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## Closed vitrification system and egg donation: Predictive factors of oocyte survival and pregnancy

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# Title page

**Full title:** Closed vitrification system and egg donation: predictive factors of oocyte survival and pregnancy

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1

2 [Abstract](#)

3         Although many studies have demonstrated the superiority of ultra-fast freezing  
4 compared with slow freezing, the debate is still ongoing concerning the best type of  
5 vitrification method: direct exposure to liquid nitrogen (i.e., open systems), or sterile system  
6 without contact with liquid nitrogen (i.e., closed systems). The aims of this study were to  
7 share our experience on closed vitrification systems in the framework of our egg donation  
8 programme with fully asynchronous cycles, and to identify predictive factors of successful  
9 outcome in this context. Logistic regression analysis indicated that the number of vitrified  
10 oocytes was the only factor predictive of the oocyte survival rate and of clinical pregnancy.  
11 The addition of one vitrified oocyte increased by 15% the odds of oocyte survival. When the  
12 oocyte survival rate was considered as a continuous variable, the following results were  
13 obtained: 7% of clinical pregnancy probability for 50% survival rate, 15% for 75% survival  
14 rate, and 32% for 100% survival rate. The rates of oocyte survival and fertilization, embryo  
15 implantation, and clinical pregnancy were in agreement with the recommended values  
16 established by ALPHA Scientists in Reproductive Medicine in 2012. On the basis of these  
17 results, and according to the European directives on safety, we validate the routine use of  
18 closed oocyte vitrification systems for egg donation programmes. These results must be  
19 confirmed in larger samples before extrapolation to all patient types.

20

21 [Key words](#)

22 Egg donation, oocyte vitrification, closed system

23

## 24 Background

25 To date, few data are available on fully closed vitrification systems. There is an urgent need  
26 to assess efficiency of this systems as oocyte cryoconservation is dramatically increasing for  
27 both donation and fertility preservation.

28

## 29 Introduction

30

31 The use of ultra-fast oocyte freezing has been rapidly expanding in assisted  
32 reproductive technologies (ART). Indeed, the situations in which egg freezing is required are  
33 increasing and diversifying (1). Egg vitrification has always been proposed by ART centres in  
34 the case of sperm collection failure on the day of oocyte retrieval, or as an alternative to  
35 embryo freezing. Some ART centres also propose egg freezing to increase the number of  
36 available oocytes and to optimize the chances of pregnancy for patients considered at risk of  
37 poor response to ovarian stimulation (2,3).

38 In France, the number of patients who benefitted from egg freezing in the framework  
39 of premature infertility was multiplied by three between 2012 and 2015 (202 in 2012 and  
40 784 in 2015), according to the last report by the French Agence de la Biomédecine  
41 (Biomedicine agency) (4). Although not authorized in France yet, the possibility of egg  
42 freezing/storage for women who wish to postpone having a child cannot be fully excluded in  
43 the future due to social changes (Opinion of the French ethics committee, September 2018).

44 Moreover, egg freezing is used to constitute oocyte banks for future egg donations  
45 (i.e., asynchronous donation). Asynchronous donation guarantees the donor anonymity,  
46 simplifies the management of donors and recipients, and optimizes the endometrium

47 preparation. In addition, the French decree of 13 October 2015 (5), which allows nulliparous  
48 women to store part of their oocytes for themselves when they make a donation,  
49 contributes to increasing the indications for egg freezing.

50 Since the first successful birth starting from a vitrified oocyte (6), many articles have  
51 confirmed the superiority of ultra-fast freezing compared with slow freezing (7–9). However,  
52 the discussion is still open on the best freezing system: direct exposure to liquid nitrogen  
53 (i.e., open systems), or sterile system without contact with liquid nitrogen (i.e., closed  
54 systems). There are many literature data on open systems (10,11). Conversely, very little is  
55 known about closed systems.

56 In our ART centre, we have been using a fully sterile freezing system since July 2011,  
57 and our egg donation programme is carried out in a completely asynchronous manner since  
58 May 2012. We became rapidly aware that the outcome varied from one donation to the  
59 other. Some egg warming cycles did not lead to embryo transfer due to fertilization failure,  
60 embryo cleavage defect, or poor embryo quality. Therefore, we decided to assess the  
61 situation by comparing our performance indicators and donation outcome data with those  
62 from the literature.

