaro, I,, Brunetti, D., Duchi, R., and
838 Galli, C. (2010). Short-term and long-term effects of embryo culture in the surrogate sheep
839 oviduct versus in vitro culture for different domestic species. *Theriogenology* **73**,748–57.
840

841 Lee, K.F., and Yeung, W.S.(2006). Gamete/embryo - oviduct interactions: implications on in vitro
842 culture. *Hum. Fertil. (Camb).* 9, 137–43.

843

844 Lee, Y., El Andaloussi, S., and Wood, M. J. A. (2012). Exosomes and microvesicles: extracellular
845 vesicles for genetic information transfer and gene therapy. *Hum. Mol. Genet.* **21**, 125–134.

846

847 Lee, Y.L., Lee, K.F., Xu, J.S., He, Q.Y., Chiu, J.F., Lee, W.M., Luk, J.M., Yeung, W.S. (2004) The
848 embryotrophic activity of oviductal cell-derived complement C3b and iC3b, a novel function of
849 complement protein in reproduction. *J. Biol. Chem.* **279**, 12763–8.

850

851 Lee, Y.L., Lee, K.F., Xu, J.S., Wang, Y.L., Tsao, S.W., and Yeung, W.S. (2001). Establishment and
852 characterization of an immortalized human oviductal cell line. *Mol. Reprod. Dev.* **59**, 400–9.

853

854 Leese, H.J. (1988). The formation and function of oviduct fluid. *J. Reprod. Fertil.* **82**, 843–56.

855

856 Leese, H.J., Hugentobler, S.A., Gray, S.M., Morris, D.G., Sturmey, R.G., Whitear, S.L., and Sreenan,
857 J.M. (2008). Female reproductive tract fluids: composition, mechanism of formation and
858 potential role in the developmental origins of health and disease. *Reprod. Fertil. Dev.* **20**, 1–8.
859

860 Legge, M. (1995). Oocyte and zygote zona pellucida permeability to macromolecules. *J. Exp. Zool*.
861 **271**, 145–50.

862

863 Lenassi, M., Cagney, G., Liao, M., Vaupotic, T., Bartholomeeusen, K., Cheng, Y., Krogan, N.J.,
864 Plemenitas, A., and Peterlin, B.M. (2010). HIV Nef is secreted in exosomes and triggers apoptosis
865 in bystander CD4 T cells. *Traffic* **11**, 110–122.

866

867 Li, G.P., Bunch, T.D., White, K.L., Aston, K.I., Meerdo, L.N., Pate, B.J., and Sessions, B.R. (2004a)
868 Development, chromosomal composition, and cell allocation of bovine cloned blastocyst derived
869 from chemically assisted enucleation and cultured in conditioned media. *Mol. Reprod. Dev.* 68,
870 189–97.

871

872

873 Li, G.P., White, K.L., Aston, K.I., Meerdo, L.N., and Bunch, T.D. (2004b). Conditioned medium
874 increases the polyploid cell composition of bovine somatic cell nuclear-transferred blastocysts.
875 *Reproduction* **127**, 221–8.

876

877 Liu, J.L., Wang, M.K., Sun, Q.Y., Zhang, X.R., Jiang, L.K., and Chen, D.Y. (2001). Refrigeration of
878 donor cells in preparation for bovine somatic nuclear transfer. *Reprod.* **122**, 801–808.

879

880 Lloyd, A., Pratt, K., Siebrasse, E., Moran, M.D., and Duina, A.A. (2009). Uncoupling of the patterns
881 of chromatin association of different transcription elongation factors by a histone H3 mutant in
882 Saccharomyces cerevisiae. *Eukaryot Cell* **8**, 257–60.
883

884 Lobb, R.J., Becker, M., Wen, S.W., Wong, C.S., Wiegmans, A.P., Leimgruber, A., and Möller, A.
885 (2015). Optimized exosome isolation protocol for cell culture supernatant and human plasma. *J.*
886 *Extracell. Vesicles* **4**, 27031.

887

888 Lopera-Vasquez, R., Hamdi, M., Fernandez-Fuertes, B., Maillo, V., Beltrán-Breña, P., Calle, A.,
889 Redruello, A., López-Martín, S., Gutierrez-Adán, A., Yañez-Mó, M., Ramirez, M.Á., and Rizos, D.
890 (2016a). Extracellular Vesicles from BOEC in In Vitro Embryo Development and Quality. *PLOS ONE*
891 **11**, e0148083.
892

893 Lopera-Vasquez, R., Hamdi, M., Maillo, V., Lloreda, V., Coy, P., Gutierrez-Adan, A., Bermejo-
894 Alvarez, P., and Rizos, D. (2015). Effect of bovine oviductal fluid on development and quality of
895 bovine embryos produced in vitro. *Reprod. Fertil. Dev.* doi: 10.1071/RD15238.
896
897 Lopera-Vasquez, R., Hamdi, M., Maillo, V., Nunez, C., Yanez-Mo, M., Ramirez, M.A., Gutierrez-
898 Adan, A., Bermejo-Alvarez, P., and Rizos, D. (2016b). Extracellular Vesicles of bovine oviductal
899 fluid modify the gene expression on bovine in vitro-derived embryos. *Reprod. Fertil. Dev.* **28**, 179.
900
901 Machtinger, R., Laurent, L.C., and Baccarelli, A.A. (2015). Extracellular vesicles: roles in gamete
902 maturation, fertilization and embryo implantation. *Hum. Reprod. Update* **22**, 182–93.
903
904 Mantel, P.Y., and Marti, M. (2014). The role of extracellular vesicles in Plasmodium and other
905 protozoan parasites. *Cell Microbiol.* **16**, 344–54.
906
907 Marzesco, A.M., Janich, P., Wilsch-Bräuninger, M., Dubreuil, V., Langenfeld, K., Corbeil, D.,
908 Huttner, W.B. (2005). Release of extracellular membrane particles carrying the stem cell marker
909 prominin-1 (CD133) from neural progenitors and other epithelial cells. *J. Cell Sci.* **118**, 2849–2858.
910 Menezo, Y., Veiga, A., and Benkhalifa, M. (1998). Improved methods for blastocyst formation and
911 culture. *Hum Reprod* **13**, 256–65.
912
913 Mermillod, P., Vansteenbrugge, A., Wils, C., Mourmeaux, J.L., Massip, A., and Dessy, F. (1993).
914 Characterization of the embryotrophic activity of exogenous protein-free oviduct-conditioned
915 medium used in culture of cattle embryos. *Biol. Reprod*. **49**, 582–587.
916
917 Mittal, N., and Voldman, J. (2011). Nonmitogenic survival-enhancing autocrine factors including
918 cyclophilin A contribute to density-dependent mouse embryonic stem cell growth. *Stem Cell Res*.
919 **6**, 168–76.
920
921 Mondejar, I., Martinez-Martinez, I., Avilés, M., and Coy, P. (2013). Identification of potential
922 oviductal factors responsible for zona pellucida hardening and monospermy during fertilization
923 in mammals. *Biol. Reprod.* **89**, 67.
924
925 Morel, E., Fouquet, S., Chateau, D., Yvernault, L., Frobert, Y., Pincon-Raymond, M., Chambaz, J.,
926 Pillot, T., and Rousset, M. (2004). The cellular prion protein PrPc is expressed in human
927 enterocytes in cell-cell junctional domains. *J. Biol. Chem.* **279**, 1499–505.
928

929 Motta, P. M., Familiari, G., Nottola, S. A., Micara, G., and Aragona, C. (1991). Microstructural
930 events of human egg investments during in vitro fertilization. Ultrastructure of the zona pellucida
931 and cumulus oophorus. *Bull Assoc. Anat. (Nancy).* ***75***, 89–91.

932

933 Müller, G. (2012). Novel tools study cell type-specific exosomes microvesicles. *J. Bioanal. Biomed.*
934 **4**, 46–60.

