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Assessing the effect on pregnancy outcomes of artificial frozen embryo transfer cycles in women with advanced age following oocyte donation

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Abstract

Aim of the study: The optimal frozen embryo transfer (FET) preparation protocol is yet to be determined. Data have shown potentially better outcomes after FET in natural cycle (NC-FET) when compared to artificial cycle (AC-FET). However, it remains unknown whether this association still exists in women in later reproductive years. The main propose of this study was to assess whether female age could be a predictor of worse outcome after transferring frozen embryos in NC (versus AC) in women receiving donated oocytes.

Methods: This retrospective, multicentre, cohort study included women who underwent at least one FET of a single embryo at blastocyst stage from an oocyte donation cycle performed between January 2010 and December 2019. We divided the cohort into two study groups: NC-FET and AC-FET. The main objective of our study was to compare live birth delivery rates (LBR). The secondary outcomes of our study included 1) spontaneous miscarriage rates, 2) clinical pregnancy rate (CPR) and 3) human chorionic gonadotropin (hCG) positive pregnancy.

Results: In total 62530 embryo transfers were analysed. Results showed that despite higher hCG positive pregnancy (62.81% vs 58.72%; OR 0.842 CI 0.7293-0.895) and higher CPR (53.95% vs 50.52%; OR 0,872 CI 0.821-0.925), AC were associated also to a higher miscarriage rate (28.95% vs 24.32%; OR 0.788 CI 0.720-0.862) resulting in a trend towards lower LBRs with AC (41.60% vs 42.56%; OR 1.040 CI 0.979-1.105) that became significant after confounder adjustment (OR 1.303 CI 1.203-1.412). The interaction with female age was not statistically significant (aOR 1.015, 95% CI 0.994-1.036), thus rejecting the hypothesis that any eventual differences between cycles may be modified according to the age of the recipient.

Conclusion: This study showed that NC-FET is slightly better, which is in agreement with the findings of previous studies on this question. We found no evidence that the differences between the two endometrial preparations are modified by the age of the woman.

Keywords: Natural cycle; Artificial cycle; Oocyte donation; Advanced maternal age; Frozen embro transfer.

Resumo

Objetivo do estudo: O protocolo ideal de preparação endometrial para transferência de embriões congelados ainda não foi determinado. Estudos mostram resultados potencialmente melhores após a transferência em ciclo natural em comparação com ciclo artificial. No entanto, não é conhecido se essa associação se mantém em mulheres de idade avançada. O principal objetivo deste estudo foi avaliar se a idade poderia ser um preditor de pior desfecho após a transferência de embriões congelados em ciclo natural (versus ciclo artificial) num modelo de ovodoação.

Métodos: Este estudo de coorte retrospectivo e multicêntrico, incluiu mulheres que foram submetidas a pelo menos uma transferência de um único embrião no estádio de blastocisto num ciclo de ovodoação realizado entre janeiro de 2010 e dezembro de 2019. Dividimos a amostra em dois grupos de estudo: ciclo natural e ciclo artificial. O objetivo principal do estudo foi comparar as taxas de nados-vivos entre os dois grupos. Os desfechos secundários de nosso estudo incluíram 1) taxa de aborto espontâneo, 2) taxa de gravidez clínica e 3) taxa de gravidez bioquímica.

Resultados: No total foram analisadas 62530 transferências de embriões. Os resultados mostraram que apesar do ciclo artificial apresentar uma taxa de gravidez bioquímica (62.81% vs 58.72%; OR 0.842 CI 0.7293-0.895) e uma taxa de gravidez clínica superior (53.95% vs 50.52%; OR 0,872 CI 0.821-0.925), está associado a uma maior taxa de aborto (28.95% vs 24.32%; OR 0.788 CI 0.720-0.862) o que resulta numa menor taxa de nados vivos (41.60% vs 42.56%; OR 1.040 CI 0.979-1.105) que é estatisticamente significativa após ajuste de fatores de confundimento (OR 1.303 CI 1.203-1.412). A interação com a idade da mulher não foi estatisticamente significativa (aOR 1,015, IC 95% 0,994-1,036), rejeitando assim a hipótese de que eventuais diferenças entre os ciclos possam ser modificadas de acordo com a idade da recetora.

Conclusão: Este estudo mostrou que o ciclo natural parece ser ligeiramente superior, o que está de acordo com os achados de estudos anteriores. Mostrou ainda, que, as diferenças entre as duas preparações endometriais não são modificadas pela idade da mulher.

Palavras-chave: Ciclo natural; Ciclo artificial; Ovoadoação; Idade materna avançada, Transferência de embriões congelados.

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List of abbreviations

AC artificial cycle

ART assisted reproductive technology

CPR clinical pregnancy rate

eFET elective frozen embryo transfer

ET embryo transfer

FET frozen embryo transfer

GLM generalized linearized models

GnRH gonadotropin-releasing hormone

hCG human chorionic gonadotropin

HP-HMG highly purified human menopausal gonadotrophin

ICMART International Committee for Monitoring Assisted Reproductive Technologies

ICSI intracytoplasmic sperm injection

IVF in vitro fertilization

LBW low birthweight

LBR live birth delivery rate

LFDF luteal-phase dominant follicle

LH luteinizing hormone

LPS luteal phase support

MAR medically assisted reproduction

NC natural cycle

OHSS ovarian hyperstimulation syndrome

OPR ongoing pregnancy rate

OS ovarian stimulation

PGT preimplantation genetic testing

RCT randomized controlled trial

rFSH recombinant follicle-stimulating hormone

WHO World Health Organisation

Introduction

1. Infertility and its burden

Infertility is defined as “a disease characterised by the failure to establish a clinical pregnancy after 12 months of regular, unprotected sexual intercourse or due to an impairment of a person's capacity to reproduce either as an individual or with his/her partner” (Zegers-Hochschild et al., 2017) and it has been recognized as a global public health issue by the World Health Organisation (WHO) since 2006. The inability to have children affects approximately 9% of reproductive-aged couples around the globe which is approximately 72,4 million people worldwide (Boivin et al., 2007). Infertility is a condition that has important economic, demographic and medical implications. Furthermore, anxiety, depression, low self-esteem, impaired sexual satisfaction, marital distress and marital disruption are frequent in couples suffering from infertility. (Davari Tanha et al., 2014). It is also associated discrimination and ostracism (Chachamovich et al., 2010).

