Journal Pre-proof

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PII: S1472-6483(23)00671-5
DOI: https://doi.org/10.1016/j.rbmo.2023.103572
Reference: RBMO 103572

To appear in: *Reproductive BioMedicine Online*

Received date: 14 June 2023
Revised date: 14 September 2023
Accepted date: 25 September 2023

Please cite this article as: Jaime Guerrero , Juan Carlos Castillo , Jorge Ten , JA Ortiz , Belén Lledó , Domingo Orozco , Francisco Quereda , Andrea Bernabeu , Rafael Bernabeu , Initiation of ovarian stimulation independent of the menstrual cycle (random-start) in an oocyte donation programme: a large, single-center experience, *Reproductive BioMedicine Online* (2023), doi: https://doi.org/10.1016/j.rbmo.2023.103572

Initiation of ovarian stimulation independent of the menstrual cycle (random-start) in an oocyte donation programme: a large, single-center experience

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Abstract

Research Question

Do live birth rates differ between recipients matched with donors using conventional ovarian stimulation versus those using random-start protocols?

Design

Retrospective analysis of 891 ovarian stimulations in egg donors (January-December 2018) and clinical outcomes in matched recipients (n=935). Donors commenced ovarian stimulation on day 1/3 of the menstrual cycle (n=223) or in the mid/late-follicular (n=388) or luteal phase (n=280) under a conventional antagonist protocol. Live birth rate of matched recipients was the main outcome.

Results

Duration of stimulation and total gonadotropins dose were comparable between conventional versus random-start groups. The number of collected eggs were also similar: 17.6±8.8 vs 17.2±8.5, p=0.6, respectively. Sub-group analysis showed an increased stimulation length (10.2±1.8 vs 9.8±1.7 vs 10.4±1.7, p<0.001) and gonadotropin consumption (2041.5±645.3 vs 2003.2±647.3 vs 2158.2±685.7 IU, p=0.01) in the luteal phase group vs the mid/late follicular and conventional groups; respectively. In matched recipients receiving fresh oocytes and undergoing fresh embryo transfer, the biochemical pregnancy (63.8% and 63.3%; p=0.9), clinical pregnancy (54.6% and 56.1%; p=0.8) and live birth rates (47.7% and 46.6%; p=0.7) per embryo-transfer were similar between conventional versus random groups. Similar results were obtained in recipients receiving vitrified eggs. Euploidy rate was also comparable.

Conclusions

There were no notable variations in clinical outcomes using oocytes obtained from random-start protocols and those proceeding from conventional ovarian stimulation in oocyte donation treatments. However, luteal-phase stimulation seems to require longer stimulation and higher FSH consumption. Our results indicate that random-start

stimulation strategy does not impair the potential of the oocyte yield or clinical outcomes in oocyte donation cycles.

Keywords

Ovarian stimulation; Random-start IVF; Oocyte donation cycles; Follicular waves; Live birth.

Introduction

Ovarian stimulation strategies have been traditionally aimed to start in the early follicular phase, mainly as a needed condition to get a receptive endometrium for fresh embryo transfer, but also due to the widely held belief that it was the optimal time for follicular recruitment. However, the documentation of multiple follicular cohorts (or "waves") during the menstrual cycle, not only challenged the traditional theory that a single cohort of antral follicles grows only during the follicular phase of the menstrual cycle (Baerwald *et al.*, 2003) but additionally provided the knowledge and physiological basis for the so-called "non-conventional" ovarian stimulation approaches such as the "random-start" protocol, *i.e.* initiation of the stimulation process irrespective of the phase of the menstrual cycle.

The bulk of existing literature regarding random-start ovarian stimulation involves women referred to oncologic units for oocyte cryopreservation (Cakmak and Rosen, 2015). More recently, some studies have also evaluated the efficiency of this strategy in patients undergoing elective freezing of oocytes or embryos, including those to decide on social preservation to mitigate the effect of age (Pereira *et al.*, 2017) or infertile patients deferring the transfer due to the nature of the treatment ("freeze-all" practice) where a receptive endometrium is not required (Qin *et al.*, 2016). Data from these publications suggest no difference in the number of oocytes/embryos obtained regardless of the day of the cycle when compared to those obtained with conventional protocols. Nonetheless, at the moment, it remains quite difficult to translate these preliminary studies to routine clinical practice because of the low number of patients studied (Sighinolfi *et al.*, 2018).

To date, very limited evidence is available with regards to the use of this strategy in the context of another target of patients who could benefit: the egg donor. Being able to start donor ovarian stimulation at any time regardless of the day of the menstrual cycle, may imply a significant advantage in this population allowing towards a more efficient synchronisation between donor and recipient and a better adjustment of the availability window for both. Moreover, the oocyte donor model would contribute to assess the unexplored scenario of the efficacy of random ovarian stimulation when fresh embryos are transferred.

In the present study, we aim to investigate whether the clinical outcomes in recipients receiving donated oocytes after random-start are comparable to those obtained under conventional ovarian stimulation protocols.

Materials and methods

Study design

This retrospective observational cohort study reports data from the Oocyte Donation Programme at Instituto Bernabeu Alicante between January and December 2018. The data included in this study was framed in the routine clinical activity and was approved by the Institutional Review Board in 2019 (reference number IBMR16).

Eligibility criteria and ovarian stimulation

All donors included in the study were voluntary, healthy women, younger than 32 years, with body mass index (BMI) between 18 and 28 kg/m2, with regular menstrual cycles (*i.e.,* between 26 and 35 days) recruited according to the clinical and legal requirements of the Spanish Assisted Human Reproduction act (RD 9/2014), which includes a psychological interview, gynaecological examination and a rigorous screening for infectious diseases and genetic abnormalities. As routine, contraceptive pills were not necessarily prescribed in the previous cycle, nonetheless, donors were asked about any unprotected intercourse on the previous days since last menses before starting ovarian stimulation and exhorted to prevent pregnancy during treatment.