63 The aims of this study were to share our experience on our fully asynchronous egg  
64 donation programme using a closed vitrification system, and to identify candidate factors  
65 that could predict success in this context.

66

67 [Material and methods](#)

68

69 Retrospective study including the outcome of all egg donation cycles performed at  
70 our ART centre between May 2012 and May 2017. All cycles were carried out  
71 asynchronously using vitrified mature oocytes.

72

### 73 Egg collection from donors

74 The selection criteria for egg donors were: younger than 37 years of age at the  
75 moment of oocyte retrieval and normal ovarian reserve according to the classical evaluation  
76 criteria (FSH, LH, estradiol, antral follicle count, anti-Müllerian hormone).

77 An anonymized number was attributed to each donor during controlled ovarian  
78 stimulation using gonadotropins. On day 6 of stimulation, a gonadotropin-releasing  
79 hormone (GnRH) antagonist was added (Orgalutran, MSD), followed by a GnRH agonist  
80 (Decapeptyl, Ipsen) to trigger final oocyte maturation. Ultrasound-guided transvaginal egg  
81 retrieval was performed 35 hours after triggering. After decoronation, mature oocytes  
82 were transferred in culture medium (G-IVF™ PLUS Vitrolife) for at most 1 hour before  
83 vitrification.

84

### 85 Vitrification and warming procedure

86 Vitrification and warming were performed at room temperature (between 22 and  
87 24°C) using the Vit Kit®-Freeze system and the Vit Kit®-Thaw system (IrvineScientific®,  
88 California), according to the manufacturer's recommendations.

89

### 90 Egg fertilization and embryo culture

91 Egg fertilization was systematically performed by ICSI due to the risk of premature  
92 cortical reaction linked to the freezing process (12). Fertilization was monitored 16-18h after

93 micro-injection. Embryo transfer was performed at day 3 of in vitro culture. Two embryos  
94 were transferred in the absence of medical or obstetric contra-indications.

95 Supernumerary good-quality embryos were frozen by vitrification at day 3 of culture.

96

#### 97 Recipient endometrial preparation

98 Recipients underwent endometrial preparation with oral hormone replacement  
99 therapy that associated 6 mg/day of Provames<sup>®</sup> (estradiol), Toco 500 mg (vitamin E), and  
100 Pentoxifyllin LP 400 up to embryo transfer day. A pelvic ultrasound scan was performed  
101 between day 14 and 18 to determine the endometrial mucosa thickness. If this was higher  
102 than 7.5 mm, intravaginal progesterone supplementation was started (400 mg x 2/day).  
103 Embryo transfer was performed after 4 full days of progesterone treatment. In the case of  
104 pregnancy, the treatment was maintained up to week 12 of amenorrhea. The hormone  
105 replacement therapy was gradually reduced, whatever the cycle outcome.

106

#### 107 Embryo transfer and cycle outcome

108 A first quantitative  $\beta$ -HCG blood test was performed at day 12 post-embryo transfer.  
109 An intravaginal ultrasound scan was performed at week 6 after embryo transfer to confirm  
110 the clinical pregnancy by visualization of the gestational sac.

111

#### 112 Collected data

113 The rates of oocyte survival after warming, of oocyte fertilization, of 4-cell and 8-cell  
114 embryos at day 2 and 3 of in vitro culture respectively, of embryo transfer and implantation,  
115 and of biochemical and clinical pregnancy per transfer and per warming cycle were  
116 collected. The oocyte survival rate corresponded to the number of oocytes with intact

117 cytoplasm after warming. Biochemical pregnancy included all positive  $\beta$ -HCG tests  
118 performed at day 12 post-embryo transfer. Clinical pregnancy included only pregnancies for  
119 which a gestational sac was observed by ultrasound examination. The cumulative pregnancy  
120 rate included the clinical pregnancies obtained after transfer of fresh embryos and of frozen  
121 supernumerary embryos. Rates were per embryo transfer cycle, and also per oocyte  
122 warming cycle to take into account the warming cycles that did not lead to transfer.

