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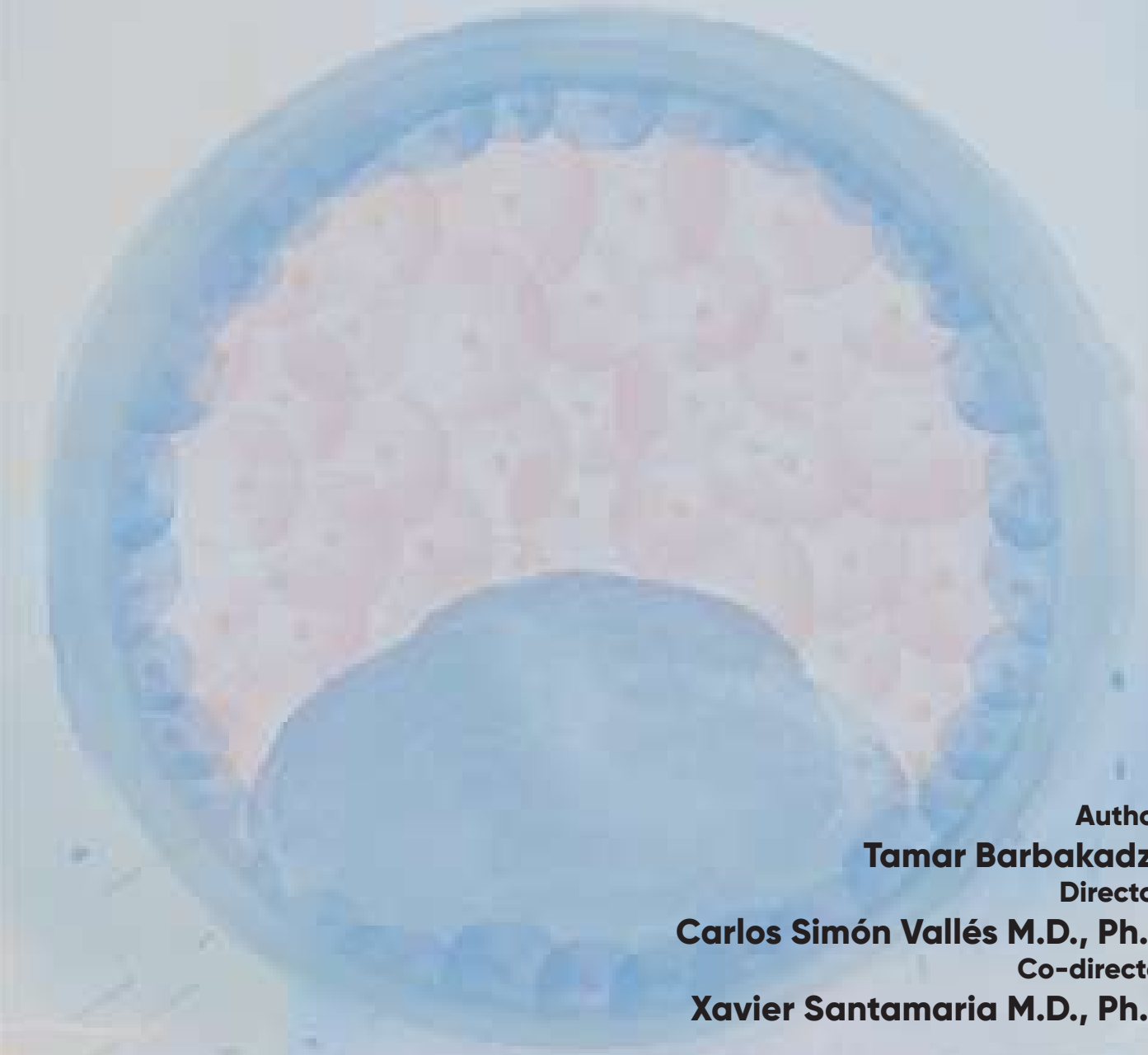
International, Randomized, and Controlled Clinical Study, to Evaluate the Clinical Benefit of the ERA Test in Infertile Patients Undergoing Assisted Reproduction Treatment and Medical Indication of PGT-A.



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International, randomized, and controlled clinical study, to evaluate the clinical benefit of the ERA test in infertile patients undergoing assisted reproduction treatment and medical indication of PGT-A.

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This dissertation is submitted for the degree of
Doctor of Medicine

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Prof. Carlos Simón Vallés, Professor of Obstetrics and Gynecology at the Faculty of Medicine at the University of Valencia and Associate Professor at Stanford University,

CERTIFY:

The research line titled: "The international, randomized, and controlled clinical study, to evaluate the clinical benefit of the ERA test in infertile patients undergoing assisted reproduction treatment and medical indication of PGT-A" has been carried out by Mrs. Tamar Barbakadze under my supervision. This thesis is completed, meets all the requirements, and is authorized for presentation and defense as a DOCTORAL THESIS for the degree of Doctor in Obstetrics, Gynecology, and Regenerative Medicine from the Faculty of Medicine at the University of Valencia, before the tribunal.

And to be known for the appropriate purposes, we sign this certification in Valencia, on December 15, 2024.

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McCulloh DH, Kutchukhidze N, Charkviani T, Zhorzholadze **T, Barbakadze** T, Munné S, Chkonia L.
Follicle size indicates oocyte maturity and blastocyst formation but not blastocyst euploidy following controlled ovarian hyperstimulation of oocyte donors. Human Reproduction. 2020 March; (35):557-564 DOI:10.1093/humrep/dez291

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Congresses

D. McCulloh, N. Kutchukhidze, T. Charkviani, T. Zhorzholadze, **T. Barbakadze**, S. Munné, L. Chkonia.
Follicle Diameter Predicts Oocyte Maturity and Blastocyst Formation but not Blastocyst Euploidy. The poster was presented at ESHRE annual meeting (Vienna, Austria, 2019).

D. McCulloh, N. Kutchukhidze, T. Charkviani, T. Zhorzholadze, **T. Barbakadze**, S. Munné, L. Chkonia.
Follicle diameter predicts oocyte maturity but not the formation of blastocysts or ploidy of blastocysts arising from mature oocytes of donors. The poster was presented at ASRM annual meeting (Philadelphia, PA, USA, 2019).