Donor ovarian stimulation was initiated when contacted by the clinic interested in carrying out the treatment irrespective of the day of menstrual cycle. Oocyte donor

cycles starting stimulation on day 1-3 of the cycle (conventional group) were compared to oocyte donor cycles initiating the ovarian stimulation independently of the menstrual cycle (from day 4 onwards, random-start group). A further sub-group analysis was performed after segregating the random-start group into: mid-late follicular phase (Day 4-14) and luteal phase (> day 14).

Donors started stimulation with an initial dose of 150-300 UI/day of FSH (Fostipur®, Angelini Pharma, Spain; Bemfola®, Gedeon Richter, Spain). The gonadotropin starting dose was selected to balance follicular recruitment optimization and minimize the risk of high response. To summarize, the suggested optimal dose was 150 IU for donors with an antral follicle count (AFC) greater than 14, while a dose of 225 IU was deemed suitable for donors with 10-14 antral follicles. In cases where fewer than 10 follicles were observed, a dose of 300 IU was determined. It's important to note that, in line with clinician discretion, these doses could be adjusted based on the donor's BMI. Donors were monitored from day 5-6 of stimulation by transvaginal ultrasounds scans every 2-3 days and underwent a standard daily fixed antagonist protocol with a GnRH antagonist (Cetrotide®, Merck-Serono, Spain) starting on day 5 of stimulation. Final oocyte maturation was induced with 0.2 mg of a GnRH agonist (GnRHa) (Decapeptyl 0.1 mg®, Ipsen Pharma, Spain) when at least three follicles larger than 17 mm were detected by ultrasound. Oocyte aspiration was performed 36 hours after induction by transvaginal ultrasound-guided needle-aspiration.

Recipients and endometrial preparation

Recipients were women under 50 years with normal uterine cavity that attended the clinic to undergo reception of donated oocytes. To assess the uterine cavity, transvaginal ultrasound was employed. Any abnormal uterine findings detected during the ultrasound underwent further evaluation via 3D scan and/or hysteroscopy. Recipients exhibiting uterine distortion due to uterine malformations or fibroids invading the cavity were subsequently excluded. In patients with regular ovarian function, a GnRH analogue (Gonapeptyl 3.75 mg®, Ipsen-Pharma, Spain) was administered in the midluteal phase of the immediate previous cycle for pituitary desensitization. Subsequently, for endometrial preparation, they were subjected to standard substitutive hormonal therapy with transdermal estrogen (Evopad 50®, Janssen-Pharmaceutica, Belgium) or oral

estradiol valerate (Progynova®, Delpharm, France) at increasing doses for at least 12 days. Endometrial thickness ≥7 mm and trilaminar appearance at ultrasound were confirmed prior to oocyte reception. Micronized progesterone supplementation started with intravaginal capsules 200 mg/8 h (Utrogestan®, SEID, Spain) as soon as optimal fertilisation was confirmed in the laboratory.

Recipients were carefully matched with donors, prioritizing shared phenotypes, blood groups, and genetic compatibility for carrier screening tests, without the use of randomization.

The laboratory and clinical outcomes per embryo transfer were assessed. Circulating β-hCG levels were determined 13 days post-donation and in case of a positive test result the presence of a gestational sac was confirmed by ultrasound after 5 weeks. In pregnant patients, the hormonal treatment was sustained for 12 weeks.

Laboratory procedures

Retrieved oocytes were denuded and metaphase II (MII) oocytes were either anonymously assigned to their matched recipients or vitrified following the Cryotop protocol with Kitazato solutions for deferred donation.

In brief, oocytes were first equilibrated in a solution containing 7.5% (v/v) ethylen glycol (EG), 7.5% (v/v) dimethylsulfoxide (DMSO) in M-199 medium. They were then transferred vitrification solution (VS) containing 15% (v/v) EG, 15% (v/v) DMSO, and 0.5 M trehalose, washed thoroughly to eliminate leftover equilibration solution, and loaded in the tip of the Cryotop before plunging in liquid nitrogen. The procedure, from exposure of the oocytes to VS until the plunge in liquid nitrogen, is completed in 50-60 seconds.

For warming, the tip of the device was submerged in thawing solution (TS, 1M trehalose) at 37ºC, as fast as possible. Oocytes were recovered from TS in one minute, and transferred to dilution solution (DS, 0.5M trehalose, room temperature) for three minutes, followed by 5 minutes in washing solution (WS, no osmotic agents, room temperature).

Oocytes were fertilized with intracytoplasmic sperm microinjection (ICSI). After 16-18 hours post-insemination, oocytes showing two pronuclei and two polar bodies were considered correctly fertilized and were disposed individually in 30 µl micro drops of pre-equilibrated continuous culture media (Global Total®, LifeGlobal) in 5% O2 6% CO2 at 37ºC and cultured to day 5-6 blastocyst stage. Blastocyst were graded according to Istanbul consensus scoring on embryo assessment (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011). Embryos of higher quality were selected to be transferred and supernumerary good quality blastocysts were cryopreserved. Embryo transfer was cancelled in the absence of viable embryos or in patients failing to reach adequate endometrium thickness, with the whole cohort of good quality embryos being cryopreserved.

Where preimplantation genetic testing for aneuploidies (PGT-A) was indicated (e.g., recurrent pregnancy losses, implantation failure or abnormal FISH in sperm), zona pellucida drilling was carried out on day 3 and laser-assisted (LYKOS, Hamilton Thorne, Beverly, MA, USA) trophoectoderm (TE) biopsy of day 5-6 hatching blastocyst was performed. Biopsied blastocysts were individually vitrified and TE cells were processed for genetic analysis. Genetic analysis was performed using Veriseq-NGS (Illumina®, San Diego, USA), with previous whole genome amplification using SurePlex DNA Amplification System (Illumina®, San Diego, USA), according to the manufacturer's protocols. In Veriseq protocol, the sequencing platform used was the MiSeq System (Illumina®, San Diego, USA). For chromosome analysis, the BlueFuse Multi software (Illumina®, San Diego, USA) was used for each corresponding technique. Embryos were reported as euploid if the analysed sample contained less than 25% of aneuploid cells, mosaic if it contained between 25% and 50% of aneuploid cells in one or more chromosomes, and aneuploid if the percentage of aneuploidy was over 50%. The detection limit for the segmental aneuploidies was 8 Mb.