935

936 Ng, Y.H., Rome, S., Jalabert, A., Forterre, A., Singh, H., Hincks, C.L., and Salamonsen, L.A. (2013).
937 Endometrial exosomes/microvesicles in the uterine microenvironment: a new paradigm for
938 embryo-endometrial cross talk at implantation. *PLoS One* **8**, e58502.

939

940 Nomura F, Akashi S, Sakao Y, Sato S, Kawai T, Matsumoto M, Nakanishi K, Kimoto M, Miyake K,
941 Takeda K, Akira S. (200). Cutting edge: endotoxin tolerance in mouse peritoneal macrophages
942 correlates with down-regulation of surface toll-like receptor 4 expression. *J. Immunol.* **164**, 3476–
943 9.

944 O'Doherty, E.M., Wade, M.G., Hill, J.L., and Boland, M.P. (1997). Effects of culturing bovine
945 oocytes either singly or in groups on development to blastocysts. *Theriogenology* **48**, 161–169.

946

947 Oliveira, D.L., Freire-De-Lima, C.G., Nosanchuk, J.D., Casadevall, A., Rodrigues, M.L., and
948 Nimrichter, L. (2010a). Extracellular vesicles from Cryptococcus neoformans modulate
949 macrophage functions. *Infect. Immun.* **78**, 1601–1609.

950

951 O'Neill, C. (2008). The potential roles for embryotrophic ligands in preimplantation embryo
952 development. *Hum. Reprod. Update*. **14**, 275–88.

953

954 Pan, B.T., and Johnstone, R. M. (1983). Fate of the transferrin receptor during maturation of
955 sheep reticulocytes *in vitro*: selective externalization of the receptor. *Cell* **33**, 967–978.

956

957 Pan, B.T., Teng, K., Wu, C., Adam, M., and Johnstone, R.M. (1985). Electron microscopic evidence
958 for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. *J. Cell Biol.*
959 **101**, 942–948.

960

961 Paria, B.C., and Dey, S.K. (1990). Preimplantation embryo development in vitro: cooperative
962 interactions among embryos and role of growth factors. *Proc. Natl. Acad. Sci.* **87**, 4756–60.

963

964 Parolini, I., Federici, C., Raggi, C., Lugini, L., Palleschi, S., De Milito, A., Coscia, C., Iessi, E., Logozzi,
965 M., Molinari, A., Colone, M., Tatti, M., Sargiacomo, M., and Fais, S. (2009). Microenvironmental
966 pH is a key factor for exosome traffic in tumor cells. *J. Biol. Chem.* **284**, 34211–22.

967

968 Piehl, L.L., Fischman, M.L., Hellman, U., Cisale, H., Miranda, P.V. (2013). Boar seminal plasma
969 exosomes: effect on sperm function and protein identification by sequencing. *Theriogenology* **79**,
970 1071–1082

971

972 Potolicchio, I., Carven, G.J., Xu, X., Stipp, C., Riese, R.J., Stern, L.J., and Santambrogio, L. (2005).
973 Proteomic analysis of microglia-derived exosomes: metabolic role of the aminopeptidase CD13
974 in neuropeptide catabolism. *J. Immunol.* **175**, 2237–2243.

975

976 Raposo, G., and Stoorvogel, W. (2013). Extracellular vesicles: exosomes, microvesicles, and
977 friends. *J Cell Biol.* **200**, 373–383.

978

979 Raposo, G., Nijman, H.W., Stoorvogel, W., Liejendekker, R., Harding, C.V., Melief, C.J.,and Geuze,
980 H.J. (1996). B lymphocytes secrete antigen-presenting vesicles. *J. Exp. Med.* **183**, 1161–1172.

981

982 Ratajczak, J., Miekus, K., Kucia, M., Zhang, J., Reca, R., Dvorak, P., and Ratajczak, M.Z. (2006)
983 Embryonic stem cell-derived microvesicles reprogram hematopoietic progenitors: evidence for
984 horizontal transfer of mRNA and protein delivery. *Leukemia* **20**, 847–856.

985

986 Ratajczak, M.Z., Kucia, M., Jadczyk, T., Greco, N.J., Wojakowski, W., Tendera, M., and Ratajczak,
987 J. (2012). Pivotal role of paracrine effects in stem cell therapies in regenerative medicine: can we
988 translate stem cell-secreted paracrine factors and microvesicles into better therapeutic
989 strategies? *Leukemia* **26**, 1166–1173.

990

991 Regente, M., Corti-Monzón, G., Maldonado, A.M., Pinedo, M., Jorrín, J., de la Canal, L. (2009).
992 Vesicular fractions of sunflower apoplastic fluids are associated with potential exosome marker
993 proteins. *FEBS. Lett.* **583**, 3363–6.

994

995

996 Rizos, D., Fair, T., Papadopoulos, S., Boland, M.P., and Lonergan, P. (2002). Developmental,
997 qualitative, and ultrastructural differences between ovine and bovine embryos produced in vivo
998 or in vitro. *Mol. Reprod. Dev*. **62**, 320–7.

999

1000 Rizos, D., Clemente, M., Bermejo-Alvarez, P., de La Fuente, J., Lonergan, P., and Gutiérrez-Adán,
1001 A. (2008). Consequences of in vitro culture conditions on embryo development and quality.
1002 *Reprod. Domest. Anim*. 43, 44–50.

1003

1004 Rizos, D., Carter, F., Besenfelder, U., Havlicek, V., and Lonergan, P. (2010). Contribution of the
1005 female reproductive tract to low fertility in postpartum lactating dairy cows. *J. Dairy Sci*. **93**,
1006 1022–9.

1007

1008 Rosenbluth, E.M., Shelton, D.N., Wells, L.M., Sparks, A.E., and Van Voorhis, B.J. (2014). Human
1009 embryos secrete microRNAs into culture media--a potential biomarker for implantation. *Fertil.*
1010 *Steril.* **101**, 1493–500.

1011

1012 Rottmayer, R., Ulbrich, S.E., Kölle, S., Prelle, K., Neumueller, C., Sinowatz, F., Meyer, H.H., Wolf,
1013 E., and Hiendleder, S. (2006). A bovine oviduct epithelial cell suspension culture system suitable
1014 for studying embryo-maternal interactions: morphological and functional characterization.
1015 *Reproduction* **132**, 637–48.

1016

1017 Rozmyslowicz, T., Majka, M., Kijowski, J., Murphy, S.L., Conover, D.O., Poncz, M., Ratajczak, J.,
1018 Gaulton, G.N., and Ratajczak, M.Z. (2003). Platelet- and megakaryocyte-derived microparticles
1019 transfer CXCR4 receptor to CXCR4-null cells and make them susceptible to infection by X4-HIV.
1020 *AIDS*. **17**, 33–42.

1021 Ruiz-Gonzalez, I., Xu, J., Wang, X., Burghardt, R.C., Dunlap, K., and Bazer, F.W. (2015). Exosomes,
1022 endogenous retroviruses and toll-like receptors: pregnancy recognition in ewes. *Reproduction*
1023 **149**, 281–291.

1024

1025 Rustom, A., Saffrich, R., Markovic, I., Walther, P., and Gerdes, H.H. (2004). Nanotubular highways
1026 for intercellular organelle transport. *Science* **303**, 1007–1010.

1027

1028 Saadeldin, I. M., Kim, S. J., Choi, Y. B., and Lee, B. C. (2014). Improvement of Cloned Embryos
1029 Development by Co-Culturing with Parthenotes: A Possible Role of Exosomes/Microvesicles for
1030 Embryos Paracrine Communication. *Cellular Reprogramming **16***, 223–234.

1031

1032 Sagirkaya, H., Misirlioglu, M., Kaya, A., First, N.L., Parrish, J.J., and Memili, E. (2007).
1033 Developmental potential of bovine oocytes cultured in different maturation and culture
1034 conditions. *Anim. Reprod. Sci.* **101**, 225–40.

