A 10-year follow-up of reproductive outcomes in women attempting motherhood after elective oocyte cryopreservation

Loreti, Sara; Darici, Ezgi; Nekkebroeck, Julie; Drakopoulos, Panagiotis; Van Landuyt, Lisbet; De Munck, Neelke; Tournaye, Herman; De Vos, Michel

Published in:
Human Reproduction

DOI:
10.1093/humrep/dead267

Publication date:
2024

License:
Unspecified

Document Version:
Accepted author manuscript

A 10-year follow-up of reproductive outcomes in women attempting motherhood after elective oocyte cryopreservation

Running title: Outcomes after elective oocyte freezing

Loreti S[1,†], Darici E[1,†], Nekkebroeck J[1,2], Drakopoulos P[1,3], Van Landuyt L[1], De Munck N[1], Tournaye H[1,2], De Vos M[1,2,*]

[†]Equally contributing authors

[1]Brussels IVF, Universitair Ziekenhuis Brussel, Laarbeeklaan 101, Brussels 1090, Belgium [2]Vrije Universiteit Brussel, Laarbeeklaan 103, Brussels 1090, Belgium [3]IVF Greece, Athens 15123, Greece

***Correspondence address:** Michel De Vos, MD PhD, Brussels IVF, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Laarbeeklaan 101 - 1090 Brussel , Mobile: +32484946965 e-mail: Michel.DeVos@uzbrussel.be. ORCID Id: 0000-0001-5019-5924

ABSTRACT

Study Question: Which reproductive treatment outcomes are observed in women who underwent elective oocyte cryopreservation (EOC) and who returned to the clinic with a desire for a child?

Summary answer: Whether to warm oocytes or to first use fresh own oocytes for ART depends on age upon returning, but both strategies result in favorable reproductive outcomes.

What Is known already: Most affluent countries have observed a trend towards postponement of childbearing, and EOC is increasingly used based on the assumption that oocytes cryopreserved at a younger age may extend a woman's reproductive lifespan and mitigate her age-related fertility decline. Although most follow-up studies after EOC have focused on women who requested oocyte warming, a substantial proportion of women who do not conceive naturally will embark on fertility treatment without using their cryopreserved oocytes. Reports on reproductive outcomes in past EOC users are scarce, and the lack of reproductive treatment algorithms in this group of women hampers counselling towards the most efficient clinical strategy.

Study design, size, duration: This retrospective observational single-center study encompasses 843 women who had elective oocyte vitrification between 2009 and 2019 at our fertility clinic. Women who underwent fertility preservation for medical or oncological reasons were excluded. This study describes the outcomes of the diverse reproductive treatment strategies performed until May 2022 in women returning to our clinic to attempt motherhood.

Participants/materials, settings, methods: Using descriptive statistics, patient characteristics and data of ovarian stimulation of EOC cycles were analyzed, as well as data related to ovarian stimulation and laboratory data of ART in women who pursued fertility treatment with and/or without using their cryopreserved oocytes. The primary outcome was live birth rate (LBR) per patient after oocyte warming and after ART using fresh oocytes. Secondary outcomes were return rate, utilization rate of the cryopreserved oocytes, laboratory outcomes upon return, and LBR per embryo transfer. A multivariable regression model was developed to identify factors associated with the decision to thaw oocytes as the primary strategy and factors associated with ongoing pregnancy upon return to the clinic.

Main results and the role of chance: A total of 1,353 EOC cycles (Mean ± SD, 1.6 ± 0.9 per patient) were performed. At the time of EOC, the mean age was 36.5 ± 2.8 years, mean AMH was 2.3 ± 2.0 ng/mL, and 174 (20.6%) women had a partner. On average, 13.9 ± 9.2 mature oocytes were cryopreserved. Two hundred and thirty-one (27.4%) women returned to the clinic, an average of 39.9 ± 23.4 months after EOC. Upon returning, their mean age was 40.4 ± 3.1 years, mean AMH was 1.5 ± 1.5 ng/mL, and 158/231 (68.3%) patients had a partner. As a primary approach, 110/231 (47.6 %) past EOC users embarked on oocyte warming, 50/231 (21.6%) had intrauterine insemination, and 71/231 (30.7%) had ART using fresh own oocytes. Cumulative LBR (CLBR) was 45.9 % (106/231) notwithstanding a miscarriage rate (MR) of 30.7% (51/166) in the entire cohort. In total, 141 women performed oocyte warming at some stage in their treatment trajectory. A subset of 90/231 (39.0%) patients exclusively had oocyte warming (41.6 ± 3.0 years, with 10.0 ± 5.2 oocytes warmed per patient). 52/231 (22.5%) patients exclusively had ART using fresh own oocytes (mean age of 39.0 ± 2.8 years, with 9.9 ± 7.4 mature oocytes retrieved per patient). CLBR was 37/90 (41.1%) in the oocyte warming-only group and 25/52 (48.1%) in the OS-only group. MR/transfer was 25.0% and 29.3% in the oocyte warming-only group and the OS-only group, respectively.

Limitations, reasons for caution: Both sample size and the retrospective design are limitations of this study. The decision to embark on a specific reproductive treatment strategy was based on patient preference, after counseling on their treatment options. This precludes direct comparison of the efficiency of reproductive treatment options in past EOC users in this study.

Wider Implications of the findings: Reporting on clinical outcomes of women who underwent EOC and returned to the clinic to embark on divergent reproductive treatment strategies is mandatory to establish guidelines for best clinical practice in this growing patient population.

Study funding/competing Interest(s): None

Clinical trial number: N/A

Key words: ICSI, Live birth, Oocyte Cryopreservation, Elective, Social freezing, Oocyte warming, reproductive options

INTRODUCTION

As a result of societal changes, for some decades most affluent countries have observed a trend in delaying parenthood. Indeed, women are getting older when they have their first pregnancy ('OECD-Social Policy Division, OECD Family Database, 2019 ') for several reasons among which the lack of a stable and committed partnership for starting a family is the most frequently cited one (Inhorn et al. 2018). This trend not only results in a reduced functional reproductive lifespan for an increasing proportion of women who will be unable to reach their desired family size but also in an increased risk of subfertility because of increasing meiotic errors in oocytes, a process which is accelerated after the age of 35 years (Jones 2008). Although ovarian stimulation and ART may compensate for fertility decline to some extent in women of advanced reproductive age who have a good ovarian reserve, the success rates of IVF in those with a low ovarian reserve are only moderate. While public awareness campaigns have been developed in recent years to promote the importance of a healthy lifestyle before conception and to make the general public more familiar with accelerated fertility decline in women of advancing age (Harper et al. 2021), these campaigns should also address the limitations of ART programs in older women. Moreover, fertility awareness campaigns are not likely to resolve the reproductive concerns of the increasing population of single women who are not in the position to start trying for having a baby.