Outcomes of the study and statistical analysis

The primary outcome of this study was the live birth rate (LBR), defined as living birth after 23 weeks of gestation per embryo transfer cycle. Secondary outcomes analysed included: fertilisation, aneuploidy rate, survival rate after warming oocytes (number of surviving oocytes divided by the number of warmed oocytes), usable embryos (defined as the total number of embryos of the cohort: transferred plus cryopreserved)

biochemical pregnancy (detection of circulating β-hCG at 13 days post-donation), clinical pregnancy (defined as the presence of a gestational sac confirmed by ultrasound after 5 weeks), implantation (number of gestational sacs observed divided by the number of embryos transferred), and early miscarriage (intrauterine pregnancy loss before 10 weeks´size on ultrasound) (Kolte *et al.*, 2015; Zegers-Hochschild *et al.*, 2017). With regard to donor ovarian stimulation, the following parameters were explored: total gonadotropin dose, stimulation length, cancellation rate, retrieved oocytes and MII.

Shapiro-Wilk test were used to assess whether the numeric variables were normally distributed. Kruskal-Wallis test and Wilcoxon rank sum test were used for comparing numeric variables between groups when appropriated, and were presented as mean and standard deviation and 95% CIs. Categorical variables were expressed as percentage and were compared using the Pearson´s chi-square test or Fisher´s exact test. In addition, multivariable binary logistic regression analysis was used to control potential factors that may confound reproductive outcomes, namely donor age, BMI, smoking habit, parity, number of donated MII, number of embryos transferred, endometrial thickness or sperm source. Crude and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. Values of $p < .05$ were considered statistically significant. The statistical analysis was performed with R Statistical Software, version 4.2.0 and the Statistical Package for the Social Sciences, version 23.0 (SPSS, Chicago, IL, EE.UU.).

Results

The participant flow in the study is shown in Figure 1. Among the 891 egg donors included in the study, 223 started ovarian stimulation on day 1-3 of the menstrual cycle while 668 began in the mid to late-follicular phase (n = 388) or luteal phase (n = 280). The distribution of the ovarian stimulations according to the starting day of the menstrual cycle was plotted (Figure 2).

Donor age ranged from 18 to 32, average 25.6 ± 4.4 and 25.6 ± 4.1 for control and random-start group ($p = 0.8$), respectively. Regarding baseline characteristics: previous treatments, BMI, parity, AFC and smoking habit; no significant differences between donors in both treatment groups were found, as presented in Table 1.

Overall, cycle characteristics were similar between conventional vs random-start stimulation cycles with regards to total dose of gonadotropins (2041.5 ± 645.3 and 2068.1 ± 667.5), and duration of stimulation (10.2 ± 1.8 and 10.1 ± 1.7), respectively. Additionally, the number of collected eggs were also comparable (17.6 ± 8.8 vs 17.2 ± 8.5, p = 0.6), as well as for MII (13.8 ± 7.1 vs 13.5 ± 7.0, p = 0.6). The treatment cancellation rates were similar between the groups (4% vs 3.5%, p = 0.8).

Within the study group, a sub-group analysis showed fewer days of stimulation (10.2 ± 1.8 vs 9.8 ± 1.7 vs 10.4 ± 1.7, p < 0.001) and gonadotropin consumption (2041.5 ± 645.3 vs 2003.2 ± 647.3 vs 2158.2 ± 685.7, p = 0.010) in the conventional and mid/late follicular groups vs luteal phase group, respectively (Table 2).

No adverse events were reported in conventional group, whereas one case of ovarian hyperstimulation syndrome was described in one donor who started stimulation in the luteal phase due to an unnoticed early pregnancy stablished just concomitant with the beginning of ovarian stimulation. In this case, the patient requested termination of the pregnancy and was discharged 9 days later for additional outpatient follow-up, during which she showed complete resolution of her OHSS (Castillo *et al.*, 2020).

With regard to matched recipients, a total of 946 recipients were initially evaluated. Of those, 11 patients were excluded due to uterine cavity abnormalities, leaving 935 patients for analysis, 710 receiving fresh oocytes and 225 receiving vitrified oocytes (Figure 1). Laboratory and clinical outcomes for each group were collected.

In recipients receiving a fresh embryo transfer after synchronized fresh egg donation (n = 561), there were no differences between groups in terms of recipient age, sperm source, endometrial thickness, or endometrial preparation. Number of donated eggs, fertilisation rate, and usable embryos were also comparable. The mean number of transferred embryos was slightly higher in random-start group (1.1 ± 0.3 vs 1.2 ± 0.4, p = 0.048) whereas the number of surplus blastocyst stage embryos suitable for freezing was comparable (3.1 ± 2.1 vs 3.0 ± 2.0, p = 0.5). The between-group comparisons showed comparable biochemical pregnancy (63.8% and 63.3%, p = 0.9), clinical pregnancy (54.6% and 56.1%, p = 0.8) and live birth rate (47.7% vs 46.6%, p = 0.7) per embryo-transfer (Table 3). After accounting for the previously mentioned confounding factors in our adjusted analysis, we found that pregnancy outcomes exhibited

consistency between the two groups. There were no statistically significant differences observed, with odds ratios (ORs) and 95% confidence intervals (CIs) as follows: 0.91 (0.60-1.38, p=0.660) for biochemical pregnancy, 0.99 (0.66-1.48, p=0.950) for clinical pregnancy, and 0.88 (0.48-1.58, p=0.681) for live birth (Table 4).