1035

1036 Savina, A., Fader, C.M., Damiani, M.T., Colombo, M.I. (2005) Rab11 promotes docking and
1037 fusion of multivesicular bodies in a calcium-dependent manner. *Traffic* **6**, 131-43.
1038
1039 Schmaltz-Panneau B., Cordova A., Dhorne-Pollet S., Hennequet-Antier C., Uzbekova S., Martinot
1040 E., Doret S., Martin P., Mermillod P., and Locatelli Y. (2014). Early bovine embryos regulate
1041 oviduct epithelial cell gene expression during in vitro co-culture. *Anim. Reprod. Sci*. **149**, 103–116.

1042

1043 Schmaltz-Panneau, B., Locatelli, Y., Uzbekova, S., Perreau, C., and Mermillod, P. (2015). Bovine
1044 Oviduct Epithelial Cells Dedifferentiate Partly in Culture, While Maintaining their Ability to
1045 Improve Early Embryo Development Rate and Quality. *Reprod. Domest. Anim.* **50**, 719–29.

1046

1047 Savina, A., Furlan, M., Vidal, M., and Caolombo, M.I. (2003). Exosome release is regulated by a
1048 calcium-dependent mechanism in K562 cells. *J. Biol. Chem*. **278**, 20083–20090.

1049

1050 Skog, J., Wurdinger, T., van Rijn, S., Meijer, D., Gainche, L., Sena-Esteves, M., and Breakefield, X.
1051 O. (2008). Glioblastoma microvesicles transport RNA and protein that promote tumor growth
1052 and provide diagnostic biomarkers. *Nat Cell Biol.* **10**, 1470–1476.

1053

1054

1055 Sohel, M.M.H., Hoelker, M., Noferesti, S.S., Salilew-Wondim, D., Tholen, E., Looft, C., Rings, F.,
1056 Uddin, M.J., Spencer, T.E., Schellander, K., and Tesfaye, D. (2013). Exosomal and Non-Exosomal
1057 Transport of Extra-Cellular microRNAs in Follicular Fluid: Implications for Bovine Oocyte
1058 Developmental Competence. *PLoS ONE* **8**, e78505.

1059

1060 Sostaric, E., Aalberts, M., Gadella, B.M., and Stout, T.A. (2008). The roles of the epididymis and
1061 prostasomes in the attainment of fertilizing capacity by stallion sperm. *Anim. Reprod. Sci*. **107**,
1062 237–48.

1063

1064 Sostaric, E., Aalberts, M., Gadella, B.M., and Stout, T.A. (2008). The roles of the epididymis and 1065 prostasomes in the attainment of fertilizing capacity by stallion sperm. *Anim. Reprod. Sci.* **107**, 1066 237–48.

1067

1068 Stepniak, E., Radice, G.L., and Vasioukhin, V.(2009). Adhesive and Signaling Functions of 1069 Cadherins and Catenins in Vertebrate Development. *Cold Spring Harb. Perspect. Biol*. **1**, 002949.

1070

1071 Sunkara, V, Woo, H.K., and Cho, Y.K. (2016). Emerging techniques in the isolation and 1072 characterization of extracellular vesicles and their roles in cancer diagnostics and prognostics. 1073 Analyst **141**, 371–381.

1074

1075 Tauro, B.J., Greening, D.W., Mathias, R.A., Ji, H., Mathivanan, S., Scott, A.M., and Simpson, R.J. 1076 (2012). Comparison of ultracentrifugation, density gradient separation, and immunoaffinity 1077 capture methods for isolating human colon cancer cell line LIM1863-derived exosomes. *Methods* 1078 **56**, 293–304.

1079

1080 Taylor, D.D., and Gerçel-Taylor, C. (2005). Tumour-derived exosomes and their role in cancer-1081 associated T-cell signalling defects. *Br. J. Cancer* **92**, 305–311.

1082

1083 Taylor, D.D., Lyons, K.S., and Gerçel-Taylor, C. (2002). Shed membrane fragment-associated 1084 markers for endometrial and ovarian cancers. *Gynecol Oncol* **84**, 443–448, 2002.

1085

1086 Théry, C., Amigorena, S., Raposo, G., and Clayton. A. (2006). Isolation and characterization of 1087 exosomes from cell culture supernatants and biological fluids. *Curr. Protoc. Cell Biol*. Chapter **3**, 1088 Unit 3.22.

1089

1090 Théry, C., Boussac, M., Véron, P., Ricciardi-Castagnoli, P., Raposo, G., Garin, J., and Amigorena, S. 1091 (2001). Proteomic analysis of dendritic cell-derived exosomes: a secreted subcellular 1092 compartment distinct from apoptotic vesicles. *J. Immunol..* ***166***, 7309–7318.

1093

1094

1095 Théry, C., Ostrowski, M., and Segura, E. (2009). Membrane vesicles as conveyors of immune
1096 responses. *Nat. Rev. Immunol.* **9**, 581–593.

1097

1098 Théry, C., Regnault, A., Garin, J., Wolfers, J., Zitvogel, L., Ricciardi-Castagnoli, P., Raposo G., and
1099 Amigorena S. (1999). Molecular characterization of dendritic cell-derived exosomes. Selective
1100 accumulation of the heat shock protein hsc73. J. *Cell Biol.* **147**, 599–610.

1101

1102 Thibodeaux, J K., Myers, M W., Goodeaux, L.L. Menezo, Y., Roussel, J.D., Broussard, J.R., and
1103 Godke, R.A. (1992). Evaluating an in vitro culture system of bovine utenne and oviduct epithelial
1104 cells for subsequent embryo co-culture *Reprod. Fertil. Dev.* **4**, 573–583.

1105

1106

1107 Timmers, L., Lim, S.K., Arslan, F., Armstrong, J.S., Hoefer, I.E., Doevendans, P.A., Piek, J.J., El
1108 Oakley, R.M., Choo, A., Lee, C.N., Pasterkamp, G., and de Kleijn, D.P.(2007). Reduction of
1109 myocardial infarct size by human mesenchymal stem cell conditioned medium. *Stem Cell Res.* **1**,
1110 129–137.

1111

1112 Timmers, L., Lim, S.K., Hoefer, I.E., Arslan, F., Lai, R.C., van Oorschot, A.A., Goumans, M.J., Strijder,
1113 C., Sze, S.K., Choo, A., Piek, J.J., Doevendans, P.A., Pasterkamp, G., and de Kleijn, D.P. (2011).
1114 Human mesenchymal stem cell-conditioned medium improves cardiac function following
1115 myocardial infarction. *Stem Cell Res.* **6**, 206–214.

1116

1117 Trajkovic, K., Hsu, C., Chiantia, S., Rajendran, L., Wenzel, D., Wieland, F., Schwille, P., Brügger, B.,
1118 and Simons, M. (2008). Ceramide triggers budding of exosome vesicles into multivesicular
1119 endosomes. *Science* **319**, 1244–7.

1120

1121 Trams, E.G., Lauter, C.J., Salem, N. Jr., and Heine, U. (1981). Exfoliation of membrane ecto-
1122 enzymes in the form of micro-vesicles. *Biochim. Biophys. Acta.* **645**, 63–70.

1123

1124 Turiák, L., Misják, P., Szabó, T.G., Aradi, B., Pálóczi, K., Ozohanics, O., Drahos, L., Kittel, A., Falus,
1125 A., Buzás, E.I., and Vékey, K. (2011). Proteomic characterization of thymocyte-derived
1126 microvesicles and apoptotic bodies in BALB/c mice. *J. Proteomics* **74**, 2025–2033.

1127

1128

1129 Turner, K., and Horobin, R.W. (1997). Permeability of the mouse zona pellucida: a structure-
1130 staining-correlation model using coloured probes. *J. Reprod. Fertil.* **111**, 259–65.