Since the introduction of vitrification as an efficacious technology for oocyte cryopreservation, the application of this strategy has expanded beyond the initial indications of oncofertility preservation and oocyte donation, to include elective oocyte cryopreservation (EOC) for delayed parenthood as the currently most common application (Human fertilisation and embryology authority, 2018). In spite of the increasing availability and usage of EOC, the majority of follow-up studies published to date are limited by their small sample size and/or short follow-up period. Nevertheless, robust follow-up data of reproductive outcomes several years after EOC are indispensable for proper counselling of women who consider this procedure. So far, only one large multicenter study has been published, encompassing more than 5,000 women who had their oocytes vitrified for elective reasons (Cobo et al. 2018). According to these data, live birth rates in women who used their cryopreserved oocytes to attempt pregnancy were 68.8% in 93 women who had their oocytes vitrified at an average age of 32.6 ± 5.7 years but only 25.5% in 384 women who had their oocytes

vitrified at an average age of 38.7 ± 2.8 years. Although in theory, EOC holds the potential to mitigate age-related fertility decline, the concept of EOC remains controversial. The procedure is not funded by the authorities of most countries where IVF treatment is covered, and there are currently no data to support the hypothesis that EOC truly increases the prospective chance of a live birth compared to not performing EOC. Indeed, based on the relatively low live birth rate per vitrified oocyte (Stoop et al. 2012), many women who embark on EOC will not have a baby after thawing of their limited set of cryopreserved oocytes: according to a fact sheet published in 2014, the American Society for Reproductive Medicine (ASRM) estimated that the live birth rate after EOC was only 2%–12% for women under 38 years of age, alerting that oocyte cryopreservation *per se* did not guarantee a successful pregnancy or live birth (Mature oocyte cryopreservation: a guideline, Practice Committees of American Society for Reproductive Medicine, Society for Assisted Reproductive Technology, 2013). Moreover, the cost-efficiency is not only related to the age at which the oocytes are cryopreserved and their number, but also to their ultimate utilisation rate. Several theoretical models have been developed to predict the cost-efficiency of EOC, but these models rely heavily on the actual cost of the procedure, which varies substantially across countries, and they do not consider the potential benefit of EOC on a woman's quality of life or on the sustainability of a woman's relationships. Moreover, most published follow-up studies of women after EOC to date focused on women who returned to the fertility clinic after several years and who requested oocyte warming, whereas the actual reproductive choices of past EOC users remained ill-reported. Indeed, a substantial proportion of these women attempt to conceive naturally or by ART, often without using their cryopreserved oocytes (Hodes-Wertz et al. 2013). In view of this, we set out to analyse the outcomes of reproductive treatment in women who had previously undergone EOC and who returned to the fertility clinic because of an unmet desire for motherhood.

MATERIALS AND METHODS

Study design and population

This was a single-center retrospective study in 843 women aged between 18 and 40 years who had their oocytes vitrified for the purpose of EOC during the period between 2009 and 2019 at Brussels IVF, the largest

fertility clinic in Belgium. Women who underwent fertility preservation for medical or oncological reasons were excluded. The study was approved by the ethics committee of Universitair Ziekenhuis Brussel (EC 2022/127). Data were collected from the electronic patient database of the hospital. Data analysis was performed to describe EOC cycle characteristics and clinical outcomes of reproductive treatment strategies that were chosen in those women who returned to the fertility clinic with a desire for pregnancy until May 2022 (*N*=231). Women who returned to the clinic consented to either use their cryopreserved oocytes as the first line fertility treatment, or to keep their oocytes cryopreserved until further notice and embark on fertility treatment with their own fresh oocytes. The choice of strategy was made after discussion at the outpatient clinic regarding the predicted success rates of each procedure, based on published evidence.

Ovarian stimulation protocols

GnRH antagonist and GnRH agonist protocols were used for ovarian stimulation in the context of EOC. The assessment of ovarian reserve was performed using serum anti-Müllerian hormone (AMH). Serum AMH was analysed using AMH Immunotech (IOT) kit (Beckman Coulter Inc., Marseille, France) until 24th April 2012. Between 25th April 2012 and 3rd July 2013, the Gen I kit was used (Beckman Coulter, Inc., Chaska, MN, USA); between 4th July 2013 and 17th September 2014 the modified Gen I test kit was used and since 18th September 2014 the Elecsys platform (Roche Diagnostics International AG, Rotkreuz, Switzerland) has been used. The gonadotropin dose was selected according to serum AMH levels and BMI.

GnRH Antagonist Protocol

Women who had basal concentrations of oestradiol (≤80 pg/ml) and progesterone (≤1.5 ng/ml), either at random (in women with oligo-amenorrhoea) or on day two of their menstrual cycle were instructed to start ovarian stimulation. Monotherapy ovarian stimulation was performed with either follitropin alpha (Ovaleap®, Bemfola®, Gonal-F®), follitropin beta (Puregon®), follitropin delta (Rekovelle®), corifollitropin alfa (Elonva®), or highly-purified human menopausal gonadotrophin (HP-hMG, Menopur®). The decision to administer a 3-day course of GnRH antagonist preceding the initiation of ovarian stimulation with gonadotropins was the individual discretion of the patient's physician.

According to the patient's characteristics, a daily dose of 150-300 IU of recombinant follicle-stimulating hormone (rFSH) or HP-hMG was started. The daily dose remained constant during stimulation. A fixed antagonist protocol was applied, with daily administration of 0.25mg of a GnRH antagonist (ganirelix, Orgalutran®; or cetrorelix, Cetrotide®) from day 6 of stimulation onwards. Ovulation was induced by administering either 5000 or 10.000 IU of hCG (Pregnyl®), 250µg of rhCG (Ovitrelle®), or 0.2 mg of GnRH agonist (Gonapeptyl®), or a dual trigger, as soon as three follicles measuring 17 mm or more in diameter were identified. The oocyte pick-up was planned 36 hours following ovulation trigger.

GnRH Agonist Protocol

On day 21 of the previous cycle, daily injection of the GnRH agonist triptorelin (0.1 mg/day) was started. After two weeks of pituitary desensitization, daily administration of rFSH or HP-hMG was started. Soon as the criteria for ovulation trigger were met, 5000 or 10.000 IU of hCG (Pregnyl®), or 250µg of rhCG (Ovitrelle®) were administered to induce ovulation. The time to oocyte retrieval from triggering was 36 h.