T.Barbakadze, D. McCulloh, N.Kutchukhidze, T.Zhorzholadze,T.Charkviani, L.Chkonia
First Pregnancy in Georgia Using Preimplantation Genetic Testing for Aneuploidy and Endometrial Receptivity Analysis. The poster was presented at 19th International Conference of PGDIS (Berlin, Germany 2022)

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Contemporary lifestyle modifications have postponed conception, rendering advanced maternal age a significant risk factor for female infertility. It is well established that advancing maternal age is strongly correlated with deteriorating oocyte quality and an increased risk of chromosomal abnormalities in oocytes and embryos. However, the reduction in fertility with age is not solely due to ovarian factors. Advances in assisted reproductive technology (ART), such as oocyte donation and the selection of viable embryos through preimplantation genetic testing for chromosomal abnormalities (PGT-A), have addressed several ovarian-related challenges associated with advanced maternal age. Nevertheless, other variables, particularly endometrial aging, significantly influence implantation rates, clinical pregnancy rates, live birth rates, and overall female fertility in women of advanced age.

Molecular biological techniques have significantly transformed assisted reproduction. Numerous couples facing infertility issues have successfully achieved the birth of healthy infants, even at an advanced age, through the utilization of two molecular biology methods: PGT-A and endometrial receptivity analysis (ERA). It is noteworthy that oocytes play a crucial role in the reproductive aging of women. However, given the pivotal role of the endometrium in facilitating embryo implantation and subsequent pregnancy, exploring the impact of age on endometrial assays via tests like the ERA provides valuable insights.

This underscores an important aspect of age-related fertility decline that extends beyond ovarian considerations. While advancements in ART, including oocyte donation and PGT-A, have successfully tackled some of the difficulties linked to ovarian aging, there is a growing acknowledgment that other factors, particularly the aging of the endometrium, substantially influence reproductive outcomes for women of advanced age. With advancing age, alterations in the endometrium may occur, potentially impacting implantation rates, clinical pregnancy rates, and live birth rates.

The clinical relevance of endometrial receptivity analysis (ERA) lies in its ability to guide personalized embryo transfer (pET) by aligning the embryo transfer (ET) with the patient's distinctive window of implantation (WOI). This synchronization proves to be particularly beneficial for patients experiencing implantation failure due to endometrial factors. By leveraging ERA, clinicians can tailor the timing of ET to coincide with the individual's endometrial receptivity profile, thereby optimizing the probability of successful implantation through a personalized approach and ultimately enhancing overall reproductive outcomes. Achieving comparable outcomes to those seen in patients of younger reproductive age is feasible in patients of advanced age.

Examining these effects within in-vitro fertilization (IVF) studies involving oocyte donation cycles offers valuable insights for clinicians and researchers in customizing fertility treatments. It underscores the necessity for a comprehensive approach to assisted reproduction, considering both ovarian and endometrial factors to optimize outcomes for women undergoing fertility treatments later in life.

Nonetheless, the lack of published studies on this topic indicates that further exploration is needed to enhance our understanding of methods for improving ART outcomes in advanced maternal age.

This study aims to improve ART outcomes with pET according to ERA in advanced-age patients with challenging reproductive histories and recurrent implantation failure (RIF) by utilizing donor oocytes and PGT-A for embryo testing.

Los cambios del estilo de vida actual han retrasado la concepción, convirtiendo la edad materna avanzada en un factor de riesgo considerable para la infertilidad femenina. Se ha demostrado que el aumento de la edad materna está estrechamente correlacionada con la disminución de la calidad de los ovocitos y un incremento del riesgo de anomalías cromosómicas en ovocitos y embriones. No obstante, la disminución de la fertilidad con la edad no se atribuye exclusivamente a factores ováricos. Los progresos en la tecnología de reproducción asistida (TRA), como la donación de ovocitos y la selección de embriones viables mediante pruebas genéticas preimplantacionales para anomalías cromosómicas (PGT-A), han abordado diversos desafíos relacionados con los ovarios asociados a la edad materna avanzada. Sin embargo, otros factores, especialmente el envejecimiento endometrial, afectan de manera significativa las tasas de implantación, embarazo clínico, nacidos vivos y la fertilidad general en mujeres de edad avanzada.

Las técnicas de biología molecular han revolucionado significativamente la reproducción asistida. Numerosas parejas con problemas de infertilidad han conseguido con éxito el nacimiento de bebés sanos, incluso a edades avanzadas, gracias a la aplicación de dos métodos de biología molecular: el PGT-A y el análisis de receptividad endometrial (ERA). Cabe destacar el papel crucial que desempeñan los ovocitos en el envejecimiento reproductivo de la mujer. No obstante, dado el rol fundamental del endometrio a la hora de promover la implantación del embrión y el posterior embarazo, explorar el efecto de la edad en los análisis endometriales mediante pruebas como el test ERA proporciona información valiosa.

Esto subraya un aspecto importante del descenso de la fertilidad relacionado con la edad que va más allá de las consideraciones ováricas. Si bien los avances en las TRA, como la donación de ovocitos y el PGT-A, han abordado con éxito algunas de las dificultades asociadas al envejecimiento ovárico, existe cada vez un mayor reconocimiento que otros factores, especialmente el envejecimiento del endometrio, impactan significativamente en los resultados reproductivos de mujeres de edad avanzada. A medida que avanza la edad, pueden producirse alteraciones en el endometrio que pueden afectar a las tasas de implantación, embarazo clínico y recién nacido vivo.

La importancia clínica del análisis de receptividad endometrial (ERA) radica en su capacidad para guiar la transferencia embrionaria personalizada (pET) sincronizando la transferencia de embriones (TE) con la ventana de implantación (WOI) específica de la paciente. Esta sincronización resulta especialmente beneficiosa en pacientes que experimentan fallos de implantación de origen endometrial. Al utilizar el test ERA, los médicos pueden sincronizar el momento de la TE con el perfil de receptividad endometrial específico de la paciente, optimizando así la probabilidad de éxito de la implantación mediante un enfoque personalizado y, en última instancia, mejorando los resultados reproductivos en general. Lograr resultados comparables a los observados en pacientes de menor edad reproductiva es factible en pacientes de edad avanzada.