As shown in Table 5, we also analysed 225 ICSI cycles of egg donation using oocytes vitrified after conventional (n = 59) or random-start ovarian stimulation (n = 166). No differences were observed in the average number of devitrified oocytes (11.0 ± 2.2 and 11.2 ± 2.1, p = 0.5), survival rate (86.7% and 87.8%, p = 0.6), and fertilisation rate (71.4% and 70.1%; p = 0.4). For recipients receiving a fresh embryo transfer (31 recipients in conventional and 106 recipients in random-start group), the number of embryos transferred (1.1 ± 0.3 and 1.1 ± 0.3; p = 0.6), biochemical pregnancy (61.3% and 59.4%; p = 0.8), clinical pregnancy (51.6% and 49.1%, p = 0.8), implantation (45.7% and 47.4%, p = 0.9), early miscarriage (18.8% and 21.2%, p > 0.9) and live birth rates (41.9% and 38.7%; p = 0.7) were also comparable. Likewise, these findings were consistent with the multivariable-adjusted pregnancy outcomes, as evidenced in Table 4.

In 131 treatments where PGT-A was indicated, a total of 518 blastocysts were biopsied. The reported incidence of aneuploidy (25.3% vs 26.1%, p = 0.9) and mosaicism (17.1% vs 17.2%, p = 0.9) were comparable in embryos derived from oocytes coming from conventional versus random-start stimulations (Table 6).

Discussion

Our extensive observational study suggests that the likelihood of live birth in recipients who receive oocytes from random-start ovarian stimulation protocols, initiated at any point in the menstrual cycle, is comparable to that of recipients who receive oocytes from conventional ovarian stimulation protocols initiated on days 1-3 of the cycle. Importantly, a similar euploidy rate was demonstrated in embryos derived from the random approach compared to conventional controls.

The recognition and documentation of multiple follicular cohorts or “waves” during the menstrual cycle (Baerwald *et al.*, 2003) provided new insights for understanding human ovarian follicular physiology and paved the way for ovarian stimulation approaches

such as the initiation of the stimulation process irrespective of the phase of the menstrual cycle, the so-called: "random-start" protocol. Three separate theories of follicular recruitment have been proposed (Baerwald *et al.*, 2012): (a) The single recruitment episode suggests that a dominant ovulatory follicle is selected from a single follicular cohort that emerges during the mid-follicular phase following luteal regression. (b) The follicular waves theory suggests that at least two cohorts of antral follicles emerge during the ovarian cycle, with a dominant ovulatory follicle developed in the final wave of the inter-ovulatory interval while preceding waves are anovulatory. Finally, (c) the theory of continuous recruitment suggests that small antral follicles (4-6 mm) grow and regress constantly throughout the inter-ovulatory interval and the dominant ovulatory follicle is selected, purely by chance, from the pool following luteal regression. Our findings, exploring random-start ovarian stimulation protocols in oocyte donors, appear to support the continuous recruitment theory.

The major part of medical literature regarding random-start ovarian stimulation derives from women requiring urgent ovarian stimulation before gonadotoxic therapy for oncologic conditions (von Wolff *et al.*, 2016). Additionally, smaller studies have also evaluated the efficiency of this strategy for elective fertility preservation (Pereira *et al.*, 2017) and for infertile patients undergoing a freeze-all approach for logistic reasons (Qin *et al.*, 2016). Our findings in the oocyte donor population starting ovarian stimulation on any day of the menstrual cycle allow a total disarticulation among menstrual cycle and ovarian stimulation with the generation of competent embryos. Today, oocyte donation makes up an increasingly large percentage of all ART cycles worldwide (European IVF-Monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE) *et al.*, 2021; "ART Success Rates | CDC," 2022). Much of the current knowledge about the window of implantation, freeze-all / FET protocols and trigger modalities has been learned through experience and investigations with donor egg cycles. In this way, oocyte donation rounds have played a pivotal role as a scientific tool in studying the menstrual cycle dynamics for improving ovarian stimulation protocols and implantation. The busy modern oocyte donation programs face several challenges, including the synchronization of donor/recipient cycles. Thus, starting donor ovarian stimulation irrespective of the phase of the menstrual cycle without adversely impacting oocyte yield or quality could

facilitate schedules. Nonetheless, the optimization of ovarian stimulation protocols must invariably be accomplished while maximizing donor safety.

Overall, in terms of ovarian stimulation parameters, our data showed a comparable consumption of gonadotropins, GnRH antagonist and stimulation days in random-start cycles compared to conventional controls. However, the sub-group analysis within the random group showed an increased number of days of stimulation and gonadotropin consumption when ovarian stimulation was started in the luteal phase compared to the mid/late follicular and conventional groups. Our findings are concordant with previous publications showing the same pattern in oncologic patients undergoing urgent ovarian stimulation and in own-eggs IVF/ICSI cycles. A large analysis in the oncologic group (von Wolff *et al.*, 2016) showed an increased number of days of gonadotropin stimulation (11.5 ± 2.2 vs 10.6 ± 2.7 vs 10.8 ± 2.4), and total dose of gonadotrophins (2970 ± 1145 vs 2595 ± 980 vs 2496 ± 980) in the luteal phase group vs Day 6-14 and Day 1-5 groups, respectively; while a retrospective study (Qin *et al.*, 2016) showed a similar trend in IVF/ICSI freeze-all cycles: longer ovarian stimulation (10.9 ± 3.4 vs 11.4 ± 3.1 vs 8.9 ± 1.4) and higher hMG consumption per day (169.4 ± 28.1 vs 159.9 ± 11.9 vs 149.2 ± 14.6) in the luteal phase starting group vs late follicular and conventional groups, respectively. Apparently, whether an ovarian stimulation is initiated in a "luteal / endogenous progestagenic environment" a longer stimulation and higher FSH consumption is expected, and even thought the exact mechanisms explaining these findings are still a matter of research they seem to be associated with a potent suppression of the hypophyseal activity induced by the elevated levels of progesterone in the luteal phase. In a safety note, the competence of embryos coming from oocytes generated during luteal phase stimulations have been demonstrated to be of good quality and performance in cohort followed up studies (Jiang *et al.*, 2022). However, as an additional important remark, luteal phase stimulation in a (potentially fertile) population like oocyte donors carries another potential significant risk: the initiation of a stimulation process concomitantly with an inadvertent pregnancy. During the timeframe of our study, our group reported on the occurrence of OHSS following a GnRHa trigger in the random-start protocol in an egg-donor due to the concomitant presence of an undetectable pregnancy during controlled ovarian stimulation (Castillo *et al.*, 2020). All in all, these findings suggest that luteal phase stimulation should be withhold in oocyte donors, perhaps with the exception of specific groups in which the