Oocyte vitrification/warming

Vitrification and warming were carried out as previously described (De Munck et al. 2015). Briefly, the Irvine Scientific® Vitrification Freeze Kit (Irvine Scientific, USA) was used, containing 7.5% (v/v) ethylene glycol (EG) + 7.5% (v/v) dimethylsulphoxide (DMSO) in an M-199 HEPES-buffered medium supplemented with 20% dextran serum supplement (DSS), referred to as equilibration solution (ES), with vitrification solution (VS) containing 15% (v/v) EG + 15% (v/v) DMSO + 0.5 M sucrose. Oocytes were placed in 25 µl HTF-HEPES supplemented with HSA and immediately merged with 25 µl ES for 2 min at room temperature followed by a second merging step with 25 µl ES for 2 min. Then, oocytes were transferred into a new 25 µl ES droplet for 10 min, followed by two consecutive 50 µl VS droplets, and loaded on the CBS vit straw (CryoBiosystems, France). Straws were thermosealed and plunged into liquid nitrogen (LN 2) within 60 s. The high-security closed system prevents any contact with LN_2. Depending on the number of oocytes available, oocytes were vitrified individually or in pairs. For warming, we used the Irvine Scientific® Vitrification Thaw Kit (Irvine Scientific, USA), containing a thawing solution (TS) with 1 M sucrose in an HEPES-

buffered medium supplemented with 20% DSS, a dilution solution (DS) containing 0.5 M sucrose in an HEPES-buffered medium supplemented with 20% DSS, and a washing solution (WS) containing HEPES-buffered medium supplemented with 20% DSS. Oocytes with full recovery after warming underwent intracytoplasmic sperm injection (ICSI) and were assessed 16-18h post ICSI for the presence of pronuclei. Oocytes retrieved after ovarian stimulation ware inseminated using IVF or ICSI depending on sperm quality. Embryos were cultured in individual droplets of 25 µl medium with oil overlay until transfer on day 3; in cycles with at least four embryos on day 3 that were classified as transferable or good-quality embryos according to the criteria described by Van Landuyt et al. (Van Landuyt et al. 2013), embryos were cultured until transfer on day 5. Supernumerary embryos were vitrified on day 3, 5 or 6, as previously described (Van Landuyt et al. 2015).

Study outcomes

The primary outcome was the live birth rate (LBR) per patient after fertility treatment in past EOC-users who returned to the fertility clinic with a desire for motherhood.

The secondary outcomes of the study were (i) the rate of return to the fertility clinic, (ii) the utilization rate of the oocytes cryopreserved for EOC, and (iii) the laboratory outcomes of ART treatment cycles. A positive pregnancy test was defined by a serum β-hCG > 0.1 IU/L determined 12 days after embryo transfer. Clinical pregnancy was identified as a visible fetal pole with normal fetal heartbeat observed at 7 weeks of gestation at a bidimensional (2D) transvaginal ultrasound. Ongoing pregnancy was defined as pregnancy with a detectable heart rate at 12 weeks' gestation or beyond after the completion of embryo transfer. Ongoing pregnancy was assessed in the subgroup of women who were pregnant at the moment of the construction of the database and who had not yet delivered a baby. LBR was defined as the number of births of live infants beyond viability (>24 weeks) and included the live births obtained both from fresh and frozen embryo transfers. Duration of stimulation, total doses of gonadotropins used, numbers of oocytes retrieved and vitrified, oocyte survival rates, fertilization rates, and the presence of a partner at the moment of the treatment were also described.

Statistical analysis

Descriptive statistics were used to analyze the demographic characteristics, the treatment characteristics, the laboratory data, and the reproductive outcomes of the study population focusing on the women who pursued fertility treatment with and/or without using their cryopreserved oocytes when they came back to the clinic with a desire for motherhood. Data pertaining to the different reproductive treatment strategies until May 2022 were retrieved from the electronic medical records of our hospital. Data are expressed as mean (± standard deviation, SD). ~~or total (mean ± standard deviation).~~ Categorical variables are given as raw numbers (percentages). Multivariable regression analysis was performed to identify factors that were associated with the decision to thaw oocytes as the primary strategy upon return to the clinic, and factors that were associated with ongoing pregnancy.

RESULTS

In total, 843 women underwent 1,353 elective oocyte vitrification (EOC) cycles (on average 1.6 ± 0.9 per woman) between 2009 and 2019. The baseline characteristics of the cohort of women who had EOC are shown in Table 1. The mean age at the time of vitrification was 36.5 ± 2.8 years, and 174 (20.6%) women had a partner when they had EOC. Duration of ovarian stimulation was 11.1 ± 2.1 days. The mean number of oocytes retrieved was 11.0 ± 7.4 per stimulation cycle. The mature oocyte rate (number of metaphase II oocytes per cumulus-oocyte complex (COC)) was 78.8%, resulting in a mean number of 8.7 ± 5.8 MII per cycle. On average, 13.9 ± 9.2 mature oocytes (MII) were cryopreserved in total per patient.

In total, 231 (27.4%) women returned to the clinic with a desire for motherhood, on average 39.9 ± 23.4 months after EOC and at a mean age of 40.4 ± 3.1 years. Table 2 shows the characteristics of the group of women who returned. The mean AMH of this group of women was 2.3 ± 2.0 ng/mL at the time of EOC, and 1.5 ± 1.5 ng/mL upon returning. Of women who returned to the clinic, 68.3% (158/231) had a partner and had failed to conceive naturally with this partner, or, in case of same-sex couples, requested fertility treatment with donor sperm. Sixty-eight women (68/231, 29.4%) did not have a partner and five women had a same-sex partner when they returned to the clinic. After counseling at the out-patient clinic, 47.6% (110/231) women requested oocyte warming as the primary approach to attempt pregnancy, whereas 21.6%

(50/231) embarked on intrauterine insemination (IUI) treatment, and 30.7% (71/231) decided to start fertility treatment using their own fresh oocytes while keeping their cryopreserved oocytes stored. In total, 141 (16.7%) women performed oocyte warming at some stage in their fertility treatment trajectory. Figure 1 shows the details of the various treatment strategies selected by the women when they returned to the clinic.

In total, 1,844 oocytes (9.3 ± 4.7 per woman) were warmed in 200 oocyte warming cycles (1.4 ± 0.8 cycles per woman). The average oocyte survival rate after warming was 82.6%. The mean number of usable embryos obtained per warming cycle was 2.7 ± 1.8. When considering ART cycles with fresh oocytes, 240 cycles of ovarian stimulation (OS) followed by IVF or ICSI were performed among women who returned to the clinic (2.2 ± 2.0 cycles per woman). This resulted in 2.1 ± 2.1 usable embryos per OS cycle.

Overall, partner sperm was used in 58.2% of all ART cycles (fresh and warmed oocytes), whereas donor sperm was used in 41.8%. An average of 1.3 ± 0.5 embryos were transferred per ART cycle. Per embryo transfer, the rate of positive serum human chorionic gonadotrophin (hCG) was 36.3% (196/539), clinical pregnancy rate (CPR) was 30.8% (166/539) and LBR was 17.3% (93/539). Overall, the cumulative ongoing pregnancy rate (COPR) in the cohort of women who returned after EOC was 55.4% (128/231). CLBR was 45.9 % (106/231) until May 2022, with 22 pregnancies still ongoing. The overall miscarriage rate was 30.7% (51/166). After counselling regarding the advantages and disadvantages of PGT-a, none of the patients in our study requested PGT-a.

