Clinical applications of endometrial receptivity tests in patients with recurrent implantation failure

Ioan BOLEAC[1], Manuela NEAGU[2,3], Anca CORICOVAC[1,2], Dorina CODREANU[1], Alina BUSAN-PIRVOIU[3], Bogdan MARINESCU[2,3]

[1] Gynera Fertility Center, Bucharest, Romania
[2] "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[3] Obstetrics and Gynecology Department, "Panait Sirbu" University Hospital, Bucharest, Romania

Abstract

Recurrent implantation failure is represented by the failure to achieve a clinical pregnancy after transfer of at least 4 good-quality embryos in a minimum of 3 fresh or frozen cycles in a woman under the age of 40 years. One of the recent approaches in studying the window of implantation was building the expression profile of the genes of the endometrial cells. We performed a retrospective study which investigated if endometrial receptivity tests improved the outcomes of IVF procedures in patients with recurrent implantation failure. We enrolled 47 couples with RIF and divided them in 2 groups: the first group of 22 couples performed the ERA test and the embryo transfer according to the result of the test; the second group of 27 couples had the embryo transfer done without the ERA test. Our conclusion was that the ERA test did not improve the outcomes for patients with recurrent implantation failure.

INTRODUCTION

Recurrent implantation failure (RIF) is a clinical entity which is characterized by multiple failures of obtaining a pregnancy after an embryo transfer (ET). In the last 20 years, many authors tried to define RIF, by considering the following criterias: the number and the quality of the transferred embryos, the developing stage of the transferred embryos (blastocyst or cleavage-stage embryo), the number of the in vitro fertilization (IVF) / ET cycles, the age of the patient.

According to the definition proposed by Coughlan et al. in 2014, recurrent implantation failure means failure to achieve a clinical pregnancy after transfer of at least 4 good-quality embryos in a minimum of 3 fresh or frozen cycles in a woman under the age of 40 years [1].

Implantation needs 3 factors: a receptive endometrium, a good-quality blastocyst and a synchronized dialogue between the embryo and the endometrium. The human endometrium is a hormonally regulated organ that suffers cyclic morphological and functional changes under the effect of the hypothalamic-pituitary-ovarian axis in order to prepare itself for the embryo. The period of time when the endometrium is most receptive to the human embryo is called the re-

ceptive endometrium or the window of implantation. This interval is relatively large in humans, between days 20 and 24 of an ideal 28 days menstrual cycle.

The most popular and simple way of assessing the endometrium is by transvaginal ultrasound; we can measure the endometrial thickness, echogenicity and the uterine artery blood flow indices. We may consider we have indirect proof of endometrial receptivity when the measurements show a trilaminar endometrial pattern and a thickness over 6 mm; but a meta-analysis of 14 studies published in 2011 came to the conclusion that although there is a relationship between endometrial thickness and pregnancy, the endometrial receptivity is far more complex than a simple measurement of endometrial thickness [2].

More and more articles regarding recurrent implantation failure have been written around the world, yet an international consensus regarding the management of these patients has not been reached.

AIM

Our study wanted to question if the personalized embryo transfer (PET) according to the result of the ERA test (Endometrial Receptivity Analysis test) had statistical significant different outcomes than the frozen or fresh embryo transfer in patients with recurrent implantation failure.

MATERIALS AND METHODS

We enrolled in our retrospective study 47 cases of couples with recurrent implantation failure which adressed Gynera Fertility Clinic between 2015 and 2018. We divided the patients into 2 groups: the first group included 22 cases of RIF which performed an ERA test and after that had an PET according to the result of the test; the second group was the control group and it included 25 cases of couples with RIF that had a fresh or frozen ET, without performing an ERA test.

We had the following inclusion criterias: age of the woman under 40 years; failure of obtaining clinical pregnancy after performing at least 3 fresh or frozen ET; the transfer of at least 4 good-quality embryos, and this meant for the cleavage stage embryos having 7 or 8 cells, with symmetrical blastomeres and having fragmentations below 10% of their volume; a good-quality blastocyst meant a blastocyst graded AA, AB or BA, which reached stage 3, 4 or 5 of expansion of the blastocoel cavity by the fifth culture day. We also had the following exclusion criterias: age of the woman over 40 years; failure of obtaining clinical pregnancy after performing only 1 or 2 fresh or frozen ET; the transfer of less than 4 good-quality embryos; the lack of the necessary documents to prove the quality of the embryos

previously transferred; the transfer of low-quality embryos; donor oocyte cycles; obtaining clinical pregnancy after ET.