probability of pregnancy becomes negligible (*i.e.*, tubal blockage, carriers of intra-uterine device / implant contraceptive, same-sex or azoospermic partners). The same recommendations could be extrapolated to the group of ladies seeking for elective "social" fertility preservation. On the contrary, initiating an ovarian stimulation process at any moment during the follicular phase up to the pre-ovulatory period (*i.e.*, < day 14) appears to be safe, efficient, and convenient for egg donors with the additional advantages of facilitating scheduling and synchronization with the recipient, and avoidance of the use of oral contraceptives for this purpose.

Our study exhibits some limitations. Foremost among these is its retrospective nature, which opens the possibility of inadvertently including confounding factors, introducing selection bias, and challenges in maintaining precise experimental controls. Consequently, it is important to exercise caution when interpreting the data. Moreover, certain variables, such as the AMH levels of donors, were unavailable for our analysis and merit investigation in prospective trials. Additionally, we must acknowledge that our donor classification relied on the commencement of menstruation. Unfortunately, the ovulation status in the luteal phase subgroup was not consistently documented, implying that an indeterminate number of donors in this category may not have been in a genuine luteal phase.

In matched recipients, while acknowledging some variations in the stimulation protocols across trials, our data provide additional support for the viability of oocytes obtained from random-start protocols, as previously described in oncologic patients (von Wolff *et al.*, 2016) and in the freeze-all IVF/ICSI population (Qin *et al.*, 2016). In recipients receiving fresh embryos for transfer after a fresh oocyte donation, the inter-group comparisons showed similar biochemical pregnancy, clinical pregnancy, and live birth rates per embryo-transfer in the conventional vs random-start group. After adjusting for confounding factors, the odds of pregnancy outcomes did not exhibit significant differences, suggesting that random start protocols had no discernible impact on oocyte competence. Of note, the number of surplus good quality blastocyst stage embryos suitable for freezing was also similar. In a further reassurance note, the yield of frozen eggs derived from random-start protocols showed comparable results to those generated after a conventional ovarian stimulation in terms of post-thawing tolerance, fertilisation rate and reproductive outcomes in recipients. Furthermore, the reproductive

outcomes from frozen eggs vs fresh eggs derived from random-start protocols showed also similar performance. Of remark, and in opposite to previous publications on the subject in which oocytes/embryos were frozen following random-start, our study is, to our knowledge, the first to provide data on the performance after the transfer of fresh embryos derived from random-start protocols. Finally, when analysing PGT-A cycles derived from random-start protocols, our data showed a similar rate of euploid embryos versus conventional protocols. Taken altogether, and even acknowledging the inherent limitations associated to a retrospective data analysis, our findings provide reassurance of a comparable reproductive outcome of oocytes derived from random-start protocols and support the notion that the cohort of follicles recruited after exogenous FSH exposure demonstrate optimal competence finally providing a rationale for the notion that ovarian stimulation therapy can be initiated at different times during the menstrual cycle. However, long-term studies need to be conducted in the future in order to assess peri and post-natal outcomes to confirm the safety of random-start protocols.

In conclusion, in this large observational study, no significant differences were observed in clinical outcomes using oocytes coming from random-start protocols compared to those proceeding from conventional ovarian stimulation in oocyte donation treatments. However, due to a longer stimulation, higher FSH consumption and implicit potential risk, caution should be adopted for luteal-phase stimulation in egg donors.

Acknowledgements

The authors would like to thank the staff at Instituto Bernabeu in Alicante, Spain who kindly used their time to make this study possible.

Abbreviations

AFC antral follicular count

BMI body mass index

DS dilution solution

DMSO dimethylsulfoxide

EG ethylen glycol

GnRHa GnRH agonist

LBR live birth rate

MII metaphase II

PGT-A preimplantation genetic testing for aneuploidies

TS thawing solution

TE trophoectoderm

VS vitrification solution

WS washing solution

Declarations

Ethics approval and consent to participate

The study conformed to the Declaration of Helsinki for Medical Research about human subjects. In addition, approval was obtained from the institutional review board at Instituto Bernabeu, reference number IBMR16**.**

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

J.G., J.C., and R.B.: study conception and design; J.G., J.C. and J.T.: collection of data; J.G., J.C., J.T, J.O., A.B.: statistical analysis and interpretation of data; J.G.: wrote the first draft of the manuscript; J.C., J.T., and B.L. wrote sections of the manuscript; J.C., J.T., J.O., B.L., D.O., F.Q., A.B. and R.B.: critical review of the article. All authors agree on the submission of the manuscript.

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Legends to figures

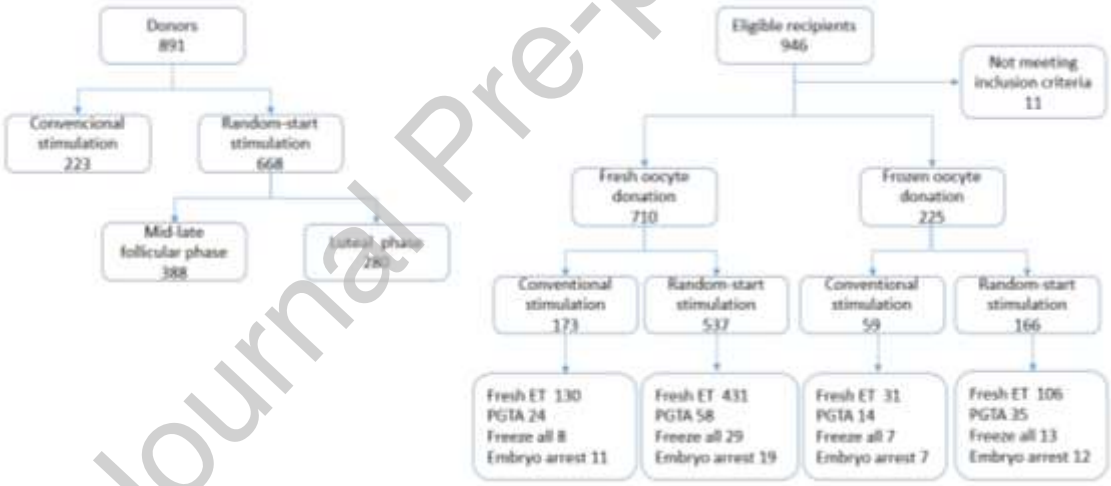

Figure 1. Flowchart. Distribution of groups, subgroups, and number of cases.