Among the reproductive treatment choices in the cohort under study, two divergent choices emerged: a subset of 90/231 (39.0%) women requesting oocyte warming cycles and not pursuing any ovarian stimulation for retrieval of (fresh) oocytes (warmed-oocytes-only group, WOO). Another distinct subgroup of 52/231 (22.5%) women decided to keep all their vitrified oocytes stored and had one or more cycles of ovarian stimulation (fresh-oocytes-only group, FOO). Table 3 lists the detailed characteristics of the EOC cycles that these women had undergone previously, as well as the outcomes of reproductive treatment in these two distinct groups, when returning to the clinic.

The age and AMH at EOC of women who successively returned to the clinic in the WOO group were 37.3± 2.3 years and 3.0 ± 2.5 ng/mL; age and AMH at EOC of women in the FOO group were 36.5 ± 3.0 years and 2.1 ± 3.0 ng/mL, respectively. In the WOO group, 19% of women had a partner, whereas 40% of women in the FOO group had a partner when they returned. When considering the characteristics of the previous EOC cycles of these subgroups, women in the WOO group had done 189 EOC cycles (2.1 ± 1.4 EOC cycles per woman), with 1,771 MII oocytes (9.1 ± 7.2 per cycle, 19.1 ± 14.6 per patient) vitrified in total, whereas patients in the FOO group had done 93 EOC cycles in total (1.8 ± 1.0 EOC cycles per woman), with 6.5 ± 4.7 MII vitrified per cycle and 11.7 ± 8.4 per patient.

The mean time interval between EOC and revisiting the clinic to start fertility treatment was 53.0 ± 21.9 months in the WOO group, at a mean age of 41.6 ± 3.0 years, and 29.8 ± 19.3 months in the FOO group, at a mean age of 39.0 ± 2.8 years. In the WOO group 61.5% (64/104) of women used partner semen to conceive, compared to 64.8% (46/71) in the FOO group. Women in the WOO group underwent 112 thawing cycles in total (1.2 ± 0.6 per woman) and had 1,155 oocytes warmed (10.0 ± 5.2 per woman). This resulted in 3.0 ± 2.0 embryos obtained per cycle. After completion of the oocyte warming cycles, 562 oocytes (6.2 ± 4.3 per woman) were still cryopreserved.

Patients in the FOO group underwent 81 OS cycles in total (1.6 ± 1.3 per woman). Overall, 663 cumulus-oocyte complexes (COCs) were retrieved (13.0 ± 10.3 per woman) and the oocyte maturation rate was 76.1% (505/663). On average, 2.4 ± 2.3 embryos were obtained per cycle in the FOO group. After completion of the oocyte warming cycles, 607 oocytes (6.5 ± 4.7 per woman) were still cryopreserved in this group.

With regard to clinical outcomes in the WOO group, the positive hCG rate per transfer was 47.4% (74/156), and CPR per transfer was 38.5% (60/156). CLBR and MR were 41.1% (37/90) and 25.0%, respectively.

In the FOO group, the positive hCG rate per transfer was 50.5% (47/93), and the CPR per transfer was 44.1 % (41/93). CLBR and MR were 48.1% (25/52) and 29.3%, respectively.

To identify characteristics that were associated with the decision to thaw oocytes as the primary strategy upon return to the clinic, a multivariable logistic regression model was developed, considering the following

confounders: age at vitrification, number of oocytes vitrified, time interval between EOC and return to the clinic, and AMH upon return to the clinic (Table 4). This model demonstrated that the total number of vitrified oocytes (OR 1.10, CI 1.00 – 1.20, p= 0.004) and the time interval between EOC and return to the clinic (OR 1.05, CI 1.02 – 1.09, p= 0.04) were associated with the decision to thaw oocytes as the primary strategy upon return to the clinic. We also developed a multivariable logistic regression model to identify factors that were associated with ongoing pregnancy upon return to the clinic (Table 5), and we considered the following confounders: age at vitrification, number of oocytes vitrified, time interval between EOC and return to the clinic, AMH upon return to the clinic, and the decision to thaw oocytes at some point during the process. Using this model, none of the above factors were identified as predictors of ongoing pregnancy, although there was a tendency towards a higher number of cryopreserved oocytes (OR 1.08, CI 1.00 – 1.17, p= 0.06) and a shorter interval between EOC and return to the clinic (OR 0.97, CI 0.94 – 1.00, p= 0.08) as factors potentially associated with ongoing pregnancy upon return to the clinic.

DISCUSSION

In this study, we report the outcomes of reproductive treatment in a cohort of 231 women who previously undertook at least one round of elective oocyte cryopreservation (EOC). To our knowledge, this is one of the first and largest European reports of reproductive outcomes in women who performed EOC at a single center, and this is the first study to provide follow-up data from actual reproductive outcomes in subfertile patients who had previously done EOC. Although oocyte cryopreservation has initially been practiced in cases where the ovarian reserve was threatened by the iatrogenic complications of cancer treatment, the indication of oocyte cryopreservation has been extended to other applications, including delaying childbearing (elective oocyte cryopreservation), threatened ovarian insufficiency in the context of benign conditions (e.g. endometriosis and benign ovarian tumours) (Santulli et al. 2023), and transgender care (Asseler et al. 2023). While the aim of EOC is to mitigate the risk of infertility, there is no evidence from prospective studies that women who have their oocytes cryopreserved in their thirties will indeed have a higher likelihood of giving birth when they attempt motherhood several years later. Although modeling studies have been developed to predict reproductive outcomes and cost-effectiveness of EOC based on age at EOC and the number of

cryopreserved oocytes, these models generally are extrapolated from published data, hence they do not provide data from actual patient outcomes (van Loendersloot et al. 2011), (Devine et al. 2015),(Mesen et al. 2015). Indeed, several observational studies have analyzed the reproductive outcomes in past EOC users who had IVF of warmed autologous oocytes; these studies generally highlighted that only a small proportion of women used their cryopreserved oocytes to attempt pregnancy. In our study, after a mean follow-up of just over three years only roughly one out of four women returned to the clinic with a desire for pregnancy, which is a small proportion. Although return rates may vary depending on the follow-up period, and higher return rates should be expected after a longer period of follow-up, a substantial proportion of oocytes will not be used, which has a negative impact on the cost-efficiency rate of long-term cryostorage. In Belgium, women who embark on EOC will have their oocytes stored for 10 years by default, unless they use them before this storage limit, although the storage can be prolonged upon the woman's request. Oocytes from women who do not contact the clinic after ten years will either be destroyed, donated for scientific research or used for altruistic donation, depending on the destiny that was selected when the cryostorage contract was signed. From the follow-up studies that have been published so far, it appears that cost-efficiency of EOC is relatively low (Hammarberg et al. 2017), due to low utilization rates. Low oocyte utilization rates from published studies are in sharp contrast with surveys among past EOC users reporting that a substantial proportion of women had children without using their cryopreserved oocytes (Hodes-Wertz et al. 2013; Balkenende et al. 2018; Wafi et al. 2020; Gürtin et al. 2019), more specifically when they attempt pregnancy before the age of 40 years (Malchau et al. 2017). Women may achieve natural pregnancies or exhaust other fertility treatments before using the cryopreserved oocytes. Although the proportion of women who will embark on medically assisted reproduction (MAR) without using their cryopreserved oocytes may be related to the woman's age at the time of their return to the clinic and their estimated reproductive potential, low utilization may also be associated with a perception of cryopreserved oocytes as an insurance and a last resort after all other possibilities for becoming a mother have been exhausted (natural conception, IUI, IVF with fresh own oocytes, etc.). A woman's decision not to have her oocytes thawed (yet), but to embark on IVF with fresh oocytes instead, will invariably depend on local coverage of MAR; in Belgium, where a woman can benefit from public coverage of six IVF cycles up until the age of 42 years, this coverage policy may favour a woman's