El análisis de estos efectos en los estudios de fecundación in vitro (FIV) que incluyan ciclos de donación de ovocitos proporciona una valiosa información a médicos e investigadores en la personalización de los tratamientos de fertilidad. Esto destaca la necesidad de un enfoque integral en la reproducción asistida, teniendo en cuenta tanto los factores ováricos como endometriales para optimizar los resultados en mujeres sometidas a tratamientos de fertilidad en etapas avanzadas de la vida.

No obstante, la escasez de estudios publicados sobre este tema indica la necesidad de una mayor investigación para profundizar en nuestra comprensión de los métodos destinados a optimizar los resultados de las TRA en mujeres de edad materna avanzada.

Este estudio tiene como objetivo mejorar los resultados de las TRA mediante la pET según el análisis de la receptividad endometrial mediante el test ERA en pacientes de edad avanzada con antecedentes reproductivos complejos y fallo de implantación recurrente (RIF), utilizando ovocitos de donante y PGT-A para el análisis de los embriones.

Introduction

Contemporary lifestyle modifications have postponed conception, rendering advanced maternal age a significant risk factor for female infertility (Utting & Bewley, 2011). It is well established that advancing maternal age is strongly correlated with deteriorating oocyte quality and an increased risk of chromosomal abnormalities in oocytes and embryos. However, the reduction in fertility with age is not solely due to ovarian factors. Advances in assisted reproductive technology (ART), such as oocyte donation and the selection of viable embryos through preimplantation genetic testing for chromosomal abnormalities (PGT-A), have addressed several ovarian-related challenges associated with advanced maternal age. Nevertheless, other variables, particularly endometrial aging, significantly influence implantation rates, clinical pregnancy rates, live birth rates, and overall female fertility in women of advanced age (Pathare et al., 2023).

The clinical relevance of the endometrial receptivity analysis (ERA) resides in its ability to guide pET by aligning the ET with the patient's distinctive window of implantation (WOI). This synchronization proves to be particularly beneficial for patients experiencing implantation failure due to endometrial factors. By leveraging ERA, clinicians can tailor the timing of ET to coincide with the individual's endometrial receptivity profile, thereby optimizing the probability of successful implantation through a personalized approach and ultimately enhancing overall reproductive outcomes. In patients of advanced age, achieving comparable outcomes to those seen in patients of younger reproductive age is feasible (Ruiz-Alonso et al., 2014).

Nonetheless, the lack of published studies on this topic shows that the field needs further exploration. We are enhancing our understanding of methods for improving ART in advanced maternal age.

The study aims to improve ART outcomes with pET according to ERA in advanced-age patients with challenging reproductive histories and recurrent implantation failure (RIF) by utilizing donor oocytes and PGT-A for embryo testing.

Hypothesis

The study addresses the following question: Does the evaluation of the endometrium through the ERA test provide additional value to egg donation and PGT-A in advanced-age infertile patients?

Based on preliminary results, our hypothesis suggests that combined utilization of egg donation, PGT-A, and ERA significantly contribute to reproductive success, improving implantation rates in patients with RIF and, generally in all patients, especially those of advanced age undergoing assisted reproductive technology (ART).

The frequency of endometrial receptivity disorders in infertile women with RIF is significantly higher in those of advanced reproductive age compared to younger women.

In advanced-age women, receptivity disorders more frequently manifest as late receptivity, whereas in younger women, they are more commonly present as early receptivity.

In patients with a history of RIF, genetic testing of the embryo and pET based on endometrial receptivity assessment significantly improve the outcomes of ART including pregnancy rate, the implantation rate, decrease the rates of miscarriage, ectopic pregnancy, preterm delivery, preeclampsia, and intrauterine growth retardation and increase the live birth rates.

Objectives

O1. To evaluate the clinical benefit of personalized embryo transfer (pET) guided by ERA in advanced-age patients with recurrent implantation failure (RIF) using egg donation and PGT-A with euploid embryos, compared to control group 1, consisting of patients with the same indication undergoing non-ERA guided frozen embryo transfer (FET). This objective is assessed by comparing the implantation rates, clinical pregnancy rates, biochemical pregnancy rates, miscarriage rates, ectopic pregnancy rates, obstetric complications, and live birth rates between the study group and control group 1.

O2. To determine whether ERA-guided pET achieves reproductive success rates in advanced age patients with RIF that are comparable to those of younger patients with RIF. This involves a comparative analysis of reproductive outcomes between the study group and control group 2, composed of younger RIF patients, to assess whether there are significant differences in the clinical outcomes indicated above.

O3. To analyze the types and frequencies of endometrial window of implantation (WOI) displacement in infertile women of various reproductive ages with RIF and conduct a comparative analysis across different age groups, particularly:
- Between women younger than 35 years old and those older than 35 years old.
- To identify the types of WOI displacement in relation to age.

This analysis aims to elucidate the impact of age on endometrial receptivity and assess the potential benefits of personalized treatment approaches.

O4. To develop recommendations for ERA-guided pET based on the obtained data regarding implantation window displacement in advanced-age patients with RIF using egg donation and PGT-A with euploid embryos, and to determine its impact on reproductive outcomes.

By addressing these objectives, the study aims to provide comprehensive insights into the effectiveness of pET guided by ERA in improving ART outcomes across different age groups with RIF.

Materials and Methods

A prospective, randomized, observational follow-up study was conducted from March 2020 to September 2023 at the Georgian-American Center for Reproductive Medicine ReproArt.

Patients were allocated to the study and control groups based on the principle of randomization and application consistency.

Reproductive, obstetrical, and neonatal outcomes were systematically documented, with the data on pregnancy outcomes collected from a secure electronic national registry.

All procedures in the study were performed according to the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study protocol and a draft consent agreement for participation in the study were approved by the Ethics Committee of the Institution Review Board of the Georgian-American Center for Reproductive Medicine ReproArt (#2-20/287; February 7; 2020). Informed consent was obtained from all individual participants included in the study.