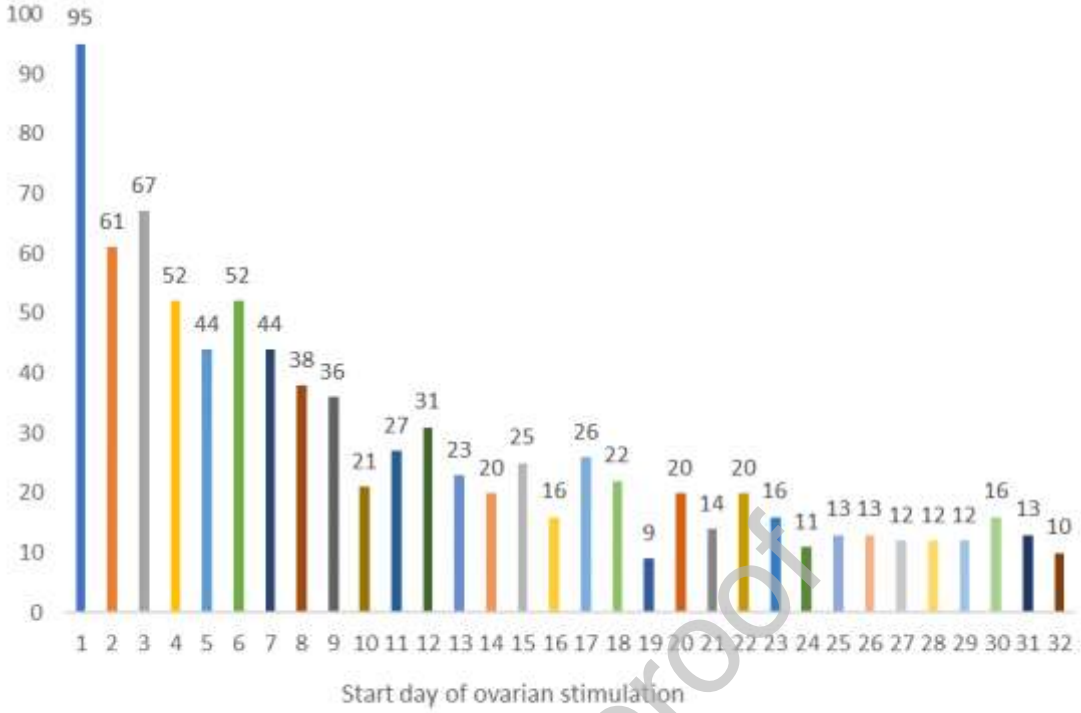

Figure 2. Case distribution among oocyte donors categorized by the day of initiating the ovarian stimulation process.

Table 1. Demographics characteristics of donors and comparison of controlled ovarian stimulation.

	Conventional, N = 223	**95% CI**	**Random start**, N = 668	**95% CI**	**Estimated difference (95% CI)**	**P-value[1]**
Age (years)	25.6 (4.4)	25, 26	25.6 (4.1)	25, 26	-0.07 (-0.73, 0.58)	0.8
Previous cycles	3.1 (2.2)	2.8, 3.4	3.0 (2.2)	2.8, 3.2	0.08 (-0.25, 0.42)	0.7
Smoking habit	92 (41.2%)	35%, 48%	309 (46.3%)	43%, 50%		0.2
Parity	112 (50.2%)	44%, 57%	370 (55.4%)	52%, 59%		0.2
AFC	17.0 (5.7)	16, 18	15.9 (4.8)	16, 16	1.1 (-0.08, 1.69)	0.070
BMI (kg/m^2)	22.2 (2.4)	22, 23	22.0 (2.6)	22, 22	0.19 (-0.19, 0.56)	0.3
Gonadotropin						0.5
Fostipur	105 (47.1%)	40%, 54%	295 (44.2%)	40%, 48%		
Bemfola	118 (52.9%)	46%, 60%	373 (55.8%)	52%, 60%		

Starting dose of gonadotropin (IU)						0.3
150	71 (31.8%)	26%, 38%	180 (26.9%)	24%, 30%		
225	107 (48.0%)	41%, 55%	323 (48.4%)	44%, 52%		
300	45 (20.2%)	15%, 26%	165 (24.7%)	22%, 28%		
Total dose of gonadotropin (IU)	2,041.5 (645.3)	1,956, 2,127	2,068.1 (667.5)	2,017, 2,119	-27 (-126, 73)	0.5
Duration of stimulation (days)	10.2 (1.8)	10, 10	10.1 (1.7)	9.9, 10	0.18 (-0.09, 0.45)	0.3
Cancellation rate	9 (4.0%)	1%, 7%	24 (3.6%)	2%, 5%		0.8
No of oocytes retrieved	17.6 (8.8)	16, 19	17.2 (8.5)	17, 18	0.33 (-1.0, 1.7)	0.6
No of mature oocytes (MII)	13.8 (7.1)	13, 15	13.5 (7.0)	13, 14	0.18 (-0.79, 1.4)	0.6

[1] *Wilcoxon rank sum test; Fisher's exact test; Pearson's Chi-squared test*

Data are presented as mean (SD) or %

AFC= Antral follicular count

BMI= Body mass index

CI = Confidence Interval

Table 2. Comparison of controlled ovarian stimulation, sub-group analysis.