choice for funded IVF cycles with fresh oocytes at almost no cost instead of IVF cycles with their expensive non-funded cryopreserved oocytes. Finally, it is not unreasonable to assume that EOC may facilitate a mental state that is more conducive to the creation of a stable relationship, once a suitable partner has been found. Therefore, in order to appraise the effectiveness of oocyte cryopreservation, it is important to conduct surveys among past EOC users, and to collect data of reproductive treatment in past EOC users who return to the fertility clinic when they attempt motherhood.

In our study, the mean age at which women performed EOC was 36.5 ± 2.8 years. Although there are insufficient data to advise women on the optimal age to undergo EOC, the age group between 35 and 37 appears optimal according to published models of cost-efficiency; Mesen et al. showed that EOC offers the highest gain of probability of childbirth when women cryopreserve their oocytes at an age between 35 and 37 years (Practice Committee of ASRM, 'Evidence-based outcomes after oocyte cryopreservation for donor oocyte in vitro fertilization and planned oocyte cryopreservation: a guideline' 2021) (Mesen et al. 2015). Similarly, the data of Doyle et al. indicated that when performing EOC before 37 years of age, a live birth rate of approx. 70–80% can be achieved with 15–20 cryopreserved oocytes (Doyle et al. 2016). The age of our cohort is slightly lower compared to other published series of 5289 women in Spain (37.2 ± 4.9 years; (Cobo et al. 2018)) 254 women in Sweden (36.9 years; (Wennberg, Schildauer, and Brännström 2019)), 373 women in the UK (38.3 years; (Kasaven et al. 2022)), 517 women in Turkey (37.4 ± 5.2 years; (Cil et al. 2019)), 543 women in the USA (38.3 years; (Cascante et al. 2022)), a further series of 1079 women in the USA (36.6 years; (Leung et al. 2021)), and a series of 446 women in Israel (37.9 ± 2.0 years; (Tsafrir et al. 2022)).

The mean age of 40.4 ± 3.1 at which the women in our cohort returned to the fertility clinic was comparable to that in previous reports. Although 27.4% of women returned to the clinic, the utilization rate of cryopreserved autologous oocytes was relatively low at 16.7% (141/843 women), but again very similar to that in other published series.

From the total group of 843 women who performed EOC in our study, 79.4% were not in a relationship at the time of EOC. Intriguingly, the proportion of women who were still single upon returning to our clinic was relatively high: in the group of women who had ART when they returned, 29.4% (68/231) used donor sperm, mainly because they had no partner. Five women (5/231) used donor sperm because they were in a same-

sex relationship. Kasaven et al. reported the use of donor sperm in 25% (9/36) of single UK women, i.e. data in line with our results while, in the USA, Leung et al. reported that 42.4% of women used donor sperm (Kasaven et al. 2022) (Leung et al. 2021). As oocyte cryopreservation needs to be considered as the purchase of extra time to find the right partner and to postpone the pursuit of motherhood, the consistently high rate of past EOC users requesting donor sperm for ART is surprising, because one of the main reasons why women embark on EOC is the lack of a partner (Inhorn et al. 2018). The observation that a substantial proportion of these women decided to attempt motherhood without a partner, more than three years after EOC on average, illustrates a gradual shift, at least in a subset of women in their late thirties and early forties, from the search for a partner to the acceptance of the pursuit of single motherhood.

Overall, the cumulative live birth rate in our cohort of past EOC users who had fertility treatment was 45.9%. In the WOO subgroup of 90 women who used their cryopreserved oocytes at a mean age of 41.6 ± 3.0 years, the cumulative ongoing pregnancy rate from 10.0 ± 5.2 warmed oocytes were high, at 52.2%. The CLBR in this group was at least 41.1%, although several women were still pregnant at the time of writing. These figures illustrate the favorable prospects of women who decide to perform EOC, even at a relatively advanced mean age of 37 years. In the largest cohort of past EOC-users published so far, the use of 10 warmed oocytes in a group of 518 women who performed EOC after the age of 35 years resulted in a CLBR of 25.2% (Cobo et al. 2021). In view of the high cost of EOC for the individual woman, especially in countries where there is no public funding, it seems intuitive to advocate that EOC should at best be offered only in centers with sufficient expertise in fertility preservation, and with a laboratory that can achieve the best possible outcomes for oocyte cryopreservation.

The early pregnancy loss rate of 25% in women in our study who used thawed oocytes was relatively low, considering their age. For reference, the miscarriage rate in a large cohort of women who had ART using fresh oocytes after the age of 40 years was 35.8% in a group of 1,380 women at 40.4 ± 0.5 years and no less than 48.6% in a group of 833 women at 42.4 ± 0.5 years (Devesa et al. 2018). After oocyte warming, embryos were typically cultured to day three rather than day five after ICSI, for fear of the emotional impact of not reaching the blastocyst stage (Cascante et al. 2022). Based on a retrospective study comparing the outcomes of

401 autologous IVF treatment using vitrified and warmed oocytes, significantly fewer cycles resulted in
402 blastocyst-stage embryo transfer when comparing vitrified versus fresh oocytes (Doyle et al. 2016).
403 In this study we also report the reproductive outcomes in the FOO subgroup of 52 past EOC users who
404 decided not to pursue oocyte warming after counselling. The mean age of these women was 39.0 ± 2.8 years.
405 The preference by these women not to pursue oocyte warming and to perform ovarian stimulation and ART
406 using fresh oocytes instead was probably related to the modest average number of cryopreserved oocytes
407 in this group (6.6 ± 4.7 vitrified MII oocytes/woman), their normal mean serum AMH level (1.5 ± 1.5 ng/mL)
408 and the public funding of six cycles of ART for Belgian citizens until the age of 42 years (whereas public funding
409 is not available for EOC).
410 We realize that the data in our study cannot provide any evidence about the cost-efficiency of EOC; just like
411 other published reports on reproductive outcomes in past EOC. The data are observational, and the results
412 cannot be extrapolated to the increasing group of women in their thirties who request EOC at various IVF
413 centers worldwide. A further limitation of our study is the absence of embryology data. Because of
414 adaptations in the embryo culture policy in our center over the years, this study contains a heterogenous mix
415 of cleavage-stage and blastocyst transfers, which precludes correct interpretation of embryology data. The
416 main limitation of our study is its retrospective nature with a small cohort of patients especially in the FOO
417 group. Prospective data comparing long-term reproductive outcomes including from natural conception, in
418 women who performed EOC and in those who did not perform EOC, should reach a higher level of evidence.
419 Although our study lacks data on natural conceptions in past EOC users, the report of clinical outcomes from
420 divergent reproductive treatment strategies in women who underwent EOC and returned to the clinic should
421 be considered as a substantial contribution to the existing data, because most previous reports have focused
422 on the use of vitrified oocytes only. Eventually, collating the data from several cohorts of past EOC users
423 should result in algorithms for best clinical practice in this rapidly growing patient population. These
424 algorithms should also enable fertility clinics to give proper guidance to women about their future
425 reproductive trajectory options; women embarking on EOC would then be counseled upfront about possible
426 fertility treatments based on their age, number of oocytes retrieved, and public funding opportunities. For
427 these women, having this prior knowledge and awareness may be beneficial, as it may increase their