1131

1132 Ulbrich, S.E., Zitta, K., Hiendleder, S., and Wolf, E. (2010). In vitro systems for intercepting early
1133 embryo-maternal cross-talk in the bovine oviduct. *Theriogenology.* **73**, 802–16.

1134

1135 Vajta, G., Peura, T.T., Holm, P., Páldi, A., Greve, T., Trounson, A.O., and Callesen, H. (2000). New
1136 method for culture of zona-included or zona-free embryos: the Well of the Well (WOW) system.
1137 *Mol. Reprod. Dev.* **55**, 256–64.

1138

1139 Van Deun, J., Mestdagh, P., Sormunen, R., Cocquyt, V., Vermaelen, K., Vandesompele, J., Bracke,
1140 M., De Wever, O., and Hendrix, A. (2014). The impact of disparate isolation methods for
1141 extracellular vesicles on downstream RNA profiling. *J. Extracell. Vesicles* **3**, doi:
1142 10.3402/jev.v3.24858.

1143

1144 van der Wijk, T., Tomassen, S.F., Houtsmuller, A.B., de Jonge, H.R., and Tilly, B.C. (2003). Increased
1145 vesicle recycling in response to osmotic cell swelling. Cause and consequence of hypotonicity-
1146 provoked ATP release. *J. Biol. Chem.* **278**, 40020–5.

1147

1148 Van Langendonckt. A, Vansteenbrugge, A., Donnay, I., Van Soom, A., Berg, U., Semple, E., Grisart,
1149 B., Mermillod, P., Brem, G., Massip, A., and Dessy, F. (1996). Three year results of in vitro
1150 production of bovine embryos in serum-poor bovine oviduct conditioned medium. An overview.
1151 *Reprod. Nutr. Dev.* **36**, 493–502.

1152

1153 Van Soom, A., Ysebaert, M.T., Vanhoucke De Medts, A., Vande Velde, A., Merton, S., Delval, A.,
1154 Van Langendonckt, A., Donnay, I., Vanroose, G., Bols, P.E.J., and de Kruif, A. (1996). Sucrose-
1155 induced shrinkage of in vitro produced bovine morulae: effect on viability, morphology and ease
1156 of evaluation. *Theriogenology* **46**, 1131–1147.

1157

1158 Van Soom, A., Ysebaert, M.T, and de Kruif, A. (1997). Relationship between timing of
1159 development, morula morphology and cell allocation to inner cell mass and trophectoderm in in
1160 vitro produced bovine embryos. *Mol. Reprod. Dev.* **47**, 47–56.

1161

1162 Van Soom, A., Wrathall, A.E., Herrler, A., and Nauwynck, H.J. (2010). Is the zona pellucida an
1163 efficient barrier to viral infection?. *Reprod. Fertil. Dev.* **22**, 21–31.

1164

1165 Vanroose, G., de Kruif, A., and Van Soom, A. (2000). Embryonic mortality and embryo-pathogen
1166 interactions. *Anim. Reprod. Sci*. **60-61**, 131–43.

1167

1168 Vidulescu, C., Clejan, S., O'connor, K.C. (2004). Vesicle traffic through intercellular bridges in DU
1169 145 human prostate cancer cells. *J. Cell Mol. Med.* **8**, 388–396.

1170

1171 Vlassov, A.V., Magdaleno, S., Setterquist, R. and Conrad, R. (2012). Exosomes: Current knowledge
1172 of their composition, biological functions, and diagnostic and therapeutic potentials. *Biochim.*
1173 *Biophys. Acta.* **1820**, 940–948.

1174

1175 Witwer, K.W., Sarbanes, S.L., Liu J., and Clements, J.E. (2011). A plasma microRNA signature of
1176 acute lentiviral infection: biomarkers of CNS disease. *AIDS* **204**, 1104–1114.

1177

1178 Witwer, K.W., Buzás, E.I., Bemis, L.T., Bora, A., Lässer, C., Lötvall, J., Nolte-'t Hoen EN, Piper MG,
1179 Sivaraman S, Skog J, Théry, C., Wauben, M.H., and Hochberg, F. (2013). Standardization of sample
1180 collection, isolation and analysis methods in extracellular vesicle research. *J. Extracell. Vesicles* **2**,
1181 20360.

1182

1183 Wolf, P. (1967). The nature and significance of platelet products in human plasma. *Br. J.*
1184 *Haematol.* **13**, 269–288.

1185

1186 Wydooghe, E., Vandaele, L., and Van Soom, A. (2013). Autocrine communication between bovine
1187 embryos cultured in Primo Vision dishes® outweighs possible negative influences of bad
1188 embryos. *Reprod. Fertil. Dev.* **26**, 115–115.

1189

1190 Wydooghe, E., Vandaele, L., Heras, S., De Sutter, P., Deforce, D., Peelman, L., De Schauwer, C.,
1191 and Van Soom, A. (2015). Autocrine embryotropins revisited: how do embryos communicate with
1192 each other in vitro when cultured in groups?. *Biol. Rev. Camb. Philos. Soc*. doi:
1193 10.1111/brv.12241.

1194

1195 Wydooghe, E., Vandaele, L., Piepers, S., Dewulf, J., Van den Abbeel, E., De Sutter, P., and Van
1196 Soom, A. (2014b). Individual commitment to a group effect: strengths and weaknesses of bovine
1197 embryo group culture. *Reproduction* **148**, 519–29.

1198

1199 Xu, J.S., Cheung, T.M., Chan, S.T.H., Ho, P.C., and Yeung, W.S.B. (2001). Temporal effect of human
1200 oviductal cell and its derived embryotrophic factors on mouse embryo development. *Biol.*
1201 *Reprod.* **65**, 1481–1488.

1202

1203 Yáñez-Mó, M., Siljander, P.R., Andreu, Z., Zavec, A.B., Borràs, F.E., Buzas, E.I., Buzas, K., Casal, E.,
1204 Cappello, F., Carvalho, J., Colás, E., Cordeiro-da Silva, A., Fais, S., Falcon-Perez, J.M., Ghobrial,
1205 I.M., Giebel, B., Gimona, M., Graner, M., Gursel, I., Gursel, M., Heegaard, N.H., Hendrix, A.,
1206 Kierulf, P., Kokubun, K., Kosanovic, M., Kralj-Iglic, V., Krämer-Albers, E.M., Laitinen, S., Lässer, C.,
1207 Lener, T., Ligeti, E., Linē, A., Lipps, G., Llorente, A., Lötvall, J., Manček-Keber, M., Marcilla, A.,
1208 Mittelbrunn, M., Nazarenko, I., Nolte-'t Hoen, E.N., Nyman, T.A., O'Driscoll, L., Olivan, M.,
1209 Oliveira, C., Pállinger, É., Del Portillo, H.A., Reventós, J., Rigau, M., Rohde, E., Sammar, M.,
1210 Sánchez-Madrid, F., Santarém, N., Schallmoser, K., Ostenfeld, M.S., Stoorvogel, W., Stukelj, R.,
1211 Van der Grein, S., Vasconcelos, M.H., Wauben, M.H., and De Wever, O. (2015). Biological
1212 properties of extracellular vesicles and their physiological functions. *J. Extracell. Vesicles* **4**,
1213 27066.

1214

1215 Yuana, Y., Sturk, A., and Nieuwland, R.(2013). Extracellular vesicles in physiological and
1216 pathological conditions. *Blood Rev*. **27**, 31–9.