	Conventional, N = 223	**95% CI**	**Mid-late follicular**, N = 388	**95% CI**	**Luteal**, N = 280	**95% CI**	**P-value**[1]
Age (years)	25.6 (4.4)	25, 26	25.4 (4.1)	25, 26	25.9 (4.2)	25, 26	0.3
Previous cycles	3.1 (2.2)	2.8, 3.4	3.0 (2.2)	2.8, 3.2	3.0 (2.1)	2.8, 3.3	0.9
Smoking habit	92 (41.2%)	35%, 48%	184 (47.4%)	42%, 53%	125 (44.6%)	39%, 51%	0.3
Parity	112 (50.2%)	44%, 57%	205 (52.8%)	48%, 58%	165 (58.9%)	53%, 65%	0.12
AFC	17.0 (5.7)	16, 18	16.0 (5.1)	16, 17	15.7 (4.4)	15, 16	0.12
BMI (kg/m^2)	22.2 (2.4)	22, 23	22.0 (2.5)	22, 22	22.0 (2.6)	22, 22	0.5
Gonadotropin							0.3

Fostipur	105 (47.1%)	40%, 54%	180 (46.4%)	41%, 51%	116 (41.4%)	35%, 47%	
Bemfola	118 (52.9%)	46%, 60%	208 (53.6%)	49%, 59%	164 (58.6%)	53%, 65%	
Starting dose of gonadotropin (IU)							0.5
150	71 (31.8%)	26%, 38%	110 (28.4%)	24%, 33%	71 (25.3%)	20%, 31%	
225	107 (48.0%)	41%, 55%	185 (47.7%)	43%, 53%	137 (48.9%)	43%, 55%	
300	45 (20.2%)	15%, 26%	93(23.9%)	20%, 29%	72 (25.7%)	21%, 31%	
Total dose of gonadotropin (IU)	2,041.5 (645.3)	1,956, 2,127	2,003.2 (647.3)	1,939, 2,068	2,158.2 (685.7)	2,077, 2,239	0.010
Duration of stimulation (days)	10.2 (1.8)	10, 10	9.8 (1.7)	9.6, 10.0	10.4 (1.7)	10, 11	<0.001
Cancellation rate	9 (4.0%)	1%, 7%	14 (3.6%)	2%, 5%	10 (3.6%)	1%, 6%	0.9
No of oocytes retrieved	17.6 (8.8)	16, 19	17.4 (8.9)	17, 18	17.0 (8.0)	16, 18	0.9
No of mature oocytes (MII)	13.8 (7.1)	13, 15	13.5 (7.1)	13, 14	13.4 (6.7)	13, 14	0.8

[1] *Kruskal-Wallis rank sum test; Fisher's exact test; Pearson's Chi-squared test*

Data are presented as mean (SD) or %

AFC= Antral follicular count

BMI= Body mass index

CI = Confidence Interval

Table 3. Characteristics of recipients, and laboratory and clinical outcomes after donation of fresh oocytes.

	Conventional, N = 173	**95% CI**	**Random start**, N = 537	**95% CI**	**Estimated difference (95% CI)**	**P-value**[1]
Recipient age (years)	41.5 (4.2)	41, 42	41.4 (4.3)	41, 42	0.12 (-0.60, 0.85)	0.7
Endometrial thickness (mm)	8.6 (1.7)	7.9,9.2	7.9 (1.9)	7.4,8.4	0.68 (-0.13, 0.41)	0.1
Endometrial preparation (days)	18.1 (3.0)	16.8,19.2	18.7 (2.6)	18.0,19.4	-0.18 (-0.39, 0.76)	0.3
Sperm source						0.8
Partner fresh sperm	130 (75%)	68%,	380	67%,		

		81%	(71%)	75%		
Partner frozen sperm	32 (18%)	13%, 25%	112 (21%)	18%, 25%		
Surgical sperm retrieval	4 (2.3%)	0.74%, 6.2%	16 (3.0%)	1.8%, 4.9%		
Donor	7 (4.0%)	1.8%, 8.5%	29 (5.4%)	3.7%, 7.8%		
Donated MII	10.2 (1.8)	9.9, 10	10.0 (1.7)	9.9, 10	0.16 (-0.13, 0.46)	0.2
2PN	7.2 (2.0)	6.9, 7.5	7.2 (2.0)	7.0, 7.3	0.07 (-0.28, 0.42)	0.4
Fertilization rate	70.9%	68, 74	71.3 %	70, 73		0.9
Usable embryos (transferred+frozen)	4.0 (2.1)	3.6, 4.3	4.0 (2.0)	3.8, 4.1	0.01 (-0.35, 0.37)	0.8
No of subjects with fresh embryo transfer	130		431			
Transferred embryos	1.1 (0.3)	1.0, 1.1	1.2 (0.4)	1.1, 1.2	-0.07 (-0.13, -0.01)	0.048
Frozen embryos	3.1 (2.1)	2.8, 3.5	3.0 (2.0)	2.8, 3.2	0.13 (-0.23, 0.48)	0.5
Biochemical pregnancy rate	83 (63.8%)	55%, 72%	273 (63.3%)	58%, 68%		0.9
Clinical pregnancy rate	71 (54.6%)	46%, 63%	242 (56.1%)	51%, 61%		0.8
Implantation rate	79/142 (55.6%)	45%, 64%	262/501 (52.3%)	48%, 57%		0.5
Miscarriage rate	8 (11.3%)	5.3%, 22%	40 (16.5%)	12%, 22%		0.3
Sacs						0.3
1	64 (90.1%)	80%, 96%	222 (91.7%)	87%, 95%		
2	6 (8.5%)	3.5%, 18%	20 (8.3%)	5.2%, 13%		
3	1(1.4%)	0.07%, 8.7%	0 (0%)	0.00%, 1.9%		
Live birth rate	62 (47.7%)	39%,56%	201 (46.6%)	42%,52%		0.7