123

## 124 [Statistical analysis](#)

125 All statistical analyses were performed with the lme4 package in R (R development  
126 Core Team, R foundation for Statistical Computing, Vienna, Austria, version 3.5.0;  
127 <http://cran.r-project.org>) by using a logistic regression model with random intercepts.  
128 Intercepts for the results of interest (for example, the clinical pregnancy rate) were  
129 permitted to vary among donors and recipients, and predictor variables were treated as  
130 fixed effects. The significance level was set at 0.05 ( $p < 0.05$ ).

131

## 132 [Results](#)

### 133 [Descriptive data](#)

134

135 Table 1 describes the donors' characteristics and Table 2 and shows the overall  
136 results of our donation programme based on oocytes vitrified using a closed system.  
137 During the study period, 54 different recipients (mean age =  $36.4 \pm 5.2$  years) underwent  
138 one or more ART cycles using vitrified oocytes donated by 49 different donors (mean age =  
139  $31.7 \pm 3.2$  years). In total, 88 warming cycles were performed, which corresponded to 406  
140 thawed mature oocytes, and on average,  $4.6 \pm 1.5$  oocytes were used per cycle/recipient.



141 Oocytes from nulliparous donors were not used. Among the 88 warming cycles, 67 (76.1%)  
142 led to embryo transfer. At day 3 of culture, 29 supernumerary embryos were frozen, among  
143 which 9 were subsequently thawed for embryo transfer, and only one led to a clinical  
144 pregnancy. Among the 22 clinical pregnancies, 4 early miscarriages were recorded. In total,  
145 there were 18 deliveries (20 living births because two twin pregnancies).

146 The main cause of embryo transfer annulation (n=21 egg warming cycles) was total  
147 failure of fertilization (n=13), followed by poor embryo quality (n=5), absence of cleavage  
148 (absence of embryo, n=2), and lastly total absence of oocyte survival after warming (n=1)  
149 (Table 3).

150

#### 151 Influence of the donors' characteristics on the oocyte survival and clinical pregnancy rates

152 As the egg survival rate distribution was asymmetric, this information was treated as  
153 a binary variable: survival of 100% of warmed oocytes (coded as 1) and survival of less than  
154 100% of warmed oocytes (coded as 0). The mixed effects logistic regression model indicated  
155 that none of the variables analysed (i.e., donor age and body mass index, cumulative dose of  
156 gonadotropins, number of retrieved oocytes, number of vitrified mature oocytes) was  
157 predictive of oocyte survival post-warming. Nevertheless, the number of vitrified oocytes  
158 was the strongest predictive factor, although it did not reach significance. After adjusting the  
159 model by excluding all the other variables, the number of vitrified oocytes predicted  
160 significantly the survival probability of all oocytes. For each additional vitrified oocyte, the  
161 likelihood that all oocytes would survive was 1.162/1 ( $p=0.00063$ ). In other terms, the  
162 addition of one vitrified oocyte increased by 15% the odds of oocyte survival. However, the  
163 probability that all oocytes would survive by adding one vitrified oocyte (54%) was only  
164 slightly higher than the hazard rate. The probability of 100% survival increased considerably

165 only with the vitrification of 15 oocytes (75%, 2.93/1 versus 40% for 5 oocytes, 0.654/1).  
166 Conversely, no benefit was observed when six instead of five oocytes were vitrified, par  
167 example.

168 This analysis indicated that the probability of 100% survival is lower than 50% when  
169 the number of vitrified oocytes is 7.83. A smaller number reduces the survival chance,  
170 whereas freezing a larger number of oocytes will increase it.

171

172 The same model was used also to investigate the probability of obtaining a clinical  
173 pregnancy in function of the donor's characteristics. As before, the exclusion of additional  
174 variables increased the model adjustment. This suggested that higher oocyte survival rates  
175 increase the likelihood of pregnancy by 1.04/1 (51%) ( $p=0.046$ ). This was a very slight  
176 increase (4% of pregnancies for 1% increase of oocyte survival). When the model was run  
177 using the oocyte survival rate coded as 1 or 0 (i.e., survival of 100% or less than 100% of  
178 warmed oocytes, respectively), clinical pregnancy was 2.96 times (74%) ( $p=0.0806$ ) more  
179 likely in the 100% survival group. In other terms, the pregnancy likelihood increased by  
180 almost 200% if all oocytes survived compared with warming cycles where at least one oocyte  
181 did not survive. Nevertheless, the effect was not statistically significant ( $p>0.05$ ).