likelihood of having children later in life. Counseling women about their future reproductive outlook based on data-driven algorithms may also facilitate the development of a long-term partnership between these patients and the fertility clinic. Based on our data, we would suggest that past EOC users who fail to conceive naturally after the age of 40 years may prefer to use their cryopreserved oocytes instead of fresh oocytes because of the improved success rate and the lower miscarriage rate with their younger cryopreserved oocytes. Those who return to the fertility clinic before the age of 40 years may attempt motherhood with fresh oocytes as a primary strategy, depending on local policies of ART funding.

In conclusion, after elective oocyte cryopreservation, women who return to the fertility clinic with a desire for motherhood have several reproductive treatment options. When the choice of treatment options and the course of the fertility treatment trajectory is driven by a patient-tailored strategy, taking into account several parameters including the age at which the patient returns and the number of cryopreserved oocytes, favorable reproductive outcomes were observed in a large tertiary fertility clinic. Oocyte warming in women of advanced age resulted in a cumulative live birth rate of more than 40%. Our study highlights the need for further reports of return rates, oocyte utilization rates and reproductive outcomes after EOC. Collating the data from follow-up studies should ultimately lead to the development of reproductive treatment algorithms in these women.

Data availability

The data underlying this article will be shared upon reasonable request to the corresponding author.

Authors's Roles

MDV-SL-ED: study design, execution, data analysis, manuscript writing. PD: statistical analysis, interpretation of the data, and inference with literature. ND-JN-HT: interpretation of the data and inference with literature. All authors contributed to the writing and the critical revisions of the manuscript, and all authors approved the final version of the manuscript and authorized the submitted version.

Funding

No external funding was used for this study.

Conflict of interests

PD has been consultant to Merck Healthcare KGaA (Darmstadt, Germany) from April 2021 till June 2023, and is a Merck employee (Medical Director, Global Medical Affairs Fertility) with Merck Healthcare KGAaA (Darmstadt, Germany) since July 2023. He declares honoraria for lecturing from Merck KGaA, MSD, Organon and Ferring. The other authors declare no conflict of interest.

FIGURE LEGEND

Figure 1: Distribution of the reproductive treatments performed in Elective oocyte cryopreservation (EOC) patients when they came back to the fertility clinic with a desire for children. (WOO: Warmed-oocytes-only; FOO: Fresh-oocytes-only)

REFERENCES

Asseler JD, Knieriem J, Huirne JA, Goddijn M, Verhoeven MO, van Mello NM. 2023. 'Outcomes of oocyte vitrification in trans masculine individuals', *Reproductive BioMedicine Online;* 47:94-101.

Balkenende E., Dahhan T., van der Veen F., Repping S., and Goddijn M. 2018. 'Reproductive outcomes after oocyte banking for fertility preservation', *Reproductive BioMedicine Online*, 37: 425-33.

Cascante, S. D., J. K. Blakemore, S. DeVore, B. Hodes-Wertz, M. E. Fino, A. S. Berkeley, C. M. Parra, C. McCaffrey, and J. A. Grifo. 2022. 'Fifteen years of autologous oocyte thaw outcomes from a large university-based fertility center', *Fertil Steril*, 118: 158-66.

Cil A., Abali R., Boza A., Karakis L., Ceyhan M., Aksakal E., Keleş I., Oktem O., Ata B., Bahceci M., et al. 2019. 'A 5-year analysis of demographics, cycle characteristics and reproductive outcomes of 907 egg freezing cycles in patients with diminished ovarian reserve and age-related fertility decline', *Fertility and Sterility*, 112: e108.

Cobo, A., J. García-Velasco, J. Domingo, A. Pellicer, and J. Remohí. 2018. 'Elective and Onco-fertility preservation: factors related to IVF outcomes', *Hum Reprod*, 33: 2222-31.

Cobo A., García-Velasco J., Remohí J., and Pellicer A. 2021. 'Oocyte vitrification for fertility preservation for both medical and nonmedical reasons', *Fertility and Sterility*, 115: 1091-101.

De Munck, N., L. Petrussa, G. Verheyen, C. Staessen, Y. Vandeskelde, J. Sterckx, G. Bocken, K. Jacobs, D. Stoop, M. De Rycke, and H. Van de Velde. 2015. 'Chromosomal meiotic segregation, embryonic developmental kinetics and DNA (hydroxy)methylation analysis consolidate the safety of human oocyte vitrification', *Molecular Human Reproduction*, 21: 535-44.

Devesa, M., R. Tur, I. Rodríguez, B. Coroleu, F. Martínez, and N. P. Polyzos. 2018. 'Cumulative live birth rates and number of oocytes retrieved in women of advanced age. A single centre analysis including 4500 women ≥38 years old', *Hum Reprod*, 33: 2010-17.

Devine, K., S. L. Mumford, K. N. Goldman, B. Hodes-Wertz, S. Druckenmiller, A. M. Propst, and N. Noyes. 2015. 'Baby budgeting: oocyte cryopreservation in women delaying reproduction can reduce cost per live birth', *Fertil Steril*, 103: 1446-53.e1-2.

Doyle, J. O., K. S. Richter, J. Lim, R. J. Stillman, J. R. Graham, and M. J. Tucker. 2016. 'Successful elective and medically indicated oocyte vitrification and warming for autologous in vitro fertilization, with predicted birth probabilities for fertility preservation according to number of cryopreserved oocytes and age at retrieval', *Fertil Steril*, 105: 459-66.e2.

Gürtin, Z. B., T. Shah, J. Wang, and K. Ahuja. 2019. 'Reconceiving egg freezing: insights from an analysis of 5 years of data from a UK clinic', *Reprod Biomed Online*, 38: 272-82.

Hammarberg K., Kirkman M., Pritchard N., Hickey M., Peate M., McBain J., Agresta F., Bayly C., and Fisher J. 2017. 'Reproductive experiences of women who cryopreserved oocytes for non-medical reasons', *Human Reproduction*, 32: 575-81.