The patients which had a new IVF procedure followed one of the two controlled ovarian hyperstimulation protocols: short protocol with GnRH antagonists (SP) or long protocol with GnRH agonists (LP). For the LP, the patients started using GnRH agonists in the midluteal phase, meaning days 18-21 of the menstrual cycle; the administration of the GnRH agonist continued until the use of the trigger for ovulation induction. After the arrival of the bleding, the patients had an ultrasound, in order to exclude ovarian cysts or follicles already recruited; they also had blood tests, in order to check if the estradiol and the progesterone had basal values. If the results of the ultrasound and blood tests were fine, the patients started the ovarian stimulation with urinary or reombinant FSH or menotropin. The results of the stimulation were asessed by ultrasound +/- hormonal blood tests. When at least 3 follicles reached 18 mm in diameter, we used hCG or recombinant hCG for triggering.

For the SP, the patients started the admistration of the gonadotropins in the second or third day of the menstrual cycle, after the ultrasound and blood checkup. The GnRH antagonist was introduced in the fifth or sixth day of stimulation, depending on the follicles and the estradiol values. We used the same injectable medication, and also monitored the ovarian response by ultrasound +/- blood tests; we used the same triggering criterias, but we triggered with hCG or recombinant hCG or with GnRH agonist.

The egg retrieval was done 35-36 hours from the trigger administration, in the operating room, the patient being sedated by the anesthesiologist. The oocyte insemination was performed through IVF standard procedure or ICSI (intracytoplasmic sperm injection) procedure or both. After the fertilization occurred, the embryos were cultured in the laboratory between 2 and 6 days. The embryo transfer was done between 2 and 6 days, most often days 3 and 5; it was always ultrasound guided. We used for luteal support progesterone administered through the vagina.

The patients which did not have a new IVF procedure, had only a frozen embryo transfer cycle. They had the ET done on their natural cycle (CN), or they administered hormonal substitution therapy (artificial cycle CA).

The patients from the first group had the ERA test done in a hormone replacement therapy cycle. We performed the ERA test (the endometrial biopsy) on P+5 day, meaning the fifth day from the beginning of the progesterone administration. Then, we performed the

personalized ET when indicated by the ERA result, also in a hormone replacement therapy cycle.

Every patient that was enrolled in our study signed an informed consent, and the study was approved by the Ethical Committee.

The primary outcomes of the study were the serum hCG levels and the clinical pregnancy (pregnancy confirmed by ultrasound). The question of our study was if the endometrial receptivity tests improved the outcomes for patients with recurrent implantation failure. The study also wanted to search if variables like age and AMH of the patient, the stimulation protocol and the fertilization technique used for the patient, influenced the outcome of the procedures for RIF patients.

We used Microsoft Excel 2017 in order to process our data; the statistical analysis and the graphic representations of the data were performed using SPSS 20 (Statistical Package for the Social Sciences).

RESULTS

We present first the descriptive part of the statistics of this study, regarding the following variables: age, result of the ERA test, AMH classification, fertilization technique, clinical pregnancy and live birth.

Age

The mean age of our subjects was 35.21 years; the standard deviation was 2.896. The mode (the modal value) was 39 years (this means that the most frequent age for our subjects was 39 years).

We defined the following age categories: under 30 years, 30-34 years, 35-37 years and 38-39 years. We found that most of our subjects belonged to the category of 30-34 years; the category with the smallest number of subjects was that of under 30 years.
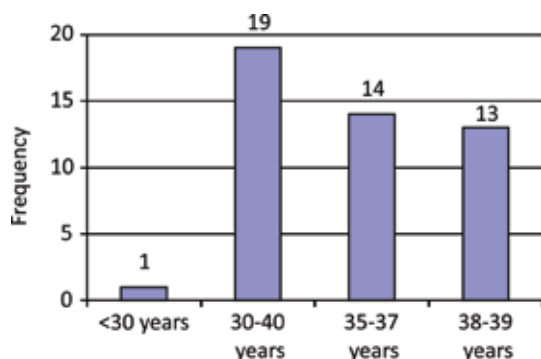