[1] *Wilcoxon rank sum test; Fisher's exact test; Pearson's Chi-squared test*

Data are presented as mean (SD) or %

CI = Confidence Interval

Table 4 Association between ovarian stimulation regimen and pregnancy outcomes on crude and adjusted analysis

Fresh oocytes	**Crude OR (95% CI) P-value**	**Adjusted OR (95% CI) P-value**
Biochemical pregnancy rate	0.98 (0.65-1.47, p=0.917)	0.91 (0.60-1.38, p=0.660)
Clinical pregnancy rate	1.06 (0.72-1.58, p=0.758)	0.99 (0.66-1.48, p=0.950)
Live birth rate	0.91 (0.51-1.58, p=0.745)	0.88 (0.48-1.58, p=0.681)
Vitrified oocytes	**Crude OR (95% CI) P-value**	**Adjusted OR (95% CI) P-value**
Biochemical pregnancy rate	0.89 (0.38-2.00, p=0.780)	0.81 (0.33-1.94, p=0.636)

Fresh oocytes	Crude OR (95% CI) P-value	Adjusted OR (95% CI) P-value
Clinical pregnancy rate	0.90 (0.40-2.02, p=0.802)	0.74 (0.31-1.76, p=0.497)
Live birth rate	0.90 (0.28-2.64, p=0.853)	0.93 (0.26-3.03, p=0.903)

OR odds ratio, *CI* confidence interval

*Adjusted for donor age, BMI, smoking habit, parity, number of donated MII, number of embryos transferred, endometrial thickness or sperm source.

Table 5. Characteristics of recipients, and laboratory and clinical outcomes after donation of vitrified oocytes.

	Conventional, N = 59	**95% CI**	**Random start**, N = 166	**95% CI**	**Estimated difference (95% CI)**	**P-value**[1]
Recipient age (years)	40.9 (4.3)	40, 42	41.6 (3.6)	41, 42	-0.70 (-1.9, 0.54)	0.5
Endometrial thickness (mm)	8.0 (1.5)	7.7,8.2	8.7 (1.8)	8.5,8.9	-0.55 (-0.9, 0.04)	0.2
Endometrial preparation (days)	13.1 (2.8)	12.0, 14.1	13.8 (3.0)	12.6,14.5	-0.75 (-1.6, 0.13)	0.5
Sperm source						>0.9
Partner fresh sperm	38 (64.4%)	51%, 76%	107 (64.5%)	57%, 72%		
Partner frozen sperm	14 (23.7%)	14%, 37%	43 (25.9%)	20%, 33%		
Surgical sperm retrieval	1 (1.7%)	0.09%, 10%	2 (1.2%)	0.21%, 4.7%		
Donor	6 (10.2%)	4.2%, 21%	14 (8.4%)	4.9%, 14%		
Oocytes thawed	11.0 (2.2)	10, 12	11.2 (2.1)	11, 12	-0.20 (-0.85, 0.45)	0.5
Oocytes injected	9.5 (1.7)	9.1, 10	9.8 (1.8)	9.6, 10	-0.29 (-0.82, 0.24)	0.2
Survival rate	86.7%	85%, 91%	87.8%	87%, 91%		0.6
2PN	6.8 (2.0)	6.3, 7.3	6.9 (1.9)	6.6, 7.2	-0.08 (-0.68, 0.52)	0.7
Fertilization rate	71.4%	67%, 76%	70.1%	68%, 72%		0.4
Usable embryos (transferred+frozen)	3.1 (1.8)	2.6, 3.5	3.4 (2.0)	3.1, 3.7	-0.38 (-0.95, 0.19)	0.3
No of subjects with fresh embryo transfer	31		106			
Transferred embryos	1.1 (0.3)	1.00, 1.25	1.1 (0.3)	1.04, 1.15	-0.11 (-0.29, 0.08)	0.6
Frozen embryos	2.7 (1.7)	2.0, 2.9	2.6 (2.0)	2.4, 3.0	-0.27 (-0.81, 0.27)	0.9

Biochemical pregnancy rate	19 (61.3%)	42%, 78%	63 (59.4%)	49%, 68%	0.8
Clinical pregnancy rate	16 (51.6%)	33%,70%	52 (49.1%)	39%,59%	0.8
Implantation rate	16/35 (45.7%)	28, 62	55/116 (47.4%)	38, 58	0.9
Miscarriage rate	3 (18.8%)	5.0%, 46%	11 (21.2%)	12%, 35%	>0.9
Sacs					>0.9
1	16 (100%)	76%, 100%	49 (94.2%)	83%, 98%	
2	0 (0%)	0.00%, 24%	3 (5.8%)	1.5%, 17%	
3	0 (0%)	0.00%, 24%	0 (0%)	0.00%, 24%	
Live birth rate	13 (41.9%)	24%,60%	41 (38.7%)	30%,49%	0.7

[1] *Wilcoxon rank sum test; Fisher's exact test; Pearson's Chi-squared test*

Data are presented as mean (SD) or %

CI = Confidence Interval

Table 6. Mosaicism and aneuploidy rates following preimplantation genetic testing.

	Conventional, N = 146	**Random start**, N = 372	**P-value**[1]
Mean biopsied embryos	3.8 (1.9)	4.0 (1.5)	0.1
Mosaicism rate	25 (17.1%)	64 (17.2%)	0.9
Aneuploidy rate	37 (25.3%)	97 (26.1%)	0.8

[1] *Wilcoxon rank sum test; Pearson's Chi-squared test*

Data are presented as mean (SD) or %

Key message: The implementation of random-start ovarian stimulation protocols in oocyte donation cycles does not negatively impact oocyte yield or clinical outcomes in recipients compared to traditional protocols.

BIOGRAPHY

Jaime Guerrero, MSc, obtained his Bachelor of Biology from the Universidad de Valencia in 2001 and has been working as an Embryologist at Instituto Bernabeu Alicante since 2002. He also holds the position of Professor in the Master's Programme in Human Reproduction at the University of Alicante. Since 2012, Jaime has been serving as the director of the Egg Donation and Cryobiology Programme.