Harper, J. C., K. Hammarberg, M. Simopoulou, E. Koert, J. Pedro, N. Massin, A. Fincham, and A. Balen. 2021. 'The International Fertility Education Initiative: research and action to improve fertility awareness', *Hum Reprod Open*, 2021: hoab031.

Hodes-Wertz, B., S. Druckenmiller, M. Smith, and N. Noyes. 2013. 'What do reproductive-age women who undergo oocyte cryopreservation think about the process as a means to preserve fertility?', *Fertil Steril*, 100: 1343-9.

Human Fertilisation and Embryology Authority, 2018. 'Egg Freezing in Fertility Treatment, Trends and Figures 2010-2016'

Inhorn, M. C., D. Birenbaum-Carmeli, L. M. Westphal, J. Doyle, N. Gleicher, D. Meirow, M. Dirnfeld, D. Seidman, A. Kahane, and P. Patrizio. 2018. 'Ten pathways to elective egg freezing: a binational analysis', *J Assist Reprod Genet*, 35: 2003-11.

Jones, K. T. 2008. 'Meiosis in oocytes: predisposition to aneuploidy and its increased incidence with age', *Hum Reprod Update*, 14: 143-58.

Kasaven, L. S., B. P. Jones, C. Heath, R. Odia, J. Green, A. Petrie, S. Saso, P. Serhal, and J. B. Nagi. 2022. 'Reproductive outcomes from ten years of elective oocyte cryopreservation', *Arch Gynecol Obstet*, 306: 1753-60.

Leung, A., Baker K., Vaughan D., Shah J., Korkidakis A., Ryley D., Sakkas D., and Toth T. 2021. 'Clinical outcomes and utilization from over a decade of planned oocyte cryopreservation', *Reproductive BioMedicine Online*, 43: 671-79.

Malchau, S. S., A. A. Henningsen, A. Loft, S. Rasmussen, J. Forman, A. Nyboe Andersen, and A. Pinborg. 2017. 'The long-term prognosis for live birth in couples initiating fertility treatments', *Hum Reprod*, 32: 1439-49.

Practice Committee of the American Society for Reproductive Medicine 'Evidence-based outcomes after oocyte cryopreservation for donor oocyte in vitro fertilization and planned oocyte cryopreservation: a guideline'. 2021. *Fertil Steril*, 116: 36-47.

Practice Committees of American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. 'Mature oocyte cryopreservation: a guideline'. 2013. *Fertil Steril*, 99: 37-43.

Mesen, T. B., J. E. Mersereau, J. B. Kane, and A. Z. Steiner. 2015. 'Optimal timing for elective egg freezing', *Fertil Steril*, 103: 1551-6.e1-4.

OECD-Social Policy Division, OECD Family Database, 2019

Santulli P., Blockeel C., Bourdon M., Coticchio G., Campbell A., De Vos M., Tryde Macklon K., Pinborg A., and Garcia-Velasco J. 2023. 'Fertility preservation in women with benign gynaecological conditions', *Human Reproduction Open*; 2023(2):hoad012

Stoop, D., B. Ermini, N. P. Polyzos, P. Haentjens, M. De Vos, G. Verheyen, and P. Devroey. 2012. 'Reproductive potential of a metaphase II oocyte retrieved after ovarian stimulation: an analysis of 23 354 ICSI cycles', *Hum Reprod*, 27: 2030-5.

Tsafrir, A., I. Ben-Ami, T. Eldar-Geva, M. Gal, N. Dekel, H. Levi, O. Schonberger, N. Srebnik, A. Weintraub, D. Goldberg, et al. 2022. 'Clinical outcome of planned oocyte cryopreservation at advanced age', *J Assist Reprod Genet*, 39: 2625-33.

Van Landuyt, L., N. P. Polyzos, N. De Munck, C. Blockeel, H. Van de Velde, and G. Verheyen. 2015. 'A prospective randomized controlled trial investigating the effect of artificial shrinkage (collapse) on the implantation potential of vitrified blastocysts', *Hum Reprod*, 30: 2509-18.

Van Landuyt, L., H. Van de Velde, A. De Vos, P. Haentjens, C. Blockeel, H. Tournaye, and G. Verheyen. 2013. 'Influence of cell loss after vitrification or slow-freezing on further in vitro development and implantation of human Day 3 embryos', *Hum Reprod*, 28: 2943-9.

van Loendersloot, L. L., L. M. Moolenaar, B. W. Mol, S. Repping, F. van der Veen, and M. Goddijn. 2011. 'Expanding reproductive lifespan: a cost-effectiveness study on oocyte freezing', *Hum Reprod*, 26: 3054-60.

Wafi A., Nekkebroeck J., Blockeel C., De Munck N., Tournaye H., and De Vos M. 2020. 'A follow-up survey on the reproductive intentions and experiences of women undergoing planned oocyte cryopreservation', *Reproductive BioMedicine Online*, 40: 207-14.

Wennberg A, Schildauer K., and Brännström M. 2019. 'Elective oocyte freezing for nonmedical reasons: a 6-year report on utilization and in vitro fertilization results from a Swedish center', *Acta Obstetricia et Gynecologica Scandinavica*, 98: 1429-34.

Table 1. Basic characteristics of women who underwent elective oocyte cryopreservation (EOC)	
Patient characteristics	
Number of women (*N*)	843
Cycles, N (mean per woman ± SD)	1,353 (1.6 ± 0.9)
Mean Age at EOC cycle (years ± SD)	36.5 ± 2.8
Antral Follicular Count (AFC) (mean ± SD)	13.5 ± 8.3
Anti-Müllerian Hormone (AMH) (ng/mL) (mean ± SD)	2.3 ± 2.0
Proportion of women who had a partner at the time of EOC	20.6% (174/843)
EOC cycle characteristics	
GnRH antagonist protocol (%)	85.3% (1,154/1,353)
GnRH agonist protocol (%)	14.7% (199/1,353)
GnRH antagonist pretreatment (%)	23.6 % (320/1,353)
Days of stimulation (*N*) (mean ± SD)	11.1 ± 2.1
Total gonadotrophin dose per cycle (IU) (mean ± SD)	2,395 ± 654
Laboratory data	
Total retrieved COC, N (mean/cycle ± SD)	14,750 (11.0 ± 7.4)
Number of retrieved COC/woman (mean ± SD)	17.6 ± 11.6
Total retrieved MII, N (mean/cycle ± SD)	11,620 (8.7 ± 5.8)
Number of MII retrieved /patient (mean ± SD)	13.9 ± 9.2
Total MII vitrified, N (mean/cycle ± SD)	11,606 (8.7 ± 5.8)
Number of MII vitrified/woman (mean ± SD)	13.9 ± 9.2
Mean oocyte maturation rate (%)	78.8% (11,620/14,750)