1217

Table 1. Studies comparing individual and group culture of bovine embryos with SOF as a basic medium, with or without serum supplementation (FCS- fetal calf serum- BSA- bovine serum albumin) *Depending on maturation conditions ; ** D10 ; ND= Not Done

Reference	IVM and IVF conditions	Protein supplement	Individual culture		Group culture	
			Embryo density	Blastocyst % D8	Embryo density	Blastocyst % D8
Carolan *et al.* 1996	Group	10 % FCS (D2)	1:1	0	1:1	32
	Individual	10 % FCS (D2)	1:20	20-35*	1:1	38
Donnay *et al.* 1997	Group	10 % FCS (D2)	1:20	0	1:1	23**
Hagemann *et al.* 1998	Individual	3.2 % BSA	1:10	23	ND	ND
		Idem + 1 µl FCS (D5)	1:10	39		
		Idem + Glutamax	1:10	24		
		Idem + glucose	1:10	24		
Fukui *et al.* 2000	Small group	0.8 % BSA	1:25	17	1:5	22
Goovaerts *et al.* 2009	Group	5 % FCS	1:20	2	1:2	25
Goovaerts *et al*. 2012	Group	5% FCS	1:20	0.4	ND	ND
		5% FCS + cumulus	1:20	40.1		
		5% FCS + ITS	1:20	2.7		
		5% FCS + ITS + BSA	1:20	18.8		
		ITS + BSA	1:20	19.4		

Table 2. Broad classification of extracellular vesicles.

Vesicle Types	Diameter(nm)	Density(g/ml)	Morphology (TEM)	Cellular Origin	Origin	Composition
Exosomes	40–150 [1-4]	1.13–1.19 [1,3]	Rounded [1–3,5]	Most cell types	Endolysosomal pathway, intraluminal budding of multivesicular bodies and fusion of multivesicular body with cell membrane, Plasma membrane, Endosomes [6–9]	mRNA, miRNA, non coding RNAs, most proteins and lipids not unique for exosomes [1,2,5,11–15,26]
Microvesicles	100–1000 [2,14–17]	Unknown	Rounded	Most cell types	Cell surface, outward budding of cell membrane , Plasma membrane	Cytoplasmic proteins and membrane proteins, including receptors[27]
Apoptotic bodies	1000–5000 [13,14,22,23]	1.16–1.28 [14]	Heterogeneous [23]	All cell types	Plasma membrane endoplasmic reticulum [24]	Histones, DNA , nuclear fractions, cell organelles [14,22–25]

[1] Escola *et al*. 1998. [2] Heijnen *et al*. 1999. [3] Raposo *et al*. 1996. [4] Trams *et al*. 1981. [5] André *et al*. 2004. [6] Booth *et al*. 2006. [7] Fang *et al*. 2007. [8] Harding *et al*. 1983. [9] Lenassi *et al*. 2010. [10] Pan *et al*. 1985.[11] Beyer and Pisetsky, 2010. [12] Taylor and Gerçel-Taylor, 2005. [13] Théry *et al*. 2009. [14] Turiák *et al*. 2011. [15] Dragovic *et al*. 2011. [16]György *et al*. 2011a. [17]Wolf, 1967. [18] Allan and Raval, 1983. [19] Crawford, 1971. [20]George *et al*. 1976, 1982. [21]Marzesco *et al*. 2005. [22] Hristov *et al*. 2004. [23]Kerr *et al*. 1972. [24]Bilyy *et al*. 2012. [25] Holmgren *et al*. 1999. [26]Kim et al. 2013. [27] Crescitelli *et al.* 2013.

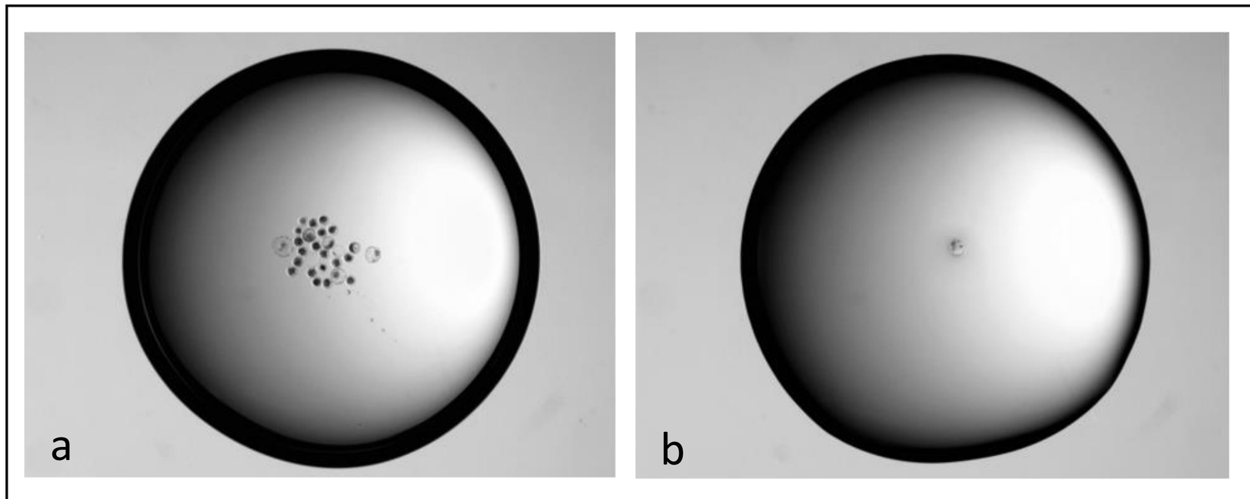

Fig. 1 Droplet of 50 µl medium containing 25 embryos (a) or a single embryo (b). It is obvious that embryonic secretions are diluted in case of single embryo culture.

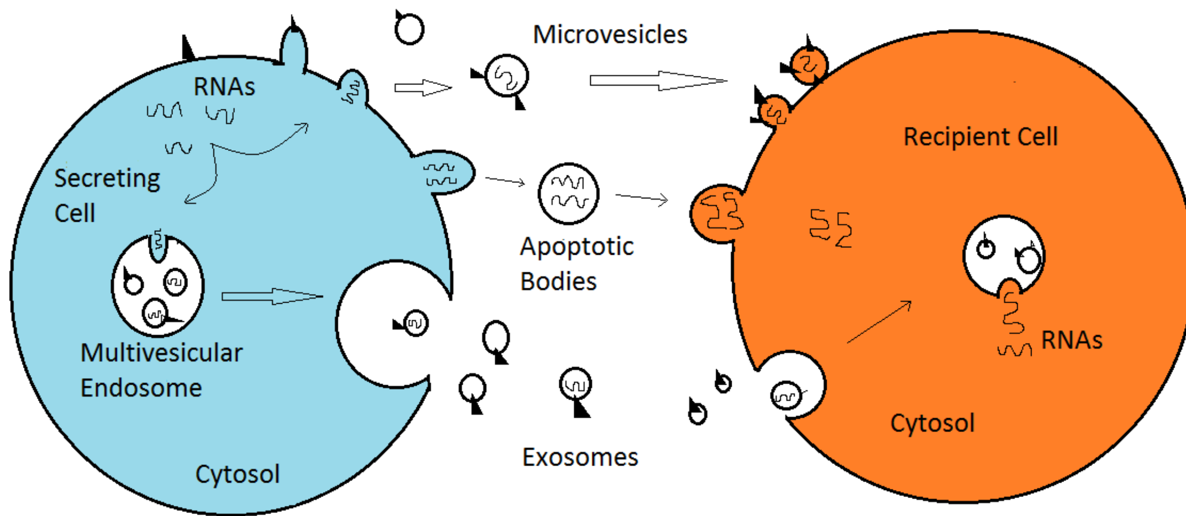

Fig. 2. Schematic illustration of extracellular vesicles: Microvesicles that are considered to be budded off from the surface of secreting cell with surface receptors attached to it, which are attached to other cell finally obtained inside the recipient cell. Exosomes were considered to be secreted by multi vesicular endosomes in which each exosomes are filled with different types of cargo, which were engulfed by the recipient cells. Apoptotic bodies are released from the cells undergoing apoptosis.