182 When the oocyte survival rate was considered as a continuous variable, the following  
183 results were obtained: 7% of clinical pregnancy probability for 50% survival rate (Odds 0.066:  
184 1), 15% for 75% survival rate (Odds 0.175: 1), and 32% for 100% survival rate (Odds 0.461:  
185 1). This prediction was quite different from what obtained by considering the oocyte survival  
186 rate as a binary variable. Also, as the survival rate was very heterogeneous, the predicted  
187 values could not be very precise. In conclusion, the expected pregnancy rate for warming

188 cycles where 100% of oocytes survived varied between 32% and 74% (when oocyte survival  
189 was considered as a binary variable).

190

#### 191 Influence of the recipient's characteristics on the clinical pregnancy rate

192

193 The retained recipient's characteristics were age, number of attributed oocytes, and  
194 number of micro-injected (ICSI) oocytes. The distribution of the attributed and micro-  
195 injected oocytes was quite normal. As the more frequent number of attributed and micro-  
196 injected oocytes was four, the considered variable was «more» or «less» than four  
197 attributed and micro-injected oocytes.

198 In this model, no predictor was significant, possibly due to the small sample. Nevertheless,  
199 the likelihood of pregnancy was slightly higher when the recipient's age increased (these  
200 results is surprising, and could be explained by hazard). Conversely, it was slightly lower  
201 when more than four oocytes were attributed to the recipient. Finally, the likelihood of  
202 pregnancy was much higher when more than four oocytes were micro-injected. However, as  
203 this was the case for only 12 recipients, this result must be taken with caution.

204

#### 205 Influence of the embryo development characteristics on clinical pregnancy

206

207 The likelihood of pregnancy increased proportionally with the oocyte fertilization  
208 rate, but this effect was quite limited for each increase of the raw percentage. The  
209 probability of pregnancy also increased proportionally with the number of 4-cell (day 2) and  
210 8-cell (day 3) embryos (0.57 vs 0.89).

211 Finally, and without surprise, the pregnancy likelihood was much higher (but not  
212 significant) when more than one embryo was transferred (4.13: 1; Odds Ratio= 0.81%)  
213 ( $p=0.094$ ).

214

215

## 216 Discussion

217

### 218 Choice of performance indicators

219 We chose to present our results by following the last Vienna consensus on indicators  
220 for ART laboratories established by the European Society of Human Reproduction and  
221 Embryology (ESHRE) experts and ALPHA Scientists in Reproductive Medicine (13).  
222 Nevertheless, as the minimum performance-level values and the target values were  
223 established for fresh oocytes, they can be used only as guidance for our centre. To assess  
224 our vitrification-specific results, our reference was the consensus on cryopreservation  
225 elaborated by the ALPHA Scientists in Reproductive Medicine in 2012 (14).

226 Concerning oocytes survival after warming, our rate of 82.3 is higher than the 70%  
227 recommended by this group. Our fertilization rate (65.2) is close to the competency value  
228 proposed by the Vienna consensus ( $\geq 65\%$ ). Moreover, according to the consensus on  
229 cryopreservation, the fertilization rate when using cryopreserved oocytes must not be lower  
230 than 10% of the mean fertilization rate of the centre (71% for our ART centre, unpublished  
231 data).

232 Concerning embryo development, we chose as criterion the number of 4-cell and 8-  
233 cell embryos at day 2 and 3 of culture. This parameter indicates whether the culture system  
234 can ensure embryo cleavage according to the expected kinetics and also gives an indication

235 of embryo viability and quality. Moreover, the development stage is the most significant  
236 predictor of pregnancy achievement (15), and has the advantage of being an objective  
237 measure (13). In our egg donation programme, the rates of 4-cell embryos at day 2 and of 8-  
238 cell embryos at day 3 of culture were 46.2 and 30.2% respectively. According to the Vienna  
239 consensus, the respective competency values for fresh oocytes are  $\geq 50\%$  and  $\geq 45\%$ . The  
240 implantation rate after freezing is acceptable if it is not lower than 10-30% of the  
241 implantation rate with fresh oocytes in the same ART laboratory for a similar population. In  
242 our centre, the day 3 embryo implantation rate was 21.5 % when using vitrified oocytes, and  
243 31% for fresh oocytes (unpublished data from the Assessment of the activity of ART centres  
244 doing in vitro fertilization in France in 2015 by the Biomedicine Agency).