FIGURE 1. Patients' distribution by age categories

Result of the ERA test

After performing the ERA test, we had the following results: for 11 subjects the endometrium was receptive, for 10 subjects the endometrium was prerecep-

tive and in case of the last subject that performed the test the endometrium was postreceptive.
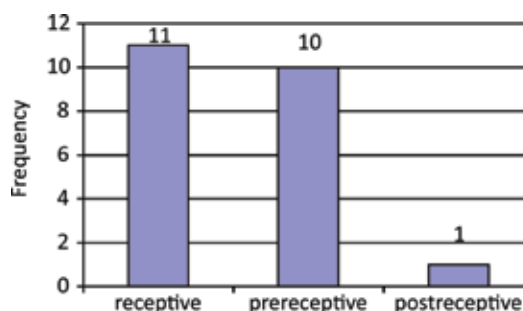

FIGURE 2. Patients' distribution by result of the ERA test

AMH classification

We defined the following AMH categories: AMH over 1 ng/ml – normal ovarian reserve; AMH between 0.4 ng/ml and 1ng/ml – low ovarian reserve; AMH under 0.4 ng/ml – very low ovarian reserve. 83.7% of our subjects had a normal ovarian reserve and only 2.3% had a very low ovarian reserve.
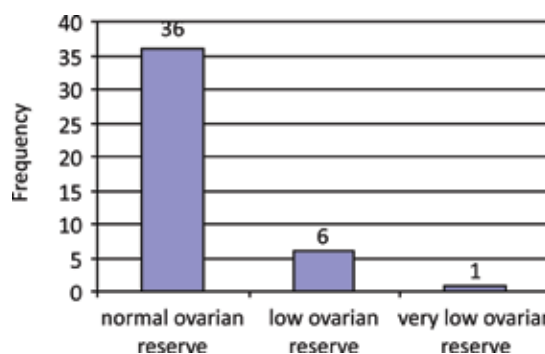

FIGURE 3. Patients' distribution by AMH categories

Fertilization technique

For 73.7% of the patients that had a new IVF procedure the fertilization technique was standard, and 26.3% of the patients had their oocytes fertilized by ICSI technique.
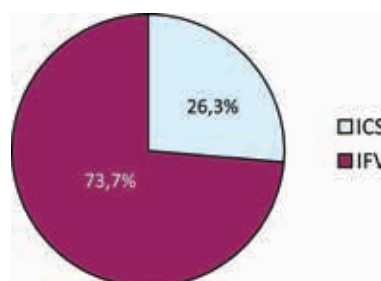

FIGURE 4. Patients' distribution by fertilization technique

Clinical pregnancy
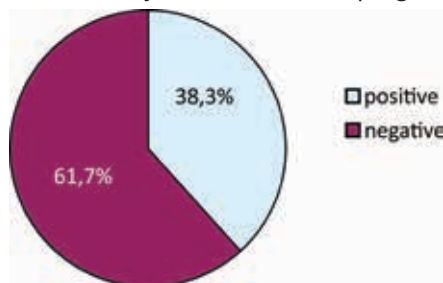
38.3% of our subjects had a clinical pregnancy.

FIGURE 5. Patients' distribution by clinical pregnancy

Live birth

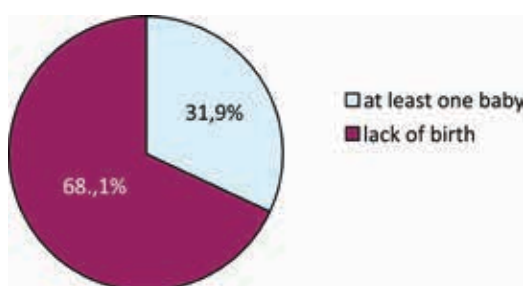31.9% of our subjects had a live birth (at least one baby).

FIGURE 6. Patients' distribution by live birth

The second part of our statistics is represented by the correlation analysis of the data.

We examined the relation between the age classification and the clinical pregnancy and found the lack of a statistically significant association between these variables. After removing from the analysis the age category of under 30 years (because we had only one subject in this category), we observed that the clinical pregnancy had a percentage of 35.7% to 42.1%.

TABLE 1. The correlation between age classification and clinical pregnancy

| | | | Clinical pregnancy | | Total |
			positive	negative	
Age classification	<30 years	Frequency	0	1	1
		%	0.0%	100.0%	100.0%
	30-34 years	Frequency	8	11	19
		%	42.1%	57.9%	100.0%
	35-37 yeasrs	Frequency	5	9	14
		%	35.7%	64.3%	100.0%
	38-39 years	Frequency	5	8	13
		%	38.5%	61.5%	100.0%
Total		Frequency	18	29	47
		%	38.3%	61.7%	100.0%

$\chi^2 = 0.777$; df = 3; p = 0.855

The results of our statistical analysis showed the lack of a statistically significant association between the result of the ERA test and the clinical pregnancy.