2. Assisted reproductive technology (ART)

ART is defined as “all interventions that include the in vitro handling of both human oocytes and sperm or of embryos for the purpose of reproduction. This includes, but is not limited to, in vitro fertilization (IVF) and embryo transfer (ET), intracytoplasmic sperm injection (ICSI), embryo biopsy, preimplantation genetic testing (PGT), assisted hatching, gamete and embryo cryopreservation, semen, oocyte and embryo donation, and gestational carrier cycles”. The broader term "medically assisted reproduction" (MAR) also includes intrauterine insemination, either with partner or donor sperm (Zegers-Hochschild et al., 2017). Indications for ART are growing as a result of societal changes and increasing desire and opportunity to preserve fertility, for example in women wishing to attempt conception at an older age, or for medical reasons such as preservation of oocytes prior to cytotoxic anticancer therapy (Bosch et al., 2020). ART has also developed vastly towards finding both more convenient and individualised treatment options, while increasing in complexity and cost. However, its success rate is

still approximately 30%, falling short of what is desirable (Kupka et al., 2014) for the 56% of infertile couples seeking medical care that eventually perform it (Boivin et al., 2007).

IVF is defined as a sequence of procedures that involves extracorporeal fertilization of gametes aiming to obtain viable embryos that will eventually be transferred to the uterus. It includes conventional in vitro insemination and ICSI, with the latter entailing the injection of a single spermatozoon into the oocyte cytoplasm (Zegers-Hochschild et al., 2017). IVF was initially designed to overcome fallopian tube blockage (Inhorn & Patrizio, 2015) and was first performed in England in 1978 by Robert Edwards following a natural cycle (NC) without ovarian stimulation (OS) and with a single embryo transferred. Since then, many improvements have been made to the process in order to increase success and safety rates, namely the use of exogenous OS (Ruth Howie & Vanessa Kay, 2018). The major steps of a IVF cycle nowadays are: 1) retrieving the woman's oocytes from the ovaries (following OS); 2) exposing the oocytes to the spermatozoa – fertilization; 3) embryo(s) culture for 2 to 7 days and 4) transferring the embryo to the uterus (Jorgensen, 2006). OS plays an important role in the success of ART, and it includes three basic components: 1) stimulation of the development of multiple follicles using exogenous gonadotrophins, 2) suppression of pituitary function to prevent premature ovulation using gonadotropin-releasing hormone (GnRH) agonist or antagonist and 3) triggering the final oocyte maturation 34-40h prior to oocyte retrieval. For this latter step, mostly human chorionic gonadotropin is used (owing to it similar biological function in the ovaries when compared to luteinizing hormone (LH), but a GnRH agonist in an antagonist protocol can also be used, since it will cause a temporary displacement of the GnRH antagonist from its receptors followed by an endogenous LH surge (Gallos et al., 2017).

With the use of OS it is possible to stimulate multiple follicles, assuring that a sufficient number of oocytes is available to obtain high quality embryos (Gallos et al., 2017). The transfer of multiple embryos at one time increases the chance that at least one would implant and produce a live birth. However, an obvious consequence of this practice was an increase in multiple gestations and related complications.

3. Cryopreservation and frozen embryo transfers in modern-day medicine

The widespread use of superovulation regimes for multiple oocyte collection frequently results in the production of multiple embryos. Therefore, there was a need to develop human embryo cryopreservation strategies to store excess embryos. Cryopreservation is defined as "the process of slow freezing or vitrification to preserve biological material (e.g. gametes, zygotes, cleavage-stage embryos, blastocysts or gonadal tissue) at extreme low temperature" (Zegers-Hochschild et al., 2017).

Since the first delivery with cryopreserved embryos, many strategies have been applied with differences such as the type and concentration of cryoprotectants, equilibration timing, cooling rates and cryopreservation devices used. The two main approaches that have been adopted were slow-freezing and vitrification. In contrast to slow-freezing, vitrification is a cryopreservation method that allows solidification of the cell(s) and the extracellular milieu into a glass-like state without the formation of ice. Vitrification is now known to be superior to slow-freezing (Rienzi et al., 2016) and, because of that, many laboratories all over the world have completely replaced slow-freezing with vitrification (Rienzi et al., 2016).

FET is defined as an ART procedure in which cycle monitoring is carried out with the intention of transferring to a woman, frozen/thawed or vitrified/warmed embryo(s) (Zegers-Hochschild et al., 2017). As a result of improvements in the performance of cryopreservation programs, the practice of freezing embryos for subsequent FET at a time that is more appropriate, led to an increase in cumulative livebirth rates (Bosch et al., 2020). The proportion of cryopreserved embryo transfer cycles compared with fresh cycles is growing in Europe and nowadays cryopreservation is no longer considered just a supplement to fresh embryo transfer, but instead it has become one increasingly important part of IVF (Doody, 2014). The main medical reasons for elective embryo cryopreservation have also expanded beyond excessive ovarian response and/or high-risk to develop ovarian hyperstimulation syndrome (OHSS) to include PGT, elevated progesterone in the late follicular phase and poor endometrial development.

4. The freeze-all strategy as a driver in the increase in frozen embryo transfers

Although fresh embryo transfer is still frequently applied in different reproductive centres worldwide, FET, and especially the "freeze all" protocol, is progressively gaining support (Shapiro et al., 2014). "Freeze-all" is a strategy to cryopreserve all good quality embryos produced in a fresh cycle and to transfer these embryos in subsequent natural or artificially prepared cycles. The major benefit of this strategy is the increased safety with less occurrence of OHSS, which is the major complication in IVF and a potentially life-threatening condition (Kawwass et al., 2015). Its clinical presentation is variable among patients, but the most common clinical signs are abdominal discomfort, nausea, vomiting and dyspnoea (Vloeberghs et al., 2009). OHSS also increases the risk of many other complications such as ovarian torsion and venous thromboembolism. To that extent, the use of GnRH antagonist co-treatment is currently the most important ovarian stimulation-related strategy to minimize the incidence of OHSS, followed by GnRH agonist triggering and the elective deferral of the embryo transfer. Together, these secondary preventive strategies have practically annulled the modern-day risk of OHSS (Santos-Ribeiro et al., 2019).