245

#### 246 [Open and closed vitrification systems](#)

247 Synchronous egg donation has major limitations in terms of organization and  
248 guarantee of donor anonymity, but allows the use of fresh oocytes that are fertilized on  
249 retrieval day. Therefore, it is crucial to ascertain that the use of vitrified oocytes in the case  
250 of asynchronous egg donation does not reduce the pregnancy chances for the patients.

251 In 2016, Papatheodorou's group demonstrated in a randomized prospective study  
252 that the closed vitrification system is an efficient alternative to fresh oocytes for egg  
253 donation because it does not affect the clinical pregnancy rate per cycle (55.4% and 58.7%,  
254 respectively). However, the number of good quality blastocysts was smaller in the frozen  
255 oocyte group compared with the fresh oocyte group (16).

256 Some studies investigated precisely the effect of vitrification by dividing sibling  
257 oocytes from the same donors in two groups. One group of oocytes is fertilized immediately  
258 (fresh oocytes), whereas the other group is frozen and stored in a donor egg bank (egg-

259 sharing donation programme). For instance, Braga (17) compared fresh oocytes fertilized  
260 with the sperm of the donor's partner, and vitrified oocytes fertilized at a later stage with  
261 the sperm of the recipient's partner. In their study, egg vitrification led to lower rates of  
262 fertilization, of high-quality embryos, and of blastocyst formation compared with fresh  
263 oocytes. Nevertheless, the authors acknowledged the possible bias of the paternal age that  
264 was higher in the recipient group. On the other hand, the clinical pregnancy rate per transfer  
265 was higher after transfer of embryos derived from vitrified oocytes [49.6% (211/425) versus  
266 39.8 (51/128);  $p < 0.01$ ]. Another study (18) limited the bias linked to the partner's age by  
267 sharing all the oocytes from one donor to recipients who received fresh or vitrified oocytes.  
268 The rates of fertilization (84.4% vs. 86.6%), of embryo cleavage, and of good quality embryos  
269 at day 3 of culture (60.4% vs. 64.9%) were not different between fresh and vitrified oocytes.

270 Other studies confirmed the efficiency of closed systems compared with open  
271 systems concerning the oocyte survival rate after warming. For instance, Gook et al  
272 (19) reported similar survival rates for in vitro matured oocytes with the closed system  
273 Rapid-i (89.7%) and the open system Cryolock (92.4%). With mature oocytes, the survival  
274 rate increased to 90.5% for the closed system. The relatively low fertilization rate (64.2%) of  
275 this study was explained by the frequent use of testicular sperm for ICSI. Similarly, Munck et  
276 al (20) did not find any difference in the survival and fertilization rates using the open system  
277 CryoTopSC and the closed system CBSVit. Nevertheless, these authors recommended  
278 avoiding extrapolating these results to all patients because they were obtained in a selected  
279 group of young donors.

280 Conversely, other works suggest a lower oocyte survival rate with closed systems. In  
281 2013, Papatheodorou et al (21) carried out a randomized prospective study in which half of  
282 the eggs of each donor were vitrified using an open system and the other half using a closed

283 system (VitriSafe). The oocyte survival rate was significantly lower with the VitriSafe system  
284 (82.9% vs 91%), but the rates of fertilization, of good quality embryos, and of pregnancy per  
285 transfer were identical. A recent french study (22) also found a significant lower oocyte  
286 survival rate with a closed vitrification system (64.5% vs 93.2% for the open system).  
287 However, in this study, immature oocytes were used. Similarly, Paffoni et al (23) compared  
288 the CryoTip (closed) and CryTop (open) systems (49 patients in each arm) and found  
289 significantly higher survival and fertilization rates for the open system (57.9% and 82.9%,  
290 and 57.6% and 73%, respectively).