Infertile patients undergoing ART, who had previously experienced RIF with euploid ETs and had at least one frozen euploid embryo, were divided into groups based on the source of oocytes: donor oocytes were used for the study group and control group 1, while patients in the control group 2 utilized their oocytes. Study participants were selected after obtaining informed consent. They were allocated into study and control groups based on consistent application and randomization principles. The inclusion criteria were as follows: the age range for the study group and control group 1 was 35-45 years, while for control group 2 was 28-34 years. Patients must have had frozen euploid blastocysts (developed to day 5/6) analyzed by PGT-A. In the study group and control group 1, patients had embryos obtained from donor oocytes fertilized via in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI), whereas in control group 2, patients had embryos obtained from their own oocytes fertilized via IVF/ICSI. The expected ET involved one (single embryo transfer – SET) or two embryos (double embryo transfer - DET) in a hormonal replacement therapy (HRT) cycle.
Patients' Body Mass Index (BMI) ranged from 18.5 to 30 kg/m^2.

The exclusion criteria for the study included the presence of uterine cavity pathologies or malformations, such as polyps, intramural myomas of 4 cm or larger in size, submucosal myomas, septum, or hydrosalpinx, identified during the patient's participation in the study. However, patients diagnosed with these conditions before or after inclusion were permitted to participate if the pathology was corrected before any study procedures. Additionally, any illness or medical condition deemed unstable or that, according to medical judgment, could compromise the patient's safety and compliance with the study was grounds for exclusion.

After the patients had at least one euploid embryo, they were suitable for randomization into the study group and control group 1 and for selection in the control group 2.

Baseline demographics, family history, personal history, harmful habits, previous surgeries, and clinical characteristics were comparable among the groups. The follicle-stimulating hormone (FSH) and anti- Müllerian hormone (AMH) values of egg donors were similar between the study group and control group 1. However, in control group 2, the FSH and AMH values showed slight variations compared to the egg donor group. Cycle characteristics and embryological data were broadly consistent across the groups, with no significant differences observed.

A comparison of the reproductive parameters means between the study group and control group 1 showed statistically no significant differences.

A comparison of reproductive parameters means such as basal FSH, AMH, Antral follicle count (AFC), and blastocyst formation rate showed a statistically significant difference between the study group and the control group 2. Between the parameters of sperm concentration and fertilization rate was not found statistically significant difference.

After a normal basal ultrasound, from the 2nd-3rd day of the menstrual cycle controlled ovarian stimulation was initiated using 375 IU recombinant FSH, with 75 IU human menopausal gonadotrophin (HMG) for the first two days, followed by 150 IU recombinant FSH and 75 IU HMG from the third day of ovarian stimulation. As for the trigger, gonadotropin-releasing hormone agonist GnRH agonist 0.2mg/2ml and human chorionic gonadotropin (HCG)1500 IU were used. Transvaginal ultrasound examination, serum oestradiol (E2), FSH, and luteinizing hormone (LH) determination were started from the day of ovarian stimulation and repeated every 48 hours. The average duration of ovarian stimulation was 10-12 days. Oocyte retrieval was performed after 36 hours after the trigger injection. ICSI was carried out, and fertilization was assessed after 17/20 hours after microinjection. Embryos were cultured according to IVF laboratory protocol. Embryo quality was evaluated according to Garndner's criteria(Gardner & Balaban, 2016a). On day 5/6 of embryo development, a trophectoderm biopsy was done. Embryos were frozen according to the IVF laboratory protocol, and biopsied samples were sent for genetic testing for aneuploidy. Samples were analyzed utilizing Next-Generation Sequencing (NGS). Only euploid embryos were selected for transfer. In the study and in the control group 1, the patient underwent one or two endometrial biopsies, and ET was performed in an HRT cycle at the timing indicated by the ERA test.

Endometrial biopsies were collected from the uterine fundus using a Pipelle catheter or office hysteroscopy under sterile conditions. After the biopsy, the endometrium tissue was transferred to a cryotube containing 1.5ml of ribonucleic acid Later (RNALater), vigorously shaken for a few seconds, and kept at 4 C or in ice for at least 4h. The samples were shipped to Igenomix at room temperature to perform the ERA test.

The endometrium was prepared for the ERA test, and pET using frozen embryo transfer (FET) cycles using HRT. On the second or third day of the menstrual cycle, a vaginal ultrasound was performed, and blood levels of estradiol and progesterone (P4) were measured. Patients received 6 mg of oral oestradiol and 3 grams of transdermal oestradiol daily. Sonographic evaluations and E2 and P4 assessments were conducted between 7 and 12 days after endometrial preparation. The mean endometrial thickness was 8.7 mm standard deviation (SD)+_0.92. On the day of progesterone introduction, P4 levels were <1 ng/mL. For luteal phase support, patients received 600 mg of vaginal progesterone and 20 mg of dydrogesterone per day.

In control, group 1, ET was performed approximately 120 hours after progesterone introduction. For the study group and control group 2, ET was conducted according to the ERA- ERA-recommended period. In all three groups, high-quality euploid embryos were transferred, with either SET or DET performed. To avoid bias the quantity of transferred embryos did not differ significantly.

Outcomes were measured based on the pregnancy rate, implantation rate, and live birth rate.

The reproductive outcomes at first ET during 1-year follow-up in the study and control groups were assessed through intention-to-treat Analysis. The results of the study were treated statistically by the software SPSS22.0. Continuous variables were expressed as mean SD. The comparison of these parameters between groups was performed by independent t-test. Categorical variables were expressed as percentages. The comparison of these parameters between groups was performed by independent t-test. The comparison of these parameters between groups was performed by odds ratio (OR) and 95% Confidence Intervals (95%CI). P-values of <0.05 were considered to indicate statistical significance.

Results

Between March 2020 and September 2023, a total of 328 patients were recruited after meeting the inclusion criteria and providing informed consent. The study group consisted of 138 patients, control group 1 consisted of 140 patients, and control group 2 consisted of 50 patients. Following the follow-up period, 16 patients were excluded from the study group due to endometrial thickness <6 mm or progesterone levels >1 ng/ml; 8 patients were excluded from control group 1 for the same reasons.

Additionally, 6 patients from control group 2 experienced spontaneous pregnancy before starting the cycle for ET. Consequently, 122 patients in the study group, 132 in control group 1, and 44 in control group 2 completed all procedures included in the study protocol.