Furthermore, the freeze-all protocol might also enhance the number of recruited oocytes and it allows a much more flexible process in which the stimulation can in theory start at any day of the cycle and the embryo transfer can be scheduled allowing better timings to the women and to the clinic (Blockeel et al., 2016). Other potential advantages of performing elective FET (eFET) instead of fresh cycles are potentially improved pregnancy rates, reduced ectopic pregnancy rates and better obstetrical and neonatal outcomes such as lower low birthweight (LBW) rate newborns, and preterm birth rates (Blockeel et al., 2016). Conversely, when compared with fresh ET, FET is thought to be associated with other adverse perinatal outcomes, including gestational hypertension, abnormal placenta formation, post-term birth and macrosomia (Jing et al., 2019).

Despite the recent raise in the use of eFET as a component of ART, the debate continues regarding whether eFET should be a standard treatment option available to

all the patients or if it is important to identify patient subgroups who are most likely to benefit from such an approach (Bosch et al., 2020).

5. Natural cycle versus artificial cycle FET

Endometrium preparation protocols for the thawed embryo includes the natural cycle and artificial cycle. Although FET is increasingly used for multiple indications, the optimal preparation protocol is yet to be determined. (Glujovsky et al., 2020)

In a NC-FET, medical intervention is mostly limited to endocrine and ultrasound monitoring during the proliferative phase, to schedule the transfer when the endometrium is synchronized to the developmental stage of the embryo (Mackens et al., 2017). For the NC-FET, proliferative phase monitoring is the key. The timing of ovulation can be estimated based either on the detection of LH surge in urine or in blood (which is the "true" NC-FET) or after exogenously triggering ovulation with hCG (which is frequently called a "modified" NC-FET) or even supporting the luteal phase with progesterone (also known as natural cycle with luteal phase support (LPS)) (Jing et al., 2019). A clear advantage of this protocol is not requiring to take as much medication for several weeks; conversely, NC-FET is also more unpredictable, entailing more frequent visits to the clinic, less cycle control and flexibility and holding a higher risk of cycle cancellation (Mackens et al., 2017). Another potential advantage is a lower miscarriage risk (Lin et al., 2020).

Due to the above-mentioned disadvantages in terms of cycle scheduling, AC-FET is also widely used. In this protocol, the endometrium is artificially prepared with exogenous steroid hormone therapy. This approach reduces the frequency of medical visits, is more flexible, can be performed based on convenience and decreases cancellation rates. Also, it is very useful in patients with irregular cycles. However, the main disadvantages of this approach are a potentially increased risk for both hypertension during pregnancy and caesarean section deliveries (Jing et al., 2019).

6. Effects of female age on the reproductive system and oocyte donation

Maternal age is still the most important factor influencing reproductive outcomes (Bosch et al., 2020). In many countries, the mean maternal age at first birth is rising associated with societal changes, namely increased education expectations for women, higher employment rates and higher access to reliable birth control. On the other hand, this has been associated with a decline in fertility (Tj & Be, 2002). Specifically, fecundability, which is the probability of achieving a pregnancy in one menstrual cycle, decreases gradually but significantly from the age of 32 and faster after the age of 37 (Female Age-Related Fertility Decline, 2014), a phenomena that is likely multifactorial. Although women are born with a predetermined number of oocytes, which declines with age, the main hindering factor is mostly thought to be the quality of oocytes, which also decline due to a higher prevalence of meiotic errors (Jones, 2008). Thus, as women age, the number of chromosomally abnormal oocytes/embryos increases, which results in lower fertility and increased rates of miscarriage. In addition to the endogenous accumulation of genetic errors in the oocyte pool over time, other factors such as smoking, other toxic environmental exposures, and certain medical and surgical treatments can compromise oocyte quality, ovarian reserve, and the chance for a healthy outcome for pregnancy as women age.

The first reported human pregnancies and births from oocyte and embryo donation were in the early 1980s (Bustillo et al., 1984). Oocyte donation was initially only offered to women with primary ovarian insufficiency or with important genetic diseases. Currently, however, it has become an important part of assisted reproductive care and is also used in women with other reproductive disorders and often by those of advanced reproductive age (Sauer & Kavic, 2006; Sauer, 2018). Studies have shown that there has been an increase in the use of donor oocytes with ART, in the percentage of cycles using frozen rather than fresh embryos and this has resulted in good perinatal outcomes, regardless of recipient age (Kawwass, 2013). However, the rates of gestational hypertension are higher in women undergoing IVF with donor oocytes when compared with those using autologous oocytes.

Oocyte donation can be accomplished other by the synchronizing the menstrual cycle of the recipient with a stimulated cycle of the woman who is donating her oocytes or via eFET. Via eFET it is possible to perform NC and AC. Conversely, whenever donor-recipient cycle synchronization is performed, AC is generally preferred. The menstrual cycle itself also undergoes changes as women age, namely owing to the decline in ovarian reserve which may lead to the reduction in hormone production by the ovaries (Gougeon et al., 1994). It is known that the prevalence of luteal-phase dominant follicles (LFDF) does not increase with age (Brink et al., 2013) but they emerge earlier, are larger, persist for longer and are associated with a decline in progesterone and inhibin A production, and with an increase in estradiol production. These variations in antral folliculogenesis may contribute to luteal insufficiency in the late reproductive years (Baerwald et al., 2018), abnormalities which may deem the NC-FET less efficient in women of more advanced age. This clinical rationale is frequently used as a justification by clinicians to perform AC-FET in women of advanced age receiving donated oocytes even when they menstruate regularly. However, robust evidence that NC-FET in such population is inferior is lacking.