TABLE 2. The correlation between the result of the ERA test and clinical pregnancy

| | | | Clinical pregnancy | | Total |
			positive	Negative	
Result of the ERA test	Receptive	Frequency	3	8	11
		%	27.3%	72.7%	100.0%
	Prereceptive	Frequency	5	5	10
		%	50.0%	50.0%	100.0%
	Postreceptive	Frequency	1	0	1
		%	100.0%	0.0%	100.0%
Total		Frequency	9	13	22
		%	40.9%	59.1%	100.0%

$\chi^2 = 2.632$; df = 2; p = 0.268

We didn't find a statistically significant association between AMH classification and the clinical pregnancy ($\chi^2 = 1.745$; df = 2; p = 0.418). The clinical pregnancy had a percentage of 33.3% to 36.1%, regardless of the level of the ovarian reserve, expressed by the AMH classification (we excluded the very low ovarian reserve category because it only had one subject).

TABLE 3. The correlation between AMH classification and clinical pregnancy

| | | | Clinical pregnancy | | Total |
			positive	negative	
AMH classification	Normal ovarian reserve	Frequency	13	23	36
		%	36.1%	63.9%	100.0%
	Low ovarian reserve	Frequency	2	4	6
		%	33.3%	66.7%	100.0%
	Very low ovarian reserve	Frequency	1	0	1
		%	100.0%	0.0%	100.0%
Total		Frequency	16	27	43
		%	37.2%	62.8%	100.0%

$\chi^2 = 1.745$; df = 2; p = 0.418

We couldn't prove the presence of a statistically significant relation between the fertilization technique and the clinical pregnancy ($\chi^2 = 0.421$; df = 1; p = 0.516).

TABLE 4. The correlation between fertilization technique and clinical pregnancy

| | | | Clinical pregnancy | | Total |
			positive	negative	
Fertilization technique	IVF	Frequency	5	9	14
		%	35.7%	64.3%	100.0%
	ICSI	Frequency	1	4	5
		%	20.0%	80.0%	100.0%
Total		Frequency	6	13	19
		%	31.6%	68.4%	100.0%

$\chi^2 = 0.421$; df = 1; p = 0.516

We analysed the relation between the age categories and the live birth and found the lack of a statistically significant association ($\chi^2=0.761$; df=3; p=0.859).

Regardless of the age category, the percentage of the women which gave birth varied from 28.6% to 36.8%.

TABLE 5. *The correlation between age classification and live birth*

| | | | Live birth | | Total |
			Lack of birth	At least one baby	
Age classi-fication	<30 ani	Frequency	1	0	1
		%	100.0%	0.0%	100.0%
	30-34 ani	Frequency	12	7	19
		%	63.2%	36.8%	100.0%
	35-37 ani	Frequency	10	4	14
		%	71.4%	28.6%	100.0%
	38-39 ani	Frequency	9	4	13
		%	69.2%	30.8%	100.0%
Total		Frequency	32	15	47
		%	68.1%	31.9%	100.0%

$\chi^2 = 0.761$; df = 3; p = 0.859

The results of the statistical analysis also showed that there wasn't any statistically significant association between the result of the ERA test and live birth ($\chi^2 = 2.200$; df = 2; p = 0.333).

TABLE 6. *The correlation between the result of the ERA test and live birth*

| | | | Live birth | | Total |
			Lack of birth	At least one baby	
Result of the ERA test	receptive	Frequency	8	3	11
		%	72.7%	27.3%	100.0%
	Prereceptive	Frequency	6	4	10
		%	60.0%	40.0%	100.0%
	Postreceptive	Frequency	0	1	1
		%	0.0%	100.0%	100.0%
Total		Frequency	14	8	22
		%	63.6%	36.4%	100.0%

$\chi^2 = 2.200$; df = 2; p = 0.333

We didn't find neither a statistically significant association between AMH classification and live birth ($\chi^2 = 2.771$; df = 2; p = 0.250).