According to the intention-to-treat analysis was statistically significant difference was found between the outcomes. The pregnancy rate was statistically significantly higher in the study group in comparison with the control group 1 ($p=0.0007$); The Implantation rate was statistically significantly higher in the study group compared to control group 1($p=0.0009$); Live birth rate was statistically significantly higher in a study group in comparison with control group 1 ($p<0.0001$); There were not statistically significant differences in biochemical pregnancies ($p=0.90$); and clinical miscarriages ($p=0.065$) between the study group and control group 1.

According to the intention-to-treat analysis, no significant difference was found between the study group and control group 2 in pregnancy rate, implantation rate, live birth rate, biochemical pregnancy, and clinical miscarriages.

The study results indicate that the WOI was adjusted more frequently in advanced-age patients compared to the younger age group. Regarding WOI diplacements, there was no significant difference in early endometrial receptivity between the two groups. However, late endometrial receptivity was more prevalent among advanced-age patients.

Discussion

Our study included patients with euploid embryos to avoid potential biases from embryonic factors. Our results show that even with egg donation, there is a risk of aneuploid embryos; in donor cycles, 68.8±22.2% were euploid, as reported (Munné et al., 2017).

Our research demonstrates statistically significant differences in pregnancy outcomes, particularly 95 pregnancies from 122 pETs (77.9%) in the study group, compared to 76 pregnancies from 132 ETs (57.6%) in control group 1. This shows the advantages of pET guided by the ERA. The implantation rates were 54.1% in the study group and 35.5% in control group 1. The live birth rates were 71.3% in the PGT-A+ERA group and 39.4% in the PGT-A group. These outcomes indicate that ERA not only supports successful implantation but also ensures normal implantation and decidualization leading to lower pregnancy complications and a lower clinical miscarriage rate in the PGT-A+ERA group (Kelleher et al., 2018a).

Furthermore, a randomized controlled trial (RCT) by Simon et al. demonstrated statistically significant improvements in pregnancy, implantation, and cumulative live birth rates with pET compared to frozen and fresh ETs (Simón et al., 2020a).

The comparison between our study group and control group 2 showed similar success rates, with higher live birth rates in the advanced maternal age (>35 years old) PGT-A+ERA group. Despite the relationship between advanced maternal age and endometrial receptivity, it is multifaceted, encompassing hormonal, cellular, and molecular dimensions (Soares et al., 2005a).

Recent studies, including ours, have sought to elucidate these complexities, particularly how aging impacts endometrial function and fertility outcomes (Duster, Keefe, & Saketos, 2023).

Further analysis of the ERA results revealed that in advanced-age patients, the WOI was more frequently altered compared to younger patients, with late receptivity being more prevalent in this demographic (Gruninger et al., 1998a). These findings underscore the clinical relevance of ERA in tailoring ART procedures to individual endometrial profiles, thereby maximizing the likelihood of successful pregnancies. Moreover, the comparison between the study group and control group 2, consisting of younger patients, showed no significant difference in reproductive outcomes, suggesting that ERA-guided pET can elevate the reproductive success rates of advanced-age patients to levels comparable to those of younger patients. The data implies that the critical determinant of successful outcomes is the alignment of ET with the individual's WOI rather than the patient's chronological age. However, it is worth noting that the study design similar to ours needs to be more broadly described in the scientific literature, and the generalizability of these results to other study populations in different countries remains to be explored.

ERA-guided pET in the advanced maternal age (>35 years old) group patients provides outcomes comparable to those of younger patients in the <35 years old group. Contrary to reports suggesting that endometrial aging leads to decreased receptivity, our findings align with those of Paulson et al. (Paulson, 2019a), indicating that endometrial receptivity might remain stable with age, particularly with high-quality embryos.

Our results showed displaced receptivity rates of 23.8% in patients <35 years and 56.6% in those >35 years, underscoring the importance of ERA in advanced reproductive age. The ERA proved critical for optimizing transfer timing and provides no significant differences in clinical outcomes between these age groups.

According to Moreno et al.'s study results, the current understanding of endometrial aging also underscores the role of the microbiome in reproductive health. Recent studies indicate that a dysbiotic microbiome in the endometrium is associated with the pre-receptive endometrium and could disrupt the WOI and fertility outcome. This finding highlights the need for a holistic approach to addressing age-related infertility, considering both the microbiome and traditional hormonal and cellular markers (Moreno- no et al., 2016a). Our study illustrated considerable improvement solely through the utilization of ERA.

Moreover, advanced molecular techniques like the ERA have shown promise in improving outcomes for patients with RIF. These tools help accurately determine the WOI and synchronize ET with the endometrium's receptive state, enhancing the chances of successful implantation and pregnancy (Gajjar et al., 2024a).

Our study demonstrates that pET guided by ERA significantly improves ART outcomes in advanced-age patients. The pregnancy rate, implantation rate, and live birth rate were significantly higher in the study group compared to control group 1, highlighting the efficacy of ERA in optimizing reproductive outcomes. The live birth rate in the study group was almost double that of control group 1, indicating a substantial improvement in outcomes through personalized approaches.

In summary, our study highlights the critical role of pET guided by ERA in improving ART outcomes for advanced-age patients. By aligning ET with the patient's unique WOI, clinicians can enhance implantation success rates, leading to higher pregnancy and live birth rates. Future research should focus on refining these personalized approaches and exploring additional factors influencing endometrial receptivity, such as the microbiome, to optimize reproductive outcomes for all ART patients (Tesarik & Mendoza-Tesarik, 2022a).

A limitation of our study may be the recruitment of participants for RCTs. The high cost of ART, which is not covered by insurance, presents substantial challenges in attracting sufficient participants. This financial barrier may impact the generalizability and scalability of our findings. Further RCTs would be more informative to increase the number of patients in the age group <35 and to broadly compare the outcomes of PGT-A tested ETs using the conventional approach versus pET according to the ERA. However, challenges such as postponing fertility for advanced reproductive age and delayed referral to fertility specialists remain significant obstacles.

Conclusions

C1. In patients with a history of RIF, pET based on endometrial receptivity assessment with euploid embryos significantly improves the outcomes of ART, such as pregnancy rates, implantation rates, and live birth rates and decreases the rates of obstetric complications, including miscarriage rates, ectopic pregnancy rates, preterm delivery, preeclampsia, and intrauterine growth retardation.

C2. In patients with a history of RIF, pET based on endometrial receptivity assessment with euploid embryos significantly decrease the rates of obstetric complications, including miscarriage rates, ectopic pregnancy rates, preterm delivery, preeclampsia, intrauterine growth retardation.