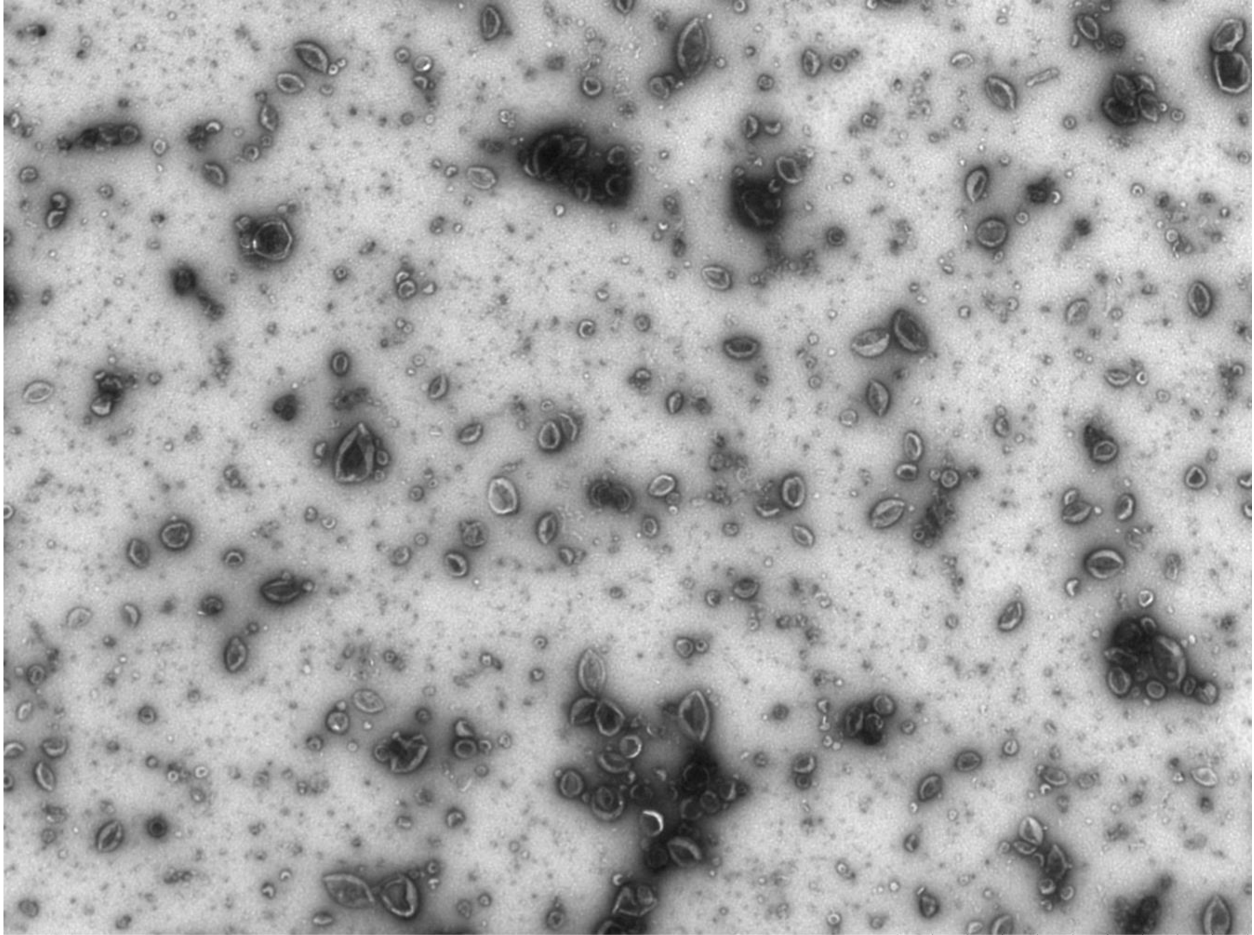

Fig. 3a Oviductal exosomes from *in vivo* origin observed by TEM after ultracentrifugation.

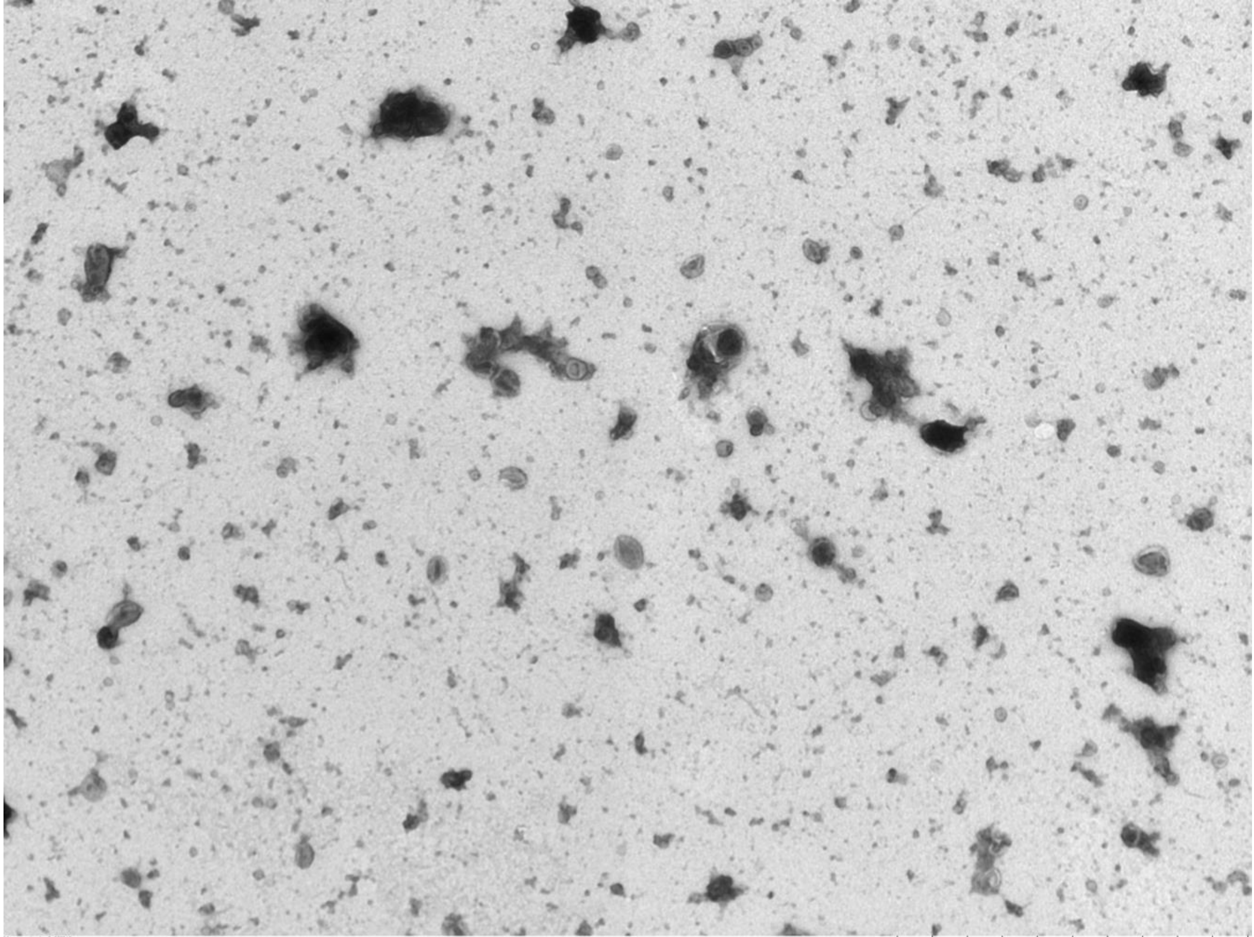

Fig. 3b Oviductal exosomes derived from Bovine Oviduct Epithelial cells (BOEC) cultured *in vitro* as observed by TEM after ultracentrifugation.