7. Gaps of knowledge and aim of this thesis

Data have shown potentially better ART outcomes after transferring frozen embryos in natural cycles when compared to transferring in an artificial cycle. However, it remains unknown whether this association still exists in women in later reproductive years. The main advantages of studying embryo implantation in the population of oocyte recipients is that one is able to exclude the potential confounding effect of lower embryo competence from older women and also to exclude the effect that ovarian stimulation may have on endometrial receptivity.

The main propose of this study was to assess whether female age could be a predictor of worse outcome after transferring frozen embryos in NC-FET (versus AC-FET) in women receiving donated oocytes.

Materials and methods

1. Population, design and selection criteria

This retrospective, multicentre, cohort study included women who underwent at least one FET of a single embryo at blastocyst stage from an oocyte donation cycle performed between January 2010 and December 2019 at one of IVI-RMA clinics in the Iberian Peninsula. We divided the cohort into two study groups, according to whether a natural or artificial FET was performed. Approval from the local ethics committee was obtained prior to performing the study.

We included oocyte recipients aged between 18 and 49 years old. The decision to include a wide span of female recipient was intentional, in order to maximize the exploration of any potential effect-modification caused by this variable on the studied outcomes according to the type of FET. Only FETs in which the embryos were cryopreserved by vitrification were included. In order to minimize confounding derived from woman with poor prognosis, we excluded cases with untreated uterine/tubal abnormalities (including congenital abnormalities, such as septate uterus and bicornuate uterus, submucosal myomas, endometrial polyps, Asherman's syndrome and hydrosalpinx). Moreover, cases in which preimplantation genetic testing was performed were also excluded. Finally, we also excluded cases with a missing result for the main exposure and outcome variables.

2. Ovarian stimulation protocol for donors and FIV

Donors were downregulated with GnRH antagonist or progesterone. Women began OS with recombinant follicle-stimulating hormone (rFSH) or highly purified human menopausal gonadotrophin (HP-HMG) to stimulate follicular recruitment and growth. The dose was adjusted to the ovarian response. Stimulation was carried out until three follicles of ≥18mm were observed. Final oocyte maturation and ovulation triggering was performed with 0.2 mg to 0.3 mg of triptorelin and oocyte retrieval was performed 35-36h later. Donors were matched with their recipients according to phenotype and blood groups.

The insemination of the collected oocytes was performed via either conventional IVF or ICSI. Fertilization was evaluated approximately 18 hours after microinjection or insemination. The embryo development was assessed daily until cryopreservation. Good quality embryos were cryopreserved on either day 5 or 6 of embryo culture using vitrification according to the AESIBIR embryo grading classification.

3. Frozen embryo transfers protocols

3.1 Natural cycle

In a natural cycle, ovulation occurred either spontaneously (true natural cycle) or artificially triggered (modified natural cycle). True natural cycle was performed after documentation of spontaneous ovulation with endocrine and/or ultrasound monitoring, while in the modified natural cycle ovulation of a dominant follicle (>16 mm) was triggered with exogenous hCG. In both instances, ovulation was followed by exogenous administration of progesterone 200 mg 12/12h. In a true natural cycle, embryo transfer was on the 6th day after LH surge, while in a modified natural cycle embryo transfer was on the 7th day after hCG injection.

3.2 Artificial cycle

In the artificial cycle, preparation consisted of oestradiol valerate administration at a dose of 6 mg/day orally (2mg, three times daily) or transdermally (100-200 ug every 48 hours). An ultrasound scan was performed after approximately an 8-to-14-day period of estrogen administration to measure endometrial thickness and exclude the presence of pre-ovulatory follicle(s), corpus luteum or luteinized endometrium. As soon as the endometrial thickness measured >7 mm, vaginal progesterone supplementation was started at a dose of 800mg/day (400mg twice a day) to promote the last phase of endometrial preparation prior to embryo transfer. Embryo transfer was scheduled on the 6th day of progesterone administration.

Over the course of the last 15 years, the clinics included in this analysis have progressively included routine monitoring of luteal phase progesterone on the day of embryo transfer, given that a low level has been shown to be associated with a

reduction of pregnancy outcomes (Melo et al., 2021). The thresholds to define low circulating progesterone and strategies to circumvent this issue varied immensely over time and was decided according at the physician's discretion.

4. Main outcomes measures

The main objective of our study was to compare LBR after cryopreserved embryo transfer between natural cycle and artificial cycle. LBR was defined in accordance to the International Committee for Monitoring Assisted Reproductive Technologies (ICMART), as the number of deliveries that resulted in at least one live birth, expressed per 100 cycle attempts. In this case the denominator refers to FETs (Zegers-Hochschild et al., 2017). Live birth was defined as such after 22 completed weeks of gestational age (Zegers-Hochschild et al., 2017).

The secondary outcomes of our study included 1) spontaneous miscarriage rate, defined as the spontaneous loss of an intra-uterine pregnancy prior to 22 completed weeks of gestational age, 2) clinical pregnancy rate, defined as the ultrasonographic visualization of one or more gestational sacs and 3) hCG positive pregnancy, defined as a pregnancy diagnosed only by the detection of beta hCG in serum or urine. (Zegers-Hochschild et al., 2017).

5. Statistical analysis

Categorical data was presented using absolute and relative frequencies, while continuous values were be summarized using means and standard deviations. Unadjusted between-group comparisons were performed using the chi-square test for categorical variables and the t-test for continuous variables. Confounder-adjustment was performed using multivariable linear/logistic generalized linearized model (GLM) regression analysis, adjusting for female donor and recipient age, recipient body mass index, number of mature oocytes donated, oocyte status (fresh versus vitrified), sperm source (fresh versus frozen), embryo status (fresh versus vitrified), endometrial thickness on the day of planning and other infertility diagnosis (beyond advanced maternal age and low ovarian reserve, subdivided in ovulatory factor infertility, tubal

factor infertility, uterine factor infertility, infertility diagnosis due endometriosis, other female infertility diagnoses and male factor infertility). In order to assess whether there was any effect modification of female age on the main outcome measure according to the type of FET, we also attempted to include an interaction variable in the final multivariable model. Finally, a sensitivity analysis was also performed to assess whether these results may not be influenced by year of treatment, to account for potential changes in treatment strategies over time.