C3. The frequency of endometrial WOI displacement in infertile women with RIF is significantly higher in those of advanced reproductive age (>35 years old) compared to younger women (<35 years old).

C4. In advanced-age women, endometrial WOI displacements more frequently manifest as late receptivity, whereas in younger women are more commonly present as early receptivity WOI shifts.

C5. Integrating molecular diagnostics such as ERA into clinical practice and developing recommendations for pET based on obtained data will enhance the effectiveness of ART programs, ensuring higher success rates and better reproductive health outcomes for patients of varying ages.

Introducción

Las transformaciones actuales en el estilo de vida han retrasado la concepción, convirtiendo la edad materna avanzada en un factor de riesgo considerable para la infertilidad femenina (Utting & Bewley, 2011). Se ha establecido que el aumento de la edad materna está estrechamente relacionado con la degradación de la calidad de los ovocitos y un incremento en el riesgo de anomalías cromosómicas en los ovocitos y embriones. No obstante, la disminución de la fertilidad con la edad no se atribuye exclusivamente a factores ováricos. Los progresos en la tecnología de reproducción asistida (TRA), como la donación de óvulos y la selección de embriones viables mediante pruebas genéticas preimplantacionales para anomalías cromosómicas (PGT-A), han enfrentado diversos desafíos vinculados a los ovarios en el contexto de la edad materna avanzada. Sin embargo, otros factores, especialmente el envejecimiento endometrial, afectan de manera significativa las tasas de implantación, las tasas de embarazo clínico, las tasas de nacidos vivos y la fertilidad femenina en general en mujeres de edad avanzada (Pathare et al., 2023).

La importancia clínica del análisis de la receptividad endometrial (ERA) reside en su habilidad para orientar la pET, sincronizando la ET con la ventana de implantación (WOI) específica de la paciente. Esta sincronización es especialmente ventajosa para las pacientes que enfrentan fracasos de implantación por causas endometriales. Al utilizar el ERA, los clínicos pueden sincronizar el momento de la transferencia de embriones con el perfil de receptividad endometrial del paciente, optimizando así la probabilidad de implantación exitosa mediante un enfoque personalizado y, en última instancia, mejorando los resultados reproductivos en general. En individuos de edad avanzada, es posible alcanzar resultados similares a los observados en pacientes de edad reproductiva más joven (Ruiz-Alonso et al., 2014).

No obstante, la escasez de investigaciones publicadas sobre este tema indica que el área requiere una exploración más profunda. Estamos perfeccionando nuestra comprensión de los métodos para optimizar la RMA en la edad materna avanzada.

El estudio tiene como objetivo mejorar los resultados de la ART con pET según el ERA en pacientes de edad avanzada con historias reproductivas desafiantes y fallos de implantación recurrentes (RIF) mediante el uso de ovocitos de donante y PGT-A para la prueba de embriones.

Hipótesis

El estudio examina la siguiente cuestión: ¿Ofrece la evaluación del endometrio mediante la prueba ERA un valor añadido a la donación de óvulos y PGT-A en pacientes infértiles de edad avanzada?

Según los resultados preliminares, nuestra hipótesis indica que la combinación de donación de óvulos, PGT-A y ERA incrementa notablemente el éxito reproductivo, optimizando las tasas de implantación en pacientes con RIF y, en general, en todos los pacientes, particularmente en aquellos de edad avanzada que se someten a tecnología de reproducción asistida (TRA).

The prevalence of endometrial receptivity disorders in infertile women with recurrent implantation failure is significantly higher in those of advanced reproductive age compared to younger women.

En mujeres de edad avanzada, los trastornos de receptividad se manifiestan con mayor frecuencia como receptividad tardía, mientras que en mujeres más jóvenes, se presentan más comúnmente como receptividad temprana.

En pacientes con antecedentes de RIF, las pruebas genéticas del embrión y la PET, fundamentadas en la evaluación de la receptividad endometrial, mejoran notablemente los resultados de la ART, incluyendo la tasa de embarazo, la tasa de implantación y reduciendo las tasas de aborto espontáneo, embarazo ectópico, parto prematuro y preeclampsia, además de incrementar las tasas de nacimientos vivos.

Objetivos

O1. Evaluar el beneficio clínico de la transferencia personalizada de embriones (pET) orientada por ERA en pacientes de edad avanzada con fallo de implantación recurrente (RIF), utilizando donación de óvulos y PGT-A con embriones euploides, en comparación con el grupo de control 1, que incluye pacientes con la misma indicación que se someten a transferencia de embriones congelados (FET) no orientada por ERA. Este objetivo se evalúa mediante la comparación de las tasas de implantación, tasas de embarazo clínico, tasas de embarazo bioquímico, tasas de aborto espontáneo, tasas de embarazo ectópico, complicaciones obstétricas y tasas de nacimientos vivos entre el grupo de estudio y el grupo de control 1.

O2. Evaluar si la PET dirigida por ERA alcanza tasas de éxito reproductivo en pacientes de edad avanzada con RIF que sean comparables a las de pacientes más jóvenes con RIF. Esto implica un análisis comparativo de los resultados reproductivos entre el grupo de estudio y el grupo de control 2, constituido por pacientes RIF más jóvenes, para determinar si existen diferencias significativas en los resultados clínicos mencionados anteriormente.

O3. Analizar los tipos y frecuencias del desplazamiento de la ventana de implantación endometrial (WOI) en mujeres infértiles de diversas edades reproductivas con RIF y llevar a cabo un análisis comparativo entre distintos grupos etarios, en particular:

Entre mujeres de menos de 35 años y aquellas de 35 años o más.

Para determinar los tipos de desplazamiento de WOI en función de la edad. Este análisis tiene como finalidad esclarecer el efecto de la edad en la receptividad endometrial y evaluar los posibles beneficios de los tratamientos personalizados.

O4. Formular recomendaciones para la pET orientada por ERA, fundamentadas en los datos recopilados sobre el desplazamiento de la ventana de implantación en pacientes de edad avanzada con RIF, utilizando donación de óvulos y PGT-A con embriones euploides, y evaluar su repercusión en los resultados reproductivos.