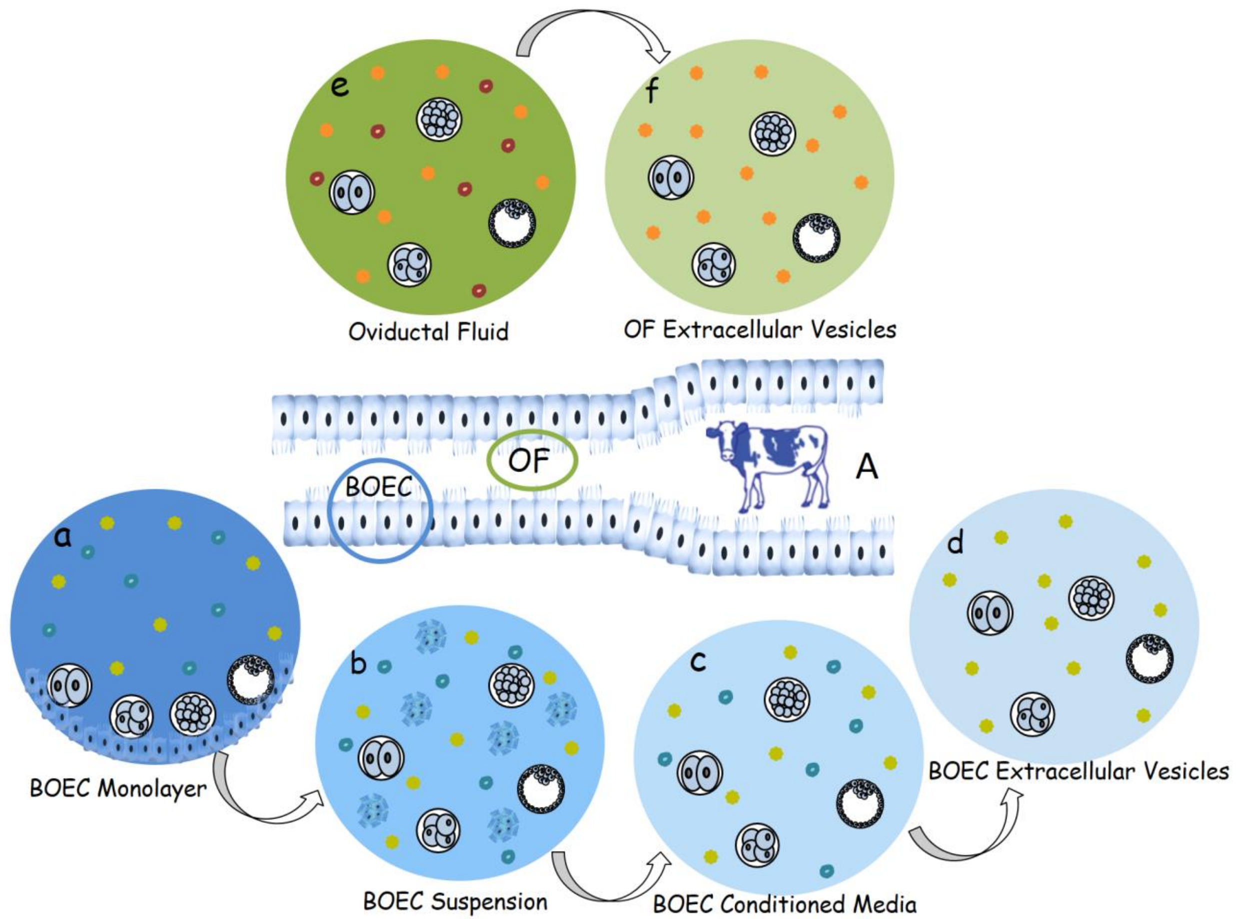

Fig. 4 *In vitro* embryo culture systems using oviduct (A) components in cattle. **(a)** Bovine oviduct epithelial cell (BOEC) monolayer ( ); **(b)** BOEC suspension; **(c)** BOEC conditioned media ( ); **(d)** Extracellular Vesicles purified from BOEC conditioned media ( ); **(e)** Oviduct Fluid (OF) supplementation; **(f)** Extracellular Vesicles purified from OF ( ).

Embryotrophic factors released from BOEC

Proteins, ions, energy substances from OF

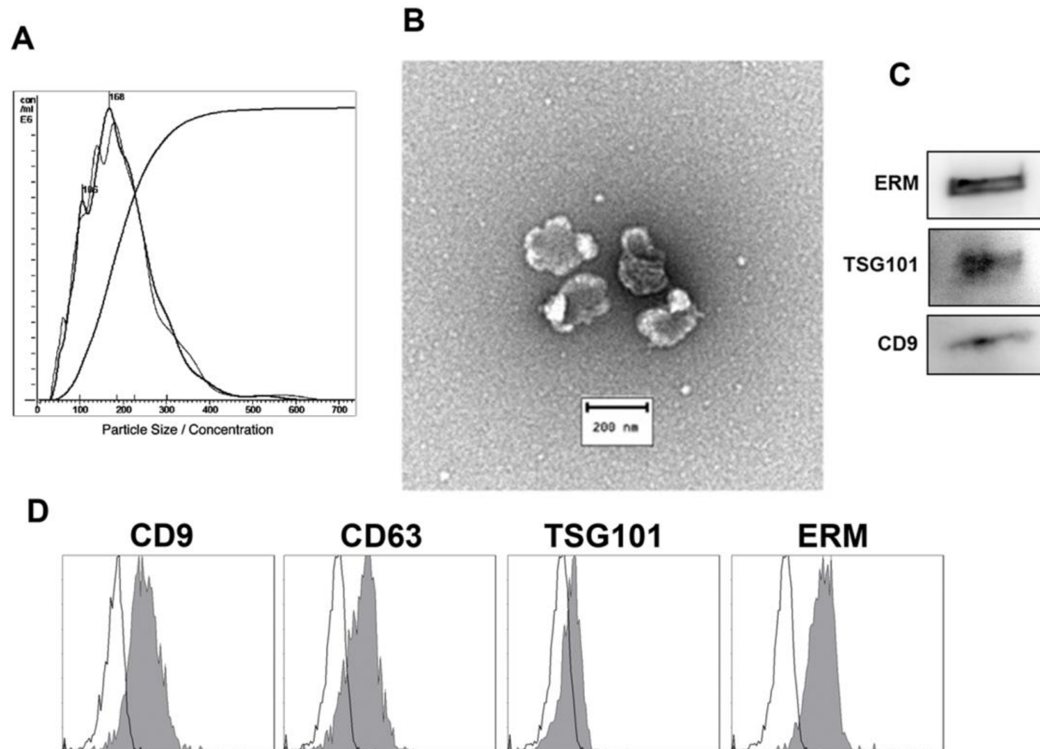

Fig. 5 Characterization of vesicles isolated from BOEC-CM.