TABLE 7. *The correlation between AMH classification and live birth*

| | | | Live birth | | Total |
			Lack of birth	At least one baby	
AMH classi-fication	Normal ovarian reserve	Frequency	24	12	36
		%	66.7%	33.3%	100.0%
	Low ovarian reserve	Frequency	5	1	6
		%	83.3%	16.7%	100.0%
	Very low ovarian reserve	Frequency	0	1	1
		%	0.0%	100.0%	100.0%
Total		Frequency	29	14	43
		%	67.4%	32.6%	100.0%

$\chi^2 = 2.771$; df = 2; p = 0.250

The fertilization technique did not statistically associate with the live birth ($\chi^2 = 0.005$; df = 1; p = 0.946); the percentage of women which gave birth was around 20%, irrespective of the used fertilization technique.

TABLE 8. *The correlation between fertilization technique and live birth*

| | | | Live birth | | Total |
			Lack of birth	At least one baby	
Fertilization technique	IVF	Frequency	11	3	14
		%	78.6%	21.4%	100.0%
	ICSI	Frequency	4	1	5
		%	80.0%	20.0%	100.0%
Total		Frequency	15	4	19
		%	78.9%	21.1%	100.0%

$\chi^2 = 0.005$; df = 1; p = 0.946

DISCUSSIONS

The window of implantation (WOI) was studied by many authors. First, it was assessed using histological criterias by Noyes et al. in 1975 [3]; the appearance of the ectoplasmic projections called pinopodes was claimed to be important for the adhesion process, but the significance of pinopodes as endometrial receptivity markers has been recently disputed [4]. Then, WOI was studied from the cellular and biochemical markers point of view. The last decade meant the discovery of several endometrial receptivity markers, such as cell adhesion molecules (integrins, selectins, mucins, immunoglobulins), transcription factors (C/EBPb, Hand2, COUP-TFII) [5], leukemia inhibitory factor (LIF), interleukins (IL-15) and growth factors (VEGF, CSF, TGFβ). Unfortunately, so far, none of these markers found its place in clinical practice. The next step was assessing the immunology of the window of implantation. One of the most studied endometrial immune cells is the uterine natural killer (uNK) cell; uNK cells infiltrate the endometrium on LH+3 day and they cluster around spiral arterioles and decidualized stroma. uNK cells have been proposed as biomarkers of endometrial receptivity; one recent study showed a significantly increased prognostic value for uNK cell count, when combined with histological dating [6], but as I said before, the classical approach has many limitations.

In the last few years, WOI was studied by building the expression profile of the genes of the endometrial cells. The group from IVI took endometrial biopsies from fertile women in different stages of the menstrual cycle: proliferative stage (between days 9 and 12 of the menstrual cycle), prereceptive stage (between LH+1 and LH+5 days), receptive stage (LH+7) and postreceptive stage (LH+9 to LH+11). They discovered 134 differentially expressed genes that represented the specific

signature of the receptive stage; this was the basis of the ERA test (Endometrial Receptivity Analysis test). This test is used like it follows: the clinician performs an endometrial biopsy 7 days after the LH surge if the patient has a natural cycle, or 5 days after the beginning of progesterone administration if the patient follows a hormone replacement cycle; the endometrial tissue is confronted to the genetic signature of the different stages of the menstrual cycle and the predictor soft classifies the probe in one of the endometrial stages. By using the ERA test, WOI was carrefully investigated; it was shown that WOI differs from woman to woman, and it may last from 12 hours to 2 days; and most important, the window of implantation is displaced in up to 30% of patients [7]. This data lead to the utility of the ERA test for the patients with recurrent implantation failure: identification of WOI displacements allows the clinician to perform a personalized embryo transfer (PET) on the specific day recommended by the result of the ERA test.

A multicenter clinical trial published in 2013 showed that in RIF patients the window of implantation is displaced in 25% of patients [8]. For these patients, by performing the embryotransfer as indicated by the result of the ERA test, the pregancy rate obtained was 50% [8]. Our study found the window of implantation to be displaced for 50% of the cases, but the result of our statistical analysis showed the lack of a statistically significant association between the result of the ERA test and the clinical pregnancy and live birth rates.

But the ERA test does not have clinical applications only for patients with RIF. Simon et al. published in 2020 a multicenter randomized controlled trial that included 458 patients that underwent IVF with blastocyst transfer and were randomized to PET guided by the result of the ERA test, frozen embryo transfer or fresh embryo transfer [9]; the study demonstrated statistically significant improvement in the implantation rate, pregnancy rate and cumulative live birth rate for PET compared with frozen and fresh embryo transfer (ET).