Al perseguir estos objetivos, el estudio busca ofrecer información exhaustiva sobre la eficacia de la pET dirigida por ERA en la mejora de los resultados de ART en diversos grupos etarios con RIF.

Materiales y métodos

Se realizó un estudio de seguimiento prospectivo, aleatorizado y observacional desde marzo de 2020 hasta septiembre de 2023 en el Centro Georgiano-Americano de Medicina Reproductiva ReproArt.

Los pacientes fueron asignados a los grupos de estudio y control según el principio de aleatorización y consistencia en la aplicación.

Los resultados reproductivos, obstétricos y neonatales fueron registrados de manera sistemática, con la información sobre los resultados del embarazo obtenida de un registro nacional electrónico seguro.

Todos los procedimientos del estudio se llevaron a cabo conforme a los estándares éticos del comité de investigación institucional y nacional, así como a la Declaración de Helsinki de 1964 y sus enmiendas posteriores o estándares éticos equivalentes. El protocolo del estudio y un borrador del consentimiento informado para la participación en el mismo fueron aprobados por el Comité de Ética de la Junta de Revisión Institucional del Centro Georgiano-Americano de Medicina Reproductiva ReproArt (#2-20/287; 7 de febrero; 2020). Se obtuvo el consentimiento informado de todos los participantes individuales involucrados en el estudio.

Pacientes infértiles sometidos a ART, que previamente habían experimentado RIF con ET euploides y tenían al menos un embrión euploide congelado, fueron divididos en grupos según la fuente de óvulos: se utilizaron óvulos de donante para el grupo de estudio y el grupo de control 1, mientras que los pacientes en el grupo de control 2 utilizaron sus propios óvulos. Los participantes del estudio fueron elegidos tras obtener el consentimiento informado. Fueron asignados a grupos de estudio y control según la aplicación sistemática y los principios de aleatorización. Los criterios de inclusión fueron los siguientes: El rango de edad para el grupo de estudio y el grupo de control 1 fue de 35 a 45 años, mientras que para el grupo de control 2 fue de 28 a 34 años. Los pacientes debían poseer blastocistos euploides criopreservados (desarrollados hasta el día 5/6) evaluados mediante PGT-A. En el grupo de estudio y el grupo de control 1, los pacientes poseían embriones derivados de ovocitos de donante fertilizados a través de fertilización in vitro (FIV)/inyección intracitoplasmática de esperma (ICSI), mientras que en el grupo de control 2, los pacientes contaban con embriones obtenidos de sus propios ovocitos fertilizados mediante FIV/ICSI. La transferencia embrionaria prevista consistió en uno (transferencia de un solo embrión - SET) o dos embriones (transferencia de dos embriones - DET) durante un ciclo de terapia de reemplazo hormonal (HRT). El índice de masa corporal (IMC) de los pacientes varió entre 18.5 y 30 kg/m2.

Los criterios de exclusión para el estudio abarcaron la existencia de patologías o malformaciones en la cavidad uterina, tales como pólipos, miomas intramurales de 4 cm o más, miomas submucosos, tabiques o hidrosálpinx, detectados durante la participación del paciente en el estudio. No obstante, se autorizó la inclusión de pacientes diagnosticadas con estas condiciones antes o después de la inclusión, siempre que la patología se corrigiera antes de cualquier procedimiento del estudio. Asimismo, cualquier enfermedad o condición médica clasificada como inestable o que, a juicio

del médico, pudiera comprometer la seguridad del paciente y su adherencia al estudio, era motivo de exclusión.

Una vez que los pacientes poseían al menos un embrión euploide, eran elegibles para la aleatorización en el grupo de estudio y en el grupo de control 1, así como para la selección en el grupo de control 2.

Las características demográficas iniciales, la historia familiar, la historia personal, los hábitos perjudiciales, las cirugías anteriores y las características clínicas fueron equivalentes entre los grupos. Los niveles de la hormona folículoestimulante (FSH) y de la hormona antimülleriana (AMH) en las donantes de óvulos fueron comparables entre el grupo de estudio y el grupo de control 1. No obstante, en el grupo de control 2, los niveles de FSH y AMH presentaron ligeras fluctuaciones en relación con el grupo de donantes de óvulos. Las características del ciclo y los datos embriológicos fueron, en términos generales, coherentes entre los grupos, sin que se evidenciaran diferencias significativas.

Una comparación de las medias de los parámetros reproductivos entre el grupo de estudio y el grupo de control 1 no mostró diferencias estadísticamente significativas.

Una comparación de parámetros reproductivos como FSH basal, AMH, recuento de folículos antrales (AFC) y tasa de formación de blastocistos mostró una diferencia estadísticamente significativa entre el grupo de estudio y el grupo de control 2. Entre los parámetros de concentración de esperma y tasa de fertilización no se encontró una diferencia estadísticamente significativa.

Tras una ecografía basal normal, se inició la estimulación ovárica controlada en el segundo o tercer día del ciclo menstrual, administrando 375 UI de FSH recombinante junto con 75 UI de gonadotropina menopáusica humana (HMG) durante los primeros dos días y continuando con 150 UI de FSH recombinante y 75 UI de HMG a partir del tercer día de la estimulación ovárica. Se empleó un agonista de la hormona liberadora de gonadotropina (GnRH) a 0.2 mg/2 ml y gonadotropina coriónica humana (HCG) a 1500 IU como desencadenante. El examen ecográfico transvaginal, la medición de estradiol sérico (E2), FSH y hormona luteinizante (LH) comenzaron el día de la estimulación ovárica y se repitieron cada 48 horas. La duración media de la estimulación ovárica fue de 10 a 12 días. La recuperación de ovocitos se llevó a cabo 36 horas tras la inyección de desencadenamiento. Se llevó a cabo ICSI y la fertilización se evaluó tras 17/20 horas de la microinyección. Los embriones fueron cultivados conforme al protocolo del laboratorio de fertilización in vitro. La calidad del embrión se evaluó según los criterios de Gardner (Gardner & Balaban, 2016a). En el día 5/6 del desarrollo del embrión, se realizó una biopsia del trofectodermo. Los embriones fueron congelados de acuerdo con el protocolo del laboratorio de FIV, y las muestras biopsiadas fueron enviadas para pruebas genéticas de aneuploidía. Las muestras fueron analizadas utilizando secuenciación de nueva generación (NGS). Solo se seleccionaron embriones euploides para la transferencia. En el estudio y en el grupo de control 1, la paciente se sometió a una o dos biopsias endometriales, y la transferencia de embriones (TE) se realizó en un ciclo de terapia de reemplazo hormonal (TRH) en el momento indicado por la prueba ERA.