Stata Software version 13.1 (StataCorp, College Station, Texas, USA) was used for statistical analysis, with a p-value <0.05 being considered as statistically significant and followed by Bonferroni-adjusted pairwise comparisons whenever warranted.

Results

1. Baseline patient and cycle characteristics

A total of 62530 embryo transfers were included in this analysis (57529 using an artificial cycle and 5001 using a natural cycle). The mean age of oocyte donors in our sample was 25.44 years old and the mean age of recipient was 41.55 years old. Infertility was mostly of a primary type (72.10%) and other infertility diagnosis beyond advanced maternal age and low ovarian reserve were male factor infertility (25.60%), endometriosis (3.30%), uterine factor (1.60%), ovulatory factor (1.30%) and tubal factor (1%). Approximately 53.60% of the embryos were fresh and 46.60% vitrified-thawed. Smoking status was unavailable in 9847 recipients and embryo quality was also unavailable in 42925 cycles. Table 1 summarizes the donor and recipient's characteristics and cycle details and table 2 refers to embryo transfer cycle characteristics.

	Total (n=62530)	Artificial cycle (AC) (n=57529)	Natural cycle (NC) (n=5001)	p-value
Baseline donor cycle characteristics				
Age at retrieval ± SD (years)	25.44 ± 4.26	25.44 ± 4.26	25.45 ± 4.21	0.878
BMI ± SD (kg/m²)	22.49 ± 3.04	22.49 ± 3.04	22.42 ± 2.95	0.084
Smoking status				0.061
Current	28250 (51.50%)	26027 (51.42%)	2223 (52.02%)	
Past	1431 (2.60%)	1343 (2.65%)	88 (2.06%)	
No	25205 (45.90%)	23243 (45.92%)	1962 (45.92%)	
Baseline ART cycle characteristics				
Sperm source				0.149
Donated	12454 (20.40%)	11489 (20.45%)	965 (19.58%)	
Partner	48649 (79.60%)	44685 (79.55%)	3964 (80.42%)	
Oocyte status				0.057
Fresh	33894 (54.20%)	31248 (54.32%)	2646 (52.91%)	
Vitrified-thawed	28636 (45.80%)	26281 (45.68%)	2355 (47.09%)	
Number of oocytes retrieved ± SD	13.06 ± 3.88	13.04 ± 3.89	13.33 ± 3.78	<0.001
Number of donated MII oocytes ± SD	11.74 ± 3.67	11.76 ± 3.65	11.52 ± 3.88	<0.001
Baseline recipient cycle characteristics				
Age at transfer ± SD (years)	41.55 ± 4.14	41.59 ± 4.17	41.08 ± 3.74	<0.001
Duration of infertility ± SD (years)	3.59 ± 2.82	3.62 ± 2.84	3.29 ± 2.65	<0.001
Type of infertility				0.771
Primary	41790 (72.10%)	38515 (72.13%)	3275 (72.34%)	

Secondary	16134 (27.90%)	14882 (27.87%)	1252 (27.66%)	
BMI ± SD (kg/m²)	23.56 ± 4.04	23.58 ± 4.06	23.28 ± 3.77	<0.001
Female smoking status				0.017
Current	8489 (16.10%)	7876 (16.18%)	613 (15.32%)	
Past	2784 (5.30%)	2604 (5.35%)	180 (4.50%)	
No	41410 (78.60%)	38202 (78.47%)	3208 (80.18%)	
Other infertility diagnoses				
Tubal factor	655 (1.00%)	576 (1.00%)	79 (1.58%)	<0.001
Ovulatory factor	804 (1.30%)	716 (1.24%)	88 (1.76%)	0.002
Endometriosis	2051 (3.30%)	1833 (3.19%)	218 (4.36%)	<0.001
Uterine factor	983 (1.60%)	919 (1.60%)	64 (1.28%)	0.094
Other female factors	1580 (2.50%)	1359 (2.36%)	221 (4.42%)	<0.001
Male factor infertility	16030 (25.60%)	14530 (25.26%)	1500 (29.99%)	<0.001

Table 1 - Baseline characteristics (per embryo transfer)

	Total (n=62530)	**Artificial cycle (AC) (n=57529)**	**Natural cycle (NC) (n=5001)**	**p-value**
Embryo status				<0.001
Fresh	33476 (53.60%)	32468 (56.53%)	1008 (20.17%)	
Vitrified-thawed	28955 (46.40%)	24965 (43.47%)	3990 (79.83%)	
Embryo quality				<0.001
A	9221 (29.90%)	8754 (30.40%)	467 (22.31%)	
B	17237 (55.80%)	16054 (55.76%)	1183 (56.52%)	
C + D + E	4427 (14.30%)	3984 (13.84%)	443 (21.17%)	
Cycle status at planning				
Endometrial thickness ± SD (mm)	8.70 ± 1.81	8.69 ± 1.81	8.82 ± 1.76	<0.001
Serum E2 ± SD (pg/mL)	288.83 ± 248.08	291.70 ± 254.73	251.72 ± 129.42	<0.001
Serum P4 ± SD (pg/mL)	0.25 ± 0.27)	0.24 ± 0.25	0.40 ± 0.37	<0.001

Table 2 – Embryo transfer cycle characteristics (per embryo transfer)

2. Pregnancy outcomes according to type of FET

Table 3 summarizes pregnancy outcomes of all transfers. The results showed that there were no major differences between cycles, even after performing confounder adjustment (figure 1), being NC-FET slightly better than AC-FET with higher LBRs (59.30% versus 52.67%, respectively) and lower miscarriage rates (17.50% versus 23.55%, respectively).