291 In 2010, Vanderzwalmen et al (24) showed that adapting the vitrification protocols  
292 allows counteracting the decrease in cooling and warming speed linked to the closed  
293 systems. Thanks to their system VitriSafe, they reached oocytes survival rates of 94%. Later,  
294 Stoop et al (25) reported oocyte survival rates of 90.2%, and fertilization rates of 77.5% for a  
295 group of young egg donors (mean age: 26.4 years). In 2013, the same centre (26) reported  
296 survival and fertilization rates of 89.6% and 81.4%, respectively, after minor changes in their  
297 vitrification and warming protocols.

298 The main results of these studies on oocyte vitrification using closed systems are  
299 summarized in Table 4. These heterogeneous results show that no formal conclusion can be  
300 drawn on the superiority of one or the other system. However, a meta-analysis of 2017  
301 showed that currently, aseptic systems cannot be considered as an efficient alternative to  
302 open systems. Indeed, the Bayesian random-effects meta-analysis indicated that the  
303 probability for closed systems to decrease the oocyte survival rate was of 83.04% compared  
304 with open systems (27).

305

306 [Hypothetical risk of contamination using open vitrification systems](#)

307           The main argument in favour of closed vitrification systems is to limit the risk of  
308 contamination through the liquid nitrogen in which gametes are stored. An interesting study  
309 of 2016 (28) showed that several species of microorganisms survive at -196°C  
310 (*Stenotrophomonas maltophilia*, *Bacillus* spp.). Nevertheless, by comparing the two  
311 vitrification systems (aseptic and in direct contact with liquid nitrogen), it seems that the risk  
312 of contamination is identical in both cases. No gamete contamination by liquid nitrogen has  
313 been reported in the literature so far. However, the theoretic risk of contamination cannot  
314 be excluded and it seems reasonable to opt for a closed system, in accordance with the  
315 requirements of the European directive of 31 March 2004 (2004) amended on 8 February  
316 2011 (29).

317

318 [Predictive factors for successful ART outcome after oocyte vitrification](#)

319           In a very large series with more than 42 000 vitrified oocytes, Cobo and colleagues  
320 (30) studied the factors predictive of egg survival after warming. Surprisingly, all the  
321 analysed variables lacked prognostic value. Moreover, they observed inter-cycle variations  
322 for the same patient because some donation cycles were associated with an excellent egg  
323 survival rate, but not others. This inter-cycle variation suggests that there are intrinsic  
324 oocyte factors that predispose or not to egg survival after warming. These authors also  
325 highlighted the recurrent low oocyte survival rates observed for some donors. Some oocytes  
326 could be more sensitive to the cooling-warming process, due to their permeability to  
327 solutes, their hydraulic conductivity, or even their energy metabolism (31,32). This different  
328 susceptibility could explain the effect «cycle» observed in our centre as well as the  
329 important percentage of warming cycles that did not result in embryo transfer (23.9%).



330 Among these cycles, the cause more often found was the total failure of fertilization (13/21  
331 cases), followed by poor embryo quality, absence of cleavage (absence of embryo), and the  
332 total absence of oocyte survival.

333 Our logistic regression analysis with random intercepts found that only the number  
334 of vitrified oocytes is a significant predictive factor of 100% oocyte survival. Below the  
335 threshold of 7.83 oocytes, the likelihood that all oocytes will survive is lower than 50%.  
336 Moreover, the likelihood of pregnancy increases with the oocyte survival rate, although this  
337 increase is rather small (4% of pregnancies for 1% increase of the oocyte survival rate).

338 Finally, as stressed by the report on oocyte vitrification and warming by the Haute  
339 Autorité de Santé (French National Health Authority), the heterogeneous results raise the  
340 question of a possible ART centre effect as well as of the learning curve and experience level  
341 of the involved health professionals. Indeed, this is a fully manual technique and therefore,  
342 operator-dependent. Consequently, the egg donor programme efficiency is intimately linked  
343 to the centre experience (33). In our centre, our results are reassuring concerning our  
344 competence.

345

#### 346 Limits of the study

347 Each cycle was analysed individually and independently. Consequently, a recipient  
348 who underwent several ART cycles with donor oocytes was considered as several recipients.  
349 A donor having given several oocytes to different recipients was analysed as several  
350 different donors.