558 COC, cumulus oocyte complex; MII, metaphase II oocytes
559

560

Table 2. ART cycle characteristics of elective oocyte cryopreservation (EOC) patients who returned to the fertility clinic

Return rate (%)	27.4% (231/843)
Time between the first EOC cycle and return visit to the clinic (months) (mean ± SD)	39.9 ± 23.4
Age at return (y) (mean ± SD)	40.4 ± 3.1
AMH at return (ng/mL) (mean ± SD)	1.5 ± 1.5
AFC at return (*N*) (mean ± SD)	10.8 ± 6.6
Patients who embarked on oocyte warming as primary approach when they returned to the clinic (%)	47.6% (110/231)
Patients who embarked on IUI as primary approach when they returned to the clinic (%)	21.6% (50/231)
Patients who embarked on ovarian stimulation as primary approach when they returned to the clinic (%)	30.7% (71/231)
Patients with a partner at return (%)	68.3% (158/231)
Partner semen utilized per ICSI cycle (%)	58.2% (256/440)
Donor semen utilized per ICSI cycle (%)	41.8% (184/440)
Number of thawing cycles (mean/patient ± SD)	200 (1.4 ± 0.8)
Total number of oocytes warmed (mean/patient ± SD)	1844 (9.3 ± 4.7)
Mean oocyte survival rate after thawing (%)	82.6% (1524/1844)
Total number of usable embryos obtained after oocyte warming (mean/cycle ± SD)	494 (2.7 ± 1.8)
Number of ovarian stimulation cycles (mean/patient ± SD)	240 (2.2 ± 2.0)
Total number of usable embryos obtained after ovarian stimulation (mean/cycle ± SD)	506 (2.1 ± 2.1)
Total number of embryos transferred (*N*) / (mean/cycle ± SD) - Day 3 - Day 5/ Day 6	761 / (1.3 ± 0.5) 74.3% (442/595) 25.7% (153/595)
Positive hCG/transfer (%)	36.3 % (196/539)
Clinical pregnancy rate/transfer (%)	30.8% (166/539)
Live birth rate/transfer (%)	17.3% (93/539)
Cumulative ongoing pregnancy rate/patient (%)	55.4% (128/231)

Cumulative live birth rate/patient (%)	45.9% (106/231)
Early miscarriage rate (%)	30.7% (51/166)
Mean neonatal weight (g) (mean ± SD)	3,197.5 ± 716.6

561
562 AMH: Anti-Mullerian Hormone; AFC: Antral follicle count
563

564 **Table 3: Elective oocyte cryopreservation (EOC) and ART cycle characteristics of warmed-oocytes-**
565 **only (WOO) patients and fresh-oocytes-only (FOO) patients**
566

	WOO	FOO
Number of patients N (N/total EOC patients) (%)	90 (90/231) (39.0%)	52 (52/231) (22.5%)
EOC cycle characteristics		
Age at EOC (y), mean ± SD	37.3 ± 2.3	36.5 ± 3.0
AMH at EOC (ng/mL), mean ± SD	3.0 ± 2.5	2.1 ± 3.0
Women with a partner at EOC, N (%)	17 (19%)	21 (40%)
Number of EOC cycles, N (mean/patient ± SD)	189 (2.1 ± 1.4)	93 (1.8 ± 1.0)
Number of COC retrieved, N (mean/patient ± SD)	2,148 (11.3 ± 9.1)	755 (8.1 ± 5.4)
Number of mature oocytes retrieved, N (mean/patient ± SD)	1,717 (9.1 ± 7.2)	612 (11.7 ± 8.4)
Number of mature oocytes vitrified, N (mean/patient ± SD)	1,717 (9.1 ± 7.2)	607 (6.5 ± 4.7)
ART cycle characteristics		
Interval between EOC and return to clinic (months), mean ± SD	53.0 ± 21.9	29.8 ± 19.3
Age at return (y), mean ± SD	41.6 ± 3.0	39.0 ± 2.8
AMH at return (ng/mL), mean ± SD	1.5 ± 2.3	1.5 ± 1.5
Partner semen/cycle (%)	62% (64/104)	65% (46/71)
Donor semen/cycle (%)	39% (40/104)	35% (25/71)
Number of oocyte warming cycles in total , N (mean/patient ± SD)	112 (1.2 ± 0.6)	----
Number of ovarian stimulation cycles in total, N (mean/patient ± SD)	----	81 (1.6 ± 1.3)
Number of COC retrieved, N (mean/patient ± SD)	----	663 (13.0 ± 10.3)
Number of mature oocytes retrieved, N (mean/patient ± SD)	----	505 (9.9 ± 7.4)
Oocytes left in storage, N (mean/patient ± SD)	562 (6.2 ± 4.3)	607 (6.5 ± 4.7)
Total number of oocytes warmed, N (mean/patient ± SD)	1,155 (10.0 ± 5.2)	---
Embryos obtained, N (mean/cycle ± SD)	329 (3.0 ± 2.0)	196 (2.4 ± 2.3)
Positive hCG /transfer, % (N/No. of transfers)	47.4% (74/156)	50.5% (47/93)

Clinical pregnancy rate/transfer, % (N/No. of transfers)	38.5% (60/156)	44.1% (41/93)
Live birth rate/transfer, % (N/No. of transfers)*	23.7% (37/156)	26.9% (25/93)
Cumulative ongoing pregnancy rate/patient, % (N/No. of patients)	52.2% (47/90)	55.7% (29/52)
Cumulative live birth rate/patient, % (N/No. of patients) *	41.1% (37/90)	48.1% (25/52)
Early miscarriage rate, % (N/No. of clinical pregnancies)	25.0% (12/60)	29.3% (12/41)

567
568 COC: Cumulus-oocyte complex; AMH: Anti-Mullerian Hormone; AFC: Antral follicle count
569 * 10 pregnant patients had not delivered at the time of writing
570

571 **Table 4:** Multivariate logistic regression models to identify the characteristics associated with the decision to
572 thaw oocytes as the primary strategy upon return to the clinic.
573
574

	Odds Ratio	**p-value**	**95% Confidence Interval**
Age (y) at EOC	1.27	0.11	0.94-1.71
Total number of oocytes cryopreserved	1.09	0.03	1.00-1.20
AMH at return	0.78	0.32	0.49-1.26
Number of months between EOC and return to the clinic	1.05	0.004	1.01-1.09

575 EOC: Elective oocyte cryopreservation; AMH: Anti-Mullerian Hormone
576
577

578 **Table 5**: Multivariate logistic regression models to identify the characteristics associated with ongoing
579 pregnancy upon return to the clinic.
580
581
582

	Odds Ratio	**p-value**	**95% Confidence Interval**
Age (y) at EOC	0.95	0.71	0.74-1.21
Total number of oocytes cryopreserved	1.08	0.06	0.99-1.17
AMH at return	0.79	0.29	0.50-1.23
Number of months between EOC and return to the clinic	0.96	0.07	0.93-1.00
Decision to thaw oocytes	0.71	0.59	0.21-2.44

583
584