	Total	Artificial cycle (AC)	Natural cycle (NC)	OR (95% CI)	Adjusted OR (95% CI)
Total					
hCG positive pregnancy	35788/57289 (62.47%)	32981/52509 (62.81%)	2807/4780 (58.72%)	0.842 (0.793-0.895)	1.048 (0.969-1.132)
Clinical pregnancy	30745/57289 (53.67%)	28330/52509 (53.95%)	2415/4780 (50.52%)	0.872 (0.821-0.925)	1.078 (0.998-1.165)
Miscarriage	10122/35404 (28.59%)	9447/32628 (28.95%)	675/2776 (24.32%)	0.788 (0.720-0.862)	0.689 (0.613-0.773)
Live birth delivery	22969/55112 (41.68%)	20976/50429 (41.60%)	1993/4683 (42.56%)	1.040 (0.979-1.105)	1.303 (1.203-1.412)
Fresh embryos					
hCG positive pregnancy	20469/29090 (70.36%)	19829/28181 (70.36%)	640/909 (70.41%)	1.002 (0.868-1.160)	4.143 (0.529-32.445)
Clinical pregnancy	17941/29090 (61.67%)	17372/28181 (61.64%)	569/909 (62.60%)	1.041 (0.909-1.194)	2.744 (0.591-12.747)
Miscarriage	5084/20230 (25.13%)	4965/19599 (25.33%)	119/631 (18.86%)	0.685 (0.557-0.835)	1.973 (0.551-7.069)
Live birth delivery	13820/27848 (49.63%)	13327/26957 (49.44%)	493/891 (55.33%)	1.267 (1.108-1.449)	1.253 (0.379-4.144)
Vitrified-thawed embryos					
hCG positive pregnancy	15319/28199 (54.32%)	13152/24328 (54.06%)	2167/3871 (55.98%)	1.081 (1.009-1.157)	1.052 (0.973-1.137)
Clinical pregnancy	12804/28199 (45.41%)	10958/24328 (45.04%)	1846/3871 (47.69%)	1.112 (1.039-1.190)	1.079 (0.998-1.166)
Miscarriage	5038/15174 (33.2%)	4482/13029 (34.40%)	556/2145 (25.92%)	0.667 (0.601-0.739)	0.679 (0.604-0.763)
Live birth delivery	9149/27264 (33.56%)	7649/23472 (32.59%)	1500/3792 (39.56%)	1.354 (1.261-1.453)	1.314 (1.212-1.424)

Table 3 - Pregnancy outcomes of all transfers (per embryo transfer)

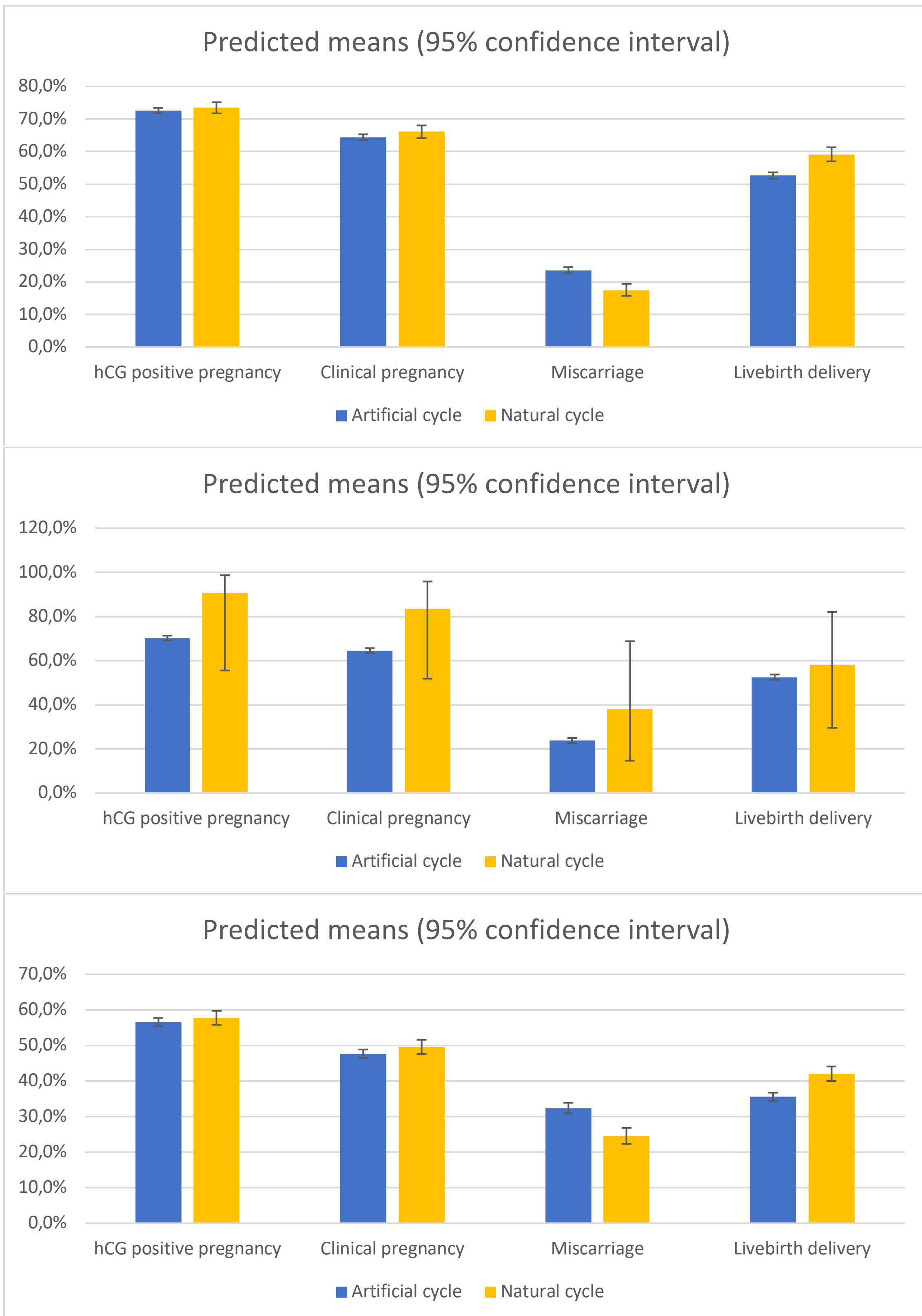

Figure 1 – Pregnancy outcomes (A) all transfers (B) fresh embryos (C) vitrified-thawed embryos

3. Effect of female age and year of treatment on livebirth according to type of FET

In order to assess whether there was any effect modification of female age on the main outcome measure according to the type of FET, we attempted to include an interaction variable in the final multivariable model. The interaction was not statistically significant (aOR 1.015, 95% CI 0.994-1.036), thus rejecting the hypothesis that any eventual differences between cycles may be modified according to the recipient's age. To aid the evaluation of the lack of this effect modification, we also plotted the distribution of FET live birth rates according to female age and type of FET in Figure 2.