A. Nanoparticle tracking analysis (NTA) of a representative EV sample. **B**- Transmission electron microscope image of negative-stained BOEC-EVs. **C-** Western-blot analysis of BOEC-EV lysates with EV markers. **D-** Bead-assisted flow cytometry analysis of EV isolated from BOEC-ECM. EV-coupled beads were stained for CD9, CD63, TSG101 and ERM EV markers. Negative control is depicted as an empty plot. (*Lopera-vasquez et al., Extracellular Vesicles from BOEC in In Vitro Embryo Development and Quality. PLoS One. 2016 Feb 4;11(2):e0148083. doi: 10.1371/journal.pone.0148083.*)

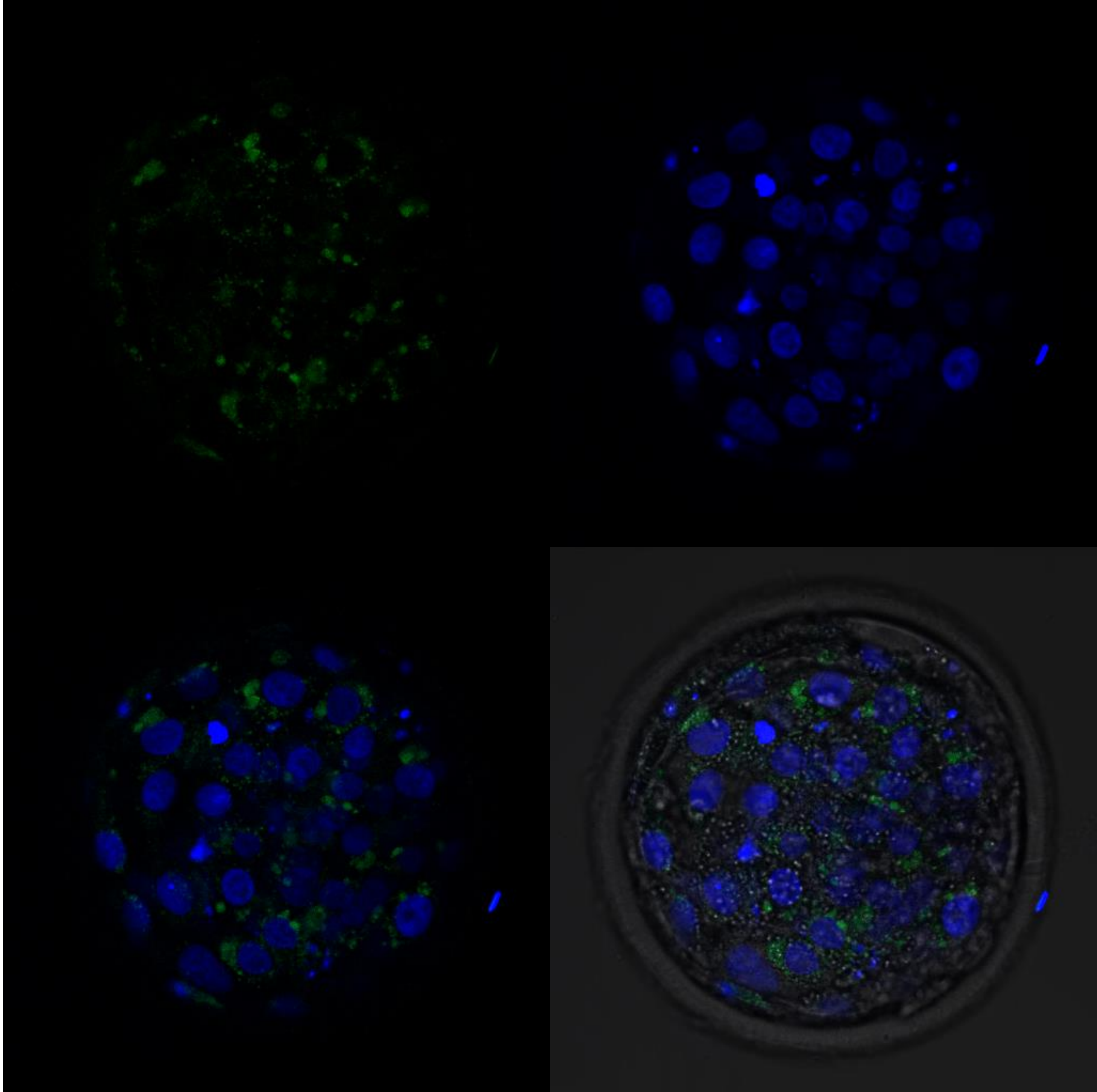

Fig. 6 Oviductal exosomes labelled with green fluorescent dye (PKH67) and internalized by embryos at blastocyst stage after 20h of co-culture. Nuclei are stained by Hoechst 33342.

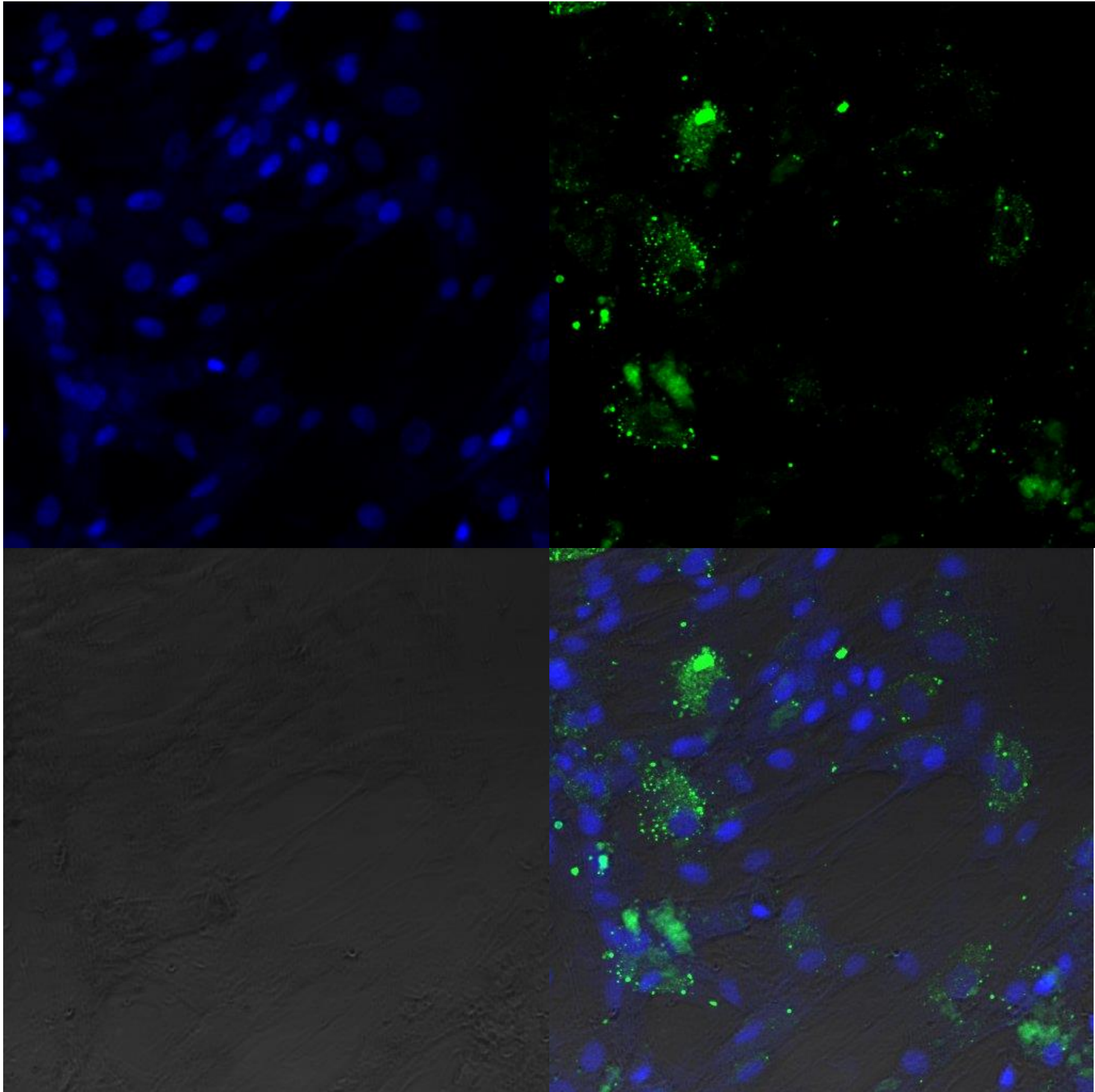

Fig. 7 Exosomes labelled with green fluorescent dye (PKH67) and internalized by partially dedifferentiated BOEC after 24 h of coculture. Nuclei are stained by Hoechst 33342.