



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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Abstract

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

Key words

Egg donation, oocyte vitrification, closed system

Background

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

Introduction

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

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

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

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

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

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

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

Material and methods

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

Egg collection from donors

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

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

Vitrification and warming procedure

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

Egg fertilization and embryo culture

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

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

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

Recipient endometrial preparation

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

Embryo transfer and cycle outcome

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

Collected data

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

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

Statistical analysis

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

Results

Descriptive data

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

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

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

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

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

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

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

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

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

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

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

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

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

Influence of the embryo development characteristics on clinical pregnancy

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

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

Discussion

Choice of performance indicators

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

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

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

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

Open and closed vitrification systems

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

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

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

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

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

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

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

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

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

Hypothetical risk of contamination using open vitrification systems

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

Predictive factors for successful ART outcome after oocyte vitrification

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

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

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

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

Limits of the study

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

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

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

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

Conclusions

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

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

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.

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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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397 commercial, or not-for-profit sectors.

398

399

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.

407

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410 for all the statistical analysis.

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412 Tables

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415 *Table 1: Donors' characteristics*

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

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

419 *of vitrification*

| | |
|------------------------------|-----|
| Number of warming cycles (n) | 88 |
| Number of warmed oocytes (n) | 406 |

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

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

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*

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

422

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 βHCG 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 | | | | | | |
|-------------------|---------------------------|-----|-------------------|-------------------|---|------------------|
| (26) | Egg donors | 253 | 93.7 (237/253) | 74.3 (176/237) | 47.3 (35/74) (positive β HCG test) | 25.4 31/122 |
| (16) | Egg donors | 984 | 92.7 (912/984) | 81.6 (744/912) | 52.2 (54/92) (gestational sac) | 38.9 (70/180) |
| (23) | Storage of own oocytes | 261 | 57.9 (151/261) | 57.6 (87/151) | 8.3 (4/48) (gestational sac) | 5.8 (4/69) |
| (25) | 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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