351 The studied population presented a selection bias because donors were healthy  
352 young women. It is important to keep in mind that there is little evidence about the

353 outcomes of IVF after fertility preservation for cancer. According to Cobo (34), cancer  
354 patients even achieve poorer clinical outcomes compared to elective fertility preservation.

355 Moreover, since 2012, our egg donation programme uses exclusively vitrified oocytes  
356 to free us from the problem of guaranteeing the donor anonymity. Consequently, we do not  
357 have recent data to form a control population who underwent ART with fresh donor  
358 oocytes. Yet, a very recent retrospective analysis of US data based on more than 30000  
359 donation cycles challenges the use of frozen oocytes (35). Indeed, the study showed that the  
360 living birth rates per donation cycle are lower for cryopreserved than fresh oocytes (39.7 %  
361 vs. 51.1%), and that the living birth rate per cycle with frozen oocytes declines over the years  
362 ( $p= 0.0094$ ). These results, although in disagreement with the previously described works,  
363 indicate that we should closely monitor our success rate over time.

364

## 365 Conclusions

366 Oocyte vitrification is an efficient tool for the management of egg donation  
367 programmes. However, oocytes might show different sensitivity to freezing, explaining the  
368 heterogeneous results between warming cycles. Our logistic regression analysis indicated  
369 that only the number of vitrified oocytes is a significant predictive factor of the likelihood of  
370 survival of all oocytes after warming, and that the chance of obtaining a clinical pregnancy is  
371 linked to the oocyte survival rate.

372 The results of our egg donor programme, which has allowed one in five recipients to  
373 have a living baby, validates the routine use of closed vitrification systems for egg donation  
374 both in terms of clinical outcome and safety. Nevertheless, these results must be confirmed  
375 in larger series and cannot be extrapolated to all patient types.

376

377 [Declarations](#)

378

379 **Ethics approval and consent to participate**

380 Patients were informed of the investigations and gave their consent before participation in  
381 the study, which was approved by the internal ethical board of the Montpellier University  
382 Hospital.

383

384

385 **Consent for publication**

386 Not applicable

387

388 **Availability of data and materiel**

389 All data are available in the ART/PGD Department and can be asked to the corresponding  
390 author on reasonable request

391

392 **Competing interests**

393 The authors declare that they have no competing interests

394

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400 **Authors' contributions**

401 Anna Gala, Samir Hamamah: conception and design of the study  
 402 Anna Gala, Alice Ferrières-Hoa, Margaux Anav, Alice Fournier, Vanessa Loup-Cabaniols,  
 403 Cécile Brunet, Sophie Bringer-Deutsch: acquisition, analysis and interpretation of data  
 404 Vanessa Loup-Cabaniols, Cécile Brunet, Alice Ferrières-Hoa, Sophie Brouillet, Noémie  
 405 Ranisavljevic: drafting the article or revising it critically for important intellectual content  
 406 All authors: final approval of the version to be submitted.

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411

412 **Tables**

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415 *Table 1: Donors' characteristics*

<b>Mean age (years <math>\pm</math> standard deviation)</b>	<b>31.7 <math>\pm</math> 3.2</b>
<b>Mean body mass index (kg/m<sup>2</sup> <math>\pm</math> standard deviation)</b>	<b>22.6 <math>\pm</math> 4.5</b>
<b>Mean cumulative dose of gonadotropins (IU <math>\pm</math> standard deviation)</b>	<b>1831 <math>\pm</math> 845</b>
<b>Mean number of collected oocytes (n <math>\pm</math> standard deviation)</b>	<b>13.9 <math>\pm</math> 10.6</b>
<b>Mean number of vitrified mature oocytes (n <math>\pm</math> standard deviation)</b>	<b>10.8 <math>\pm</math> 1.4</b>

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418 *Table 2: Overall results of the asynchronous egg donation programme using a closed system*  
 419 *of vitrification*

<b>Number of warming cycles (n)</b>	<b>88</b>
<b>Number of warmed oocytes (n)</b>	<b>406</b>