Our study couldn't prove a statistically significant variation of the clinical pregnancy rate, regardless of

the ovarian reserve expressed by the AMH categories. The same thing happened in the case of the live birth rate. AMH is strongly linked with the quantitative response in assisted reproductive techniques, but its value as a predictor of live birth rate is still unclear. A systematic review from 2014 concluded that AMH may have a limited predictive value for obtaining prenancy, if it is controlled for age [10].

If we think about the limitations of the present study, we have to take into account the short period of time the study adressed to and the limited number of recurrent implantation failure patients that we enrolled, making it difficult to reach some definitive conclusions.

CONCLUSIONS

Most of our patients belonged to the age category of 30-34 years. According to the AMH categories that we defined, the majority of our subjects had a normal ovarian reserve and only 2.3% had a very low ovarian reserve. For most of our subjects, the fertilization of the oocytes was done by the standard technique.

The results of our IVF procedures were very good, taking into account we are talking about RIF patients: the clinical pregnancy rate was 38.3%, the live birth rate was 31.9%.

Half of the subjects that performed the ERA test had the window of implantation displaced. Nevertheless, our study concluded that endometrial receptivity tests did not improve the clinical pregnancy and live birth rates for recurrent implantation failure patients.

The study also showed that for patients with recurrent implantation failure, the age of the woman did not influence the outcomes of IVF procedures. We did not find a statistically significant association between the ovarian reserve of the patient and the clinical pregnancy and live birth rates.

The analysis of our data showed the lack of an association between the variables that described the IVF procedure (like the fertilization technique) and the outcomes of IVF procedures.

REFERENCES

1. Coughlan C, Ledger W, Wang Q, Liu F, Demirol A, Gurgan T, Cutting R, Ong K, Sallam H, Li TC. Recurrent implantation failure: definition and management. *Reprod Biomed Online.* 2014 Jan;28(1):14-38.
2. Momeni M, Rahbar MH, Kovanci E. A meta-analysis of the relationship between endometrial thickness and outcome of in vitro fertilization cycles. *J Hum Reprod Sci.* 2011 Sep;4(3):130-7.

3. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. *Am J Obstet Gynecol.* 1975 May;122(2):262-3.
4. Quinn CE, Casper RF. Pinopodes: a questionable role in endometrial receptivity. *Hum Reprod Update.* 2009 Mar-Apr; 15(2):229-36.
5. Tu Z, Ran H, Zhang S, Xia G, Wang B, Wang H. Molecular determinants of uterine receptivity. *Int J Dev Biol.* 2014;58(2-4):147-54.

6. Liu B, Mariee N, Laird S, Smith J, Li J, Li TC. The prognostic value of uNK cell count and histological dating in the mid-luteal phase of women with reproductive failure. *Eur J Obstet Gynecol Reprod Biol.* 2014 Oct; 181:171-5.
7. Díaz-Gimeno P, Horcajadas JA, Martínez-Conejero JA, Esteban FJ, Alamá P, Pellicer A, Simón C. A genomic diagnostic tool for human endometrial receptivity based on the

transcriptomic signature. *Fertil Steril.* 2011 Jan;95(1):50-60, 60.e1-15.

8. Ruiz-Alonso M, Blesa D, Díaz-Gimeno P, Gómez E, Fernández-Sánchez M, Carranza F, Carrera J, Vilella F, Pellicer A, Simón C. The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure. *Fertil Steril.* 2013 Sep;100(3):818-24.

9. Simón C, Gómez C, Cabanillas S, Vladimirov I, Castillón G, Giles J, Boynukalin K, Findikli N, Bahçeci M, Ortega I, Vidal C, Funabiki M, Izquierdo A, López L, Portela S, Frantz N, Kulmann M, Taguchi S, Labarta E, Colucci F, Mackens S, Santamaría X, Muñoz E, Barrera S, García-Velasco JA, Fernández M, Ferrando M, Ruiz M, Mol BW, Valbuena D; ERA-RCT Study Consortium Group. A 5-year multicentre randomized controlled trial comparing personalized, frozen and fresh blastocyst transfer in IVF. *Reprod Biomed Online.* 2020 Sep;41(3):402-415.

10. Iliodromiti S, Kelsey TW, Wu O, Anderson RA, Nelson SM. The predictive accuracy of anti-Müllerian hormone for live birth after assisted conception: a systematic review and meta-analysis of the literature. *Hum Reprod Update.* 2014 Jul-Aug;20(4):560-70.