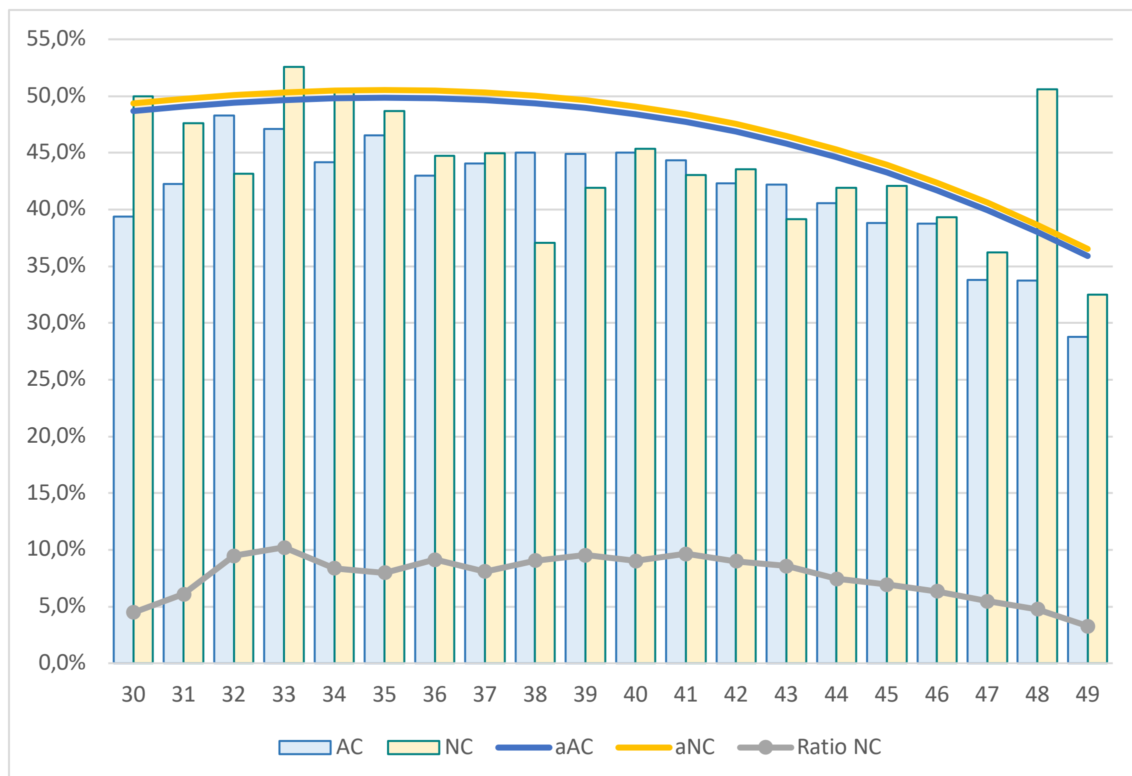

Figure 2 - Live birth rates by age: natural cycle vs artificial cycle (per embryo transfer)

Finally, a sensitivity analysis was also performed to assess whether these results may not be influenced by year of treatment, to account for potential changes in treatment strategies over time, namely the progressive introduction of luteal phase progesterone monitoring among the clinics. Figure 3 showed that serum progesterone monitoring and supplementation has not been sufficient to eliminate LBR discrepancies between

natural and artificial cycles throughout the years. In fact, year of treatment was not associated with LBR (aOR 1.000, 95% CI 0.990-1.010).