<b>Number of recipients (n)</b>	54
<b>Mean age of recipients (years <math>\pm</math> standard deviation)</b>	36.4 $\pm$ 5.2
<b>Mean number of attributed oocytes/cycle (n <math>\pm</math> standard deviation)</b> (total number of warmed oocytes/number of warming cycles)	4.6 $\pm$ 1.5 406/88
<b>Oocyte survival rate (%)</b> (number of intact oocytes/number of warmed oocytes*100)	82.3 336/406
<b>Fertilization rate (%)</b> (number of fertilized oocytes/number of micro-injected oocytes*100)	65.2 219/316
<b>Cleavage rate (%)</b> (number of obtained embryos/number of fertilized oocytes*100)	103.2 226/219
<b>Normal embryo development at day 2 (%)</b> (number of 4-cell embryos at day 2/number of 2GP2PN oocytes*100)	46.2 92/199
<b>Normal embryo development at day 3 (%)</b> (number of 8-cell embryos at day 3/ number of 2GP2PN oocytes*100)	30.2 60/199
<b>Mean number of transferred embryos/cycle with transfer (n <math>\pm</math> standard deviation)</b>	1.6 $\pm$ 0.5
<b>Embryo transfer rate per cycle (%)</b> (number of transfers/number of egg warming cycles*100)	76.1 67/88
<b>Rate of biochemical pregnancy/transfer cycles (%)</b> (number of positive $\beta$ -HCG tests/number of transfers*100)	38.8 26/67
<b>Rate of clinical pregnancy/transfer cycles (%)</b> (number of ultrasound scans with gestational sac/number of transfers*100)	31.3 21/67
<b>Rate of clinical pregnancy/warming cycles (%)</b> (number of ultrasound scans with gestational sac/number of cycles*100)	23.9 21/88
<b>Implantation rate (%)</b> (number of gestational sacs/number of transferred embryos*100)	21.5 23/107
<b>Number of supernumerary embryos frozen at day 3</b>	29
<b>Cumulative clinical pregnancy rate/donation cycle</b> (number of ultrasound scans with gestational sac after transfer of fresh and	25% 22/88

frozen supernumerary embryos/donation cycles)	
<b>Cumulative live birth rate/donation cycle</b>	<b>20.5%</b>
<b>(number of living births/donation cycles)</b>	<b>18/88</b>

420 2PB2PN oocytes, oocytes with two polar bodies and two pronuclei.

421 *Table 3: Embryo transfer annulation rate after egg warming in function of the cause*

<b>Annulation due to lysis at warming (%)</b> (number of cycles with all oocytes lysed/number of warming cycles without embryo transfer*100)	4.8 1/21
<b>Annulation due to total fertilization failure (%)</b> (number of cycles with total fertilization failure/number of warming cycles without embryo transfer*100)	61.9 13/21
<b>Annulation due to cleavage absence (%)</b> (number of cycles without cleavage/ number of warming cycles without embryo transfer*100)	9.5 2/21
<b>Annulation due to poor embryo quality (%)</b> (number of cycles with poor embryo quality/number of warming cycles without embryo transfer*100)	23.8 5/21

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423 *Table 4: Studies on egg vitrification using closed systems*

Reference	Oocyte origin	Number of warmed oocytes (n)	Oocyte survival rate (%)	Fertilization rate (%)	Pregnancy rate per transfer (%)	Embryo implantation rate (%)
(36)	Egg donors	68	97.1 (66/68)	83.3 (55/66)	66 (2/3) (positive $\beta$ HCG test)	28.6 (2/7)
(19)	Storage of own oocytes	413	90.5 (374/413)	64.2 (240/374)	44.9 (18/44)	32.7 (18/55)
(26)	Donors of eggs that gave one normal pregnancy >20 weeks of amenorrhea	793	82.8 (657/793)	76 (499/793)	NA	NA

after attribution						
<b>(26)</b>	Egg donors	253	93.7 (237/253)	74.3 (176/237)	47.3 (35/74)	25.4 31/122
					(positive $\beta$ HCG test)	
<b>(16)</b>	Egg donors	984	92.7 (912/984)	81.6 (744/912)	52.2 (54/92)	38.9 (70/180)
					(gestational sac)	
<b>(23)</b>	Storage of own oocytes	261	57.9 (151/261)	57.6 (87/151)	8.3 (4/48)	5.8 (4/69)
					(gestational sac)	
<b>(25)</b>	Egg donors	123	90.2 (111/123)	77.5 (86/111)	50 (10/20)	33.3 (12/36)

424 NA, not available.

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