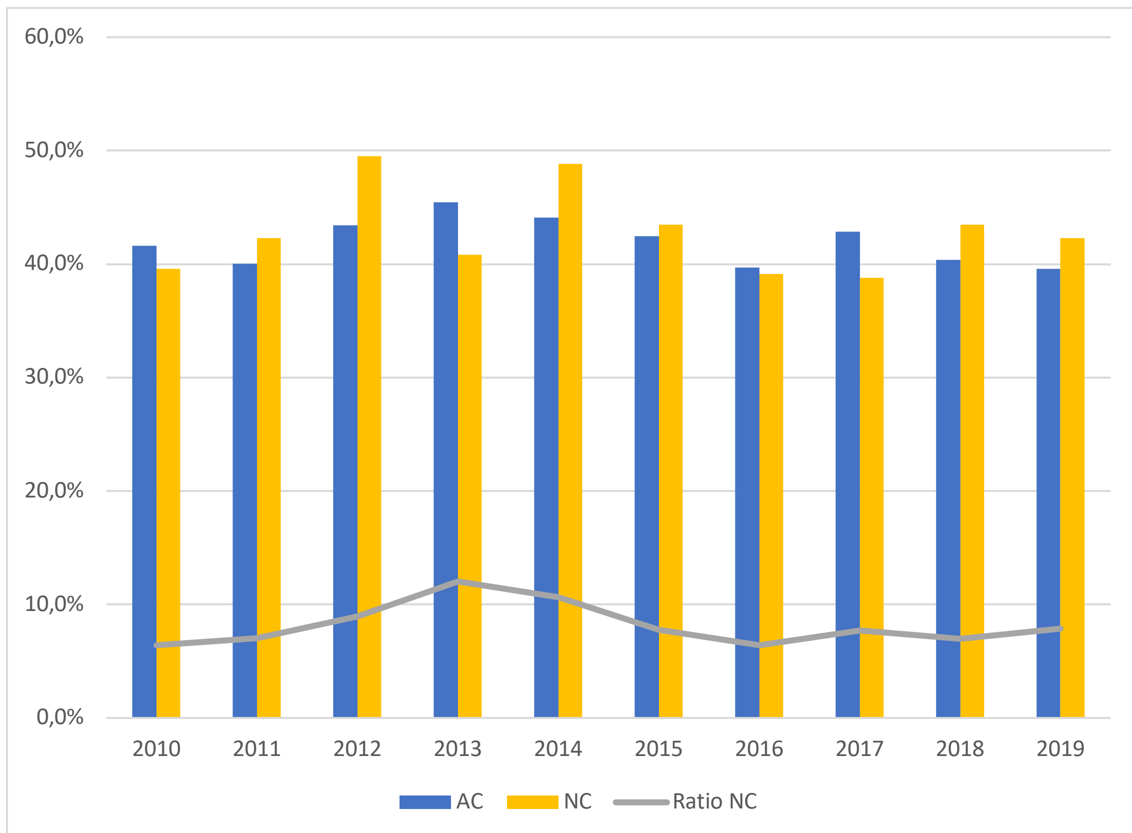

Figure 3 – LBR in NC vs AC throughout the years and proportion of NC performed

Discussion

Preceding data have shown potentially better ART outcomes after transferring frozen embryos in natural cycles when compared to transferring in an artificial cycle. However, it remained unknown whether this association may not be hindered in women in late reproductive years. The main propose of this retrospective study was to assess whether there were any significant differences in terms of LBR between transferring frozen embryos in NC-FET versus AC-FET in women receiving donated oocytes and to assess whether female age could modify the effect of this association. The results showed that there were no major differences between cycles, even after performing confounder adjustment, although transferring in NC-FET seemed to be slightly better with, lower miscarriage rates and higher LBRs. Our study also showed that these results were not significantly modified by the recipient's age.

Studies comparing NC-FET with AC-FET have shown conflicting information in terms of clinical outcomes. In order to overcome these significant gaps in the literature, a non-inferiority multicentre randomized controlled trial (RCT) was conducted to compare LBR between NC-FET and AC-FET in 1032 patients (ANTARTICA trial). This RCT has failed to show any significant differences in live birth, clinical and ongoing pregnancy rates (OPR) (Groenewoud et al., 2016). However, this study had many limitations such as lower LBR, CPR and OPR compared with those given in previous published studies, high miscarriage rates and poor cycle monitoring (Hreinsson et al., 2016).

Subsequently, numerous studies have tried to identify the optimal regimen of FET to obtain better pregnancy outcomes and several retrospective studies tend to support the natural cycle. Overall, the incidence of maternal and neonatal complications in both cycle regimens after FET are comparable, but NC-FET seems to be associated with lower miscarriage rates and gestational hypertensive disorders than the use of the AC-FET (Mubarak et al., 2019; Lin et al., 2020). Our study is in line with these results, to the extent that the reduction in miscarriage rates ultimately led to slightly superior LBR in NC-FET.

Regarding the decline of female fertility, it is well known that it's related to age, namely due to declining oocyte quality and ovarian reserve. Endometrial age has been devalued as a factor leading to reduced fertility because most studies using oocyte donation cycles showed no substantial influence of endometrial ageing on reproductive outcome. However, recent evidence showed that endometrial age affects endometrial function, namely due to changes in molecular processes, and also that those changes were more significant after 35 years of age. Those disruptions may reduce endometrial receptivity and reproductive success with women ageing (Devesa-Peiro et al., 2022). The advantage of studying embryo implantation in the population of oocyte recipients is that we are able to exclude the potential confounding effects of lower quality embryos from older women, and we can also exclude the effect that OS may have on endometrial receptivity. In our study, age apparently does not influence the differences between natural and artificial cycles.

Low serum progesterone levels on the day of embryo transfer have been related to a negative impact on pregnancy outcome in artificial endometrial preparation cycles when using micronized vaginal progesterone in either own or donated oocyte treatments. However, it is known that patients with low serum progesterone levels on the day of ET can have similar LBRs to those with adequate levels when a subcutaneous progesterone dose is added to LPS from the day of ET (Labarta et al., 2022). A recent prospective study even concluded that serum progesterone levels < 8.8ng/mL on the day of ET decreases OPR in both own or donated oocyte cycles (Labarta et al., 2021). For this reason, serum monitoring and supplementation of artificial cycles to achieve optimal LPS have become common practice, improving pregnancy outcomes. Nevertheless, our results showed that over time, apparently serum progesterone monitoring and supplementation has not been sufficient to eliminate LBR discrepancies between natural and artificial cycles throughout the years. The lack of improvement of AC-FET outcomes over time may be due to the fact that most centres were already performing some sort of routine luteal phase progesterone monitoring and off-label rescue therapy.

The main strengths of this study were its multicentre source of data, the large sample size included in the analysis and the use of a population of oocyte recipients. The vast number of cycles included in this study allowed the use of robust statistical analyses accounting for multiple potential confounding factors. Conversely, the limitations of this study are largely due to its retrospective design. Although we tried to alleviate this by including a large sample size and adjusting for potential confounding, other relevant factors may have remained unmeasured or were not accounted for due the impossibility to retrieve the data, most notably, embryo quality and female recipient smoking habits (although, regarding the latter, a recent study has shown that it is of limited importance in the donor-recipient model (Fréour et al., 2018)). Another limitation is the low numbers of natural cycles (8% of all cycles) which can be related to the low uptake to the treatment or/and to the selection of the patients itself. Finally, we cannot rule out that patients with a better prognosis may have tended to preferentially perform more natural cycles. Thus, future prospective studies with more homogeneous sample should be performed and the effect of other potential confounders that were not assessed in our study should be evaluated.

In conclusion, this study showed that these two regimens both provided high live birth rates, being NC-FET slightly better, which is in agreement with the findings of previous studies on this question. It also showed that the relative effects of the two endometrial preparations are not significantly modified by the age of the woman.

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