Las biopsias endometriales se obtuvieron del fondo uterino mediante un catéter Pipelle o histeroscopia ambulatoria en condiciones estériles. Tras la biopsia, el tejido endometrial fue transferido a un criotubo que contenía 1.5 ml de ribonucleico ácido Later (RNALater), se agitó enérgicamente durante unos segundos y se conservó a 4 °C o en hielo durante un mínimo de 4 horas. The samples were sent to Igenomix at ambient temperature for the ERA test.

El endometrio fue acondicionado para la prueba ERA y pET mediante ciclos de transferencia de embriones congelados (FET) con terapia de reemplazo hormonal (HRT). En el segundo o tercer día del ciclo menstrual, se llevó a cabo una ecografía vaginal y se cuantificaron los niveles de estradiol y progesterona (P4) en sangre. Las pacientes recibieron diariamente 6 mg de estradiol oral y 3 gramos de estradiol transdérmico. Las evaluaciones ecográficas y las mediciones de E2 y P4 se llevaron a cabo entre 7 y 12 días tras la preparación endometrial. El grosor endometrial promedio fue de 8.7 mm, con una desviación estándar de ±0.92. En el día de la introducción de la progesterona, los niveles de P4 eran inferiores a 1 ng/mL. Para el apoyo de la fase lútea, los pacientes recibieron 600 mg de progesterona vaginal y 20 mg de didrogesterona diariamente.

En el grupo de control 1, la transferencia de embriones (TE) se llevó a cabo aproximadamente 120 horas tras la administración de progesterona. Para el grupo de estudio y el grupo de control 2, la transferencia de embriones (TE) se llevó a cabo conforme al período aconsejado por el ERA. En los tres grupos, se transfirieron embriones euploides de alta calidad, ya sea a través de SET o DET. Para prevenir sesgos, la cantidad de embriones transferidos no mostró diferencias significativas.

Los resultados se evaluaron en términos de la tasa de embarazo, la tasa de implantación y la tasa de nacimientos vivos.

Los resultados reproductivos de la primera transferencia de embriones (TE) durante un seguimiento de un año en los grupos de estudio y control se evaluaron mediante un análisis por intención de tratar. Los resultados del estudio fueron analizados estadísticamente utilizando el software SPSS 22.0. Las variables continuas se presentaron como media ± desviación estándar. La comparación de estos parámetros entre grupos se llevó a cabo utilizando la prueba t independiente. Las variables categóricas se presentaron en forma de porcentajes. La comparación de estos parámetros entre grupos se llevó a cabo utilizando la prueba t independiente. La comparación de estos parámetros entre grupos se llevó a cabo utilizando la razón de momios (OR) y los intervalos de confianza del 95% (95%CI). Se consideraron significativos los valores de p menores a 0.05.

Resultados

Between March 2020 and September 2023, a total of 328 patients were recruited after meeting the inclusion criteria and providing informed permission. El grupo de estudio incluyó 138 pacientes, el grupo de control 1 incluyó 140 pacientes y el grupo de control 2 incluyó 50 pacientes. Tras el período de seguimiento, 16 pacientes fueron excluidos del grupo de estudio debido a un grosor endometrial inferior a 6 mm o niveles de progesterona superiores a 1 ng/ml; 8 pacientes fueron excluidos del grupo de control 1 por las mismas razones.

Asimismo, seis pacientes del grupo de control 2 experimentaron un embarazo espontáneo antes de iniciar el ciclo para la terapia de estimulación. Por consiguiente, 122 pacientes en el grupo de estudio, 132 en el grupo de control 1 y 44 en el grupo de control 2 finalizaron todos los procedimientos estipulados en el protocolo del estudio.

El análisis por intención de tratar reveló una diferencia estadísticamente significativa en los resultados. La tasa de embarazo fue estadísticamente superior en el grupo de estudio en comparación con el grupo de control 1 (p=0.0007); la tasa de implantación fue estadísticamente superior en el grupo de estudio en comparación con el grupo de control 1 (p=0.0009); la tasa de nacimientos vivos fue estadísticamente superior in the group de estudio en comparación con el grupo de control 1 (p<0.0001); no se observaron diferencias estadísticamente significativas en los embarazos bioquímicos (p=0.90) ni en los abortos clínicos (p=0.065) entre el grupo de estudio y el grupo de control 1.

El análisis por intención de tratar reveló que no existió una diferencia significativa entre el grupo de estudio y el grupo de control 2 en las tasas de embarazo, implantación, nacimientos vivos, embarazo bioquímico y abortos espontáneos clínicos.

Los resultados del estudio revelan que el WOI se ajustó con mayor frecuencia en pacientes de edad avanzada en comparación con el grupo de edad más joven. No se observó una diferencia significativa en la receptividad endometrial temprana entre los dos grupos en relación con los desplazamientos del WOI. No obstante, la receptividad endometrial tardía fue más común en las pacientes de edad avanzada.

Discusión

Nuestra investigación abarcó pacientes con embriones euploides para eludir sesgos potenciales relacionados con factores embrionarios. Nuestros hallazgos indican que, a pesar de la donación de óvulos, persiste un riesgo de aneuploidía en los embriones; en los ciclos de donación, el 68.8±22.2% resultaron ser euploides, según lo reportado (Munné et al., 2017). .

Nuestra investigación revela diferencias estadísticamente significativas sobre los resultados del embarazo, específicamente 95 embarazos de 122 pETs (77.9%) en el grupo de estudio, en contraste con 76 embarazos de 132 ETs (57.6%) en el grupo de control 1. Esto evidencia los beneficios de la pET orientada por el ERA. Las tasas de implantación fueron del 54.1% en el grupo experimental y del 35.5% en el grupo de control 1. Las tasas de nacimientos vivos fueron del 71.3% en el grupo PGT-A+ERA y del 39.4% en el grupo PGT-A. Estos resultados sugieren que el ERA no solo facilita la implantación exitosa, sino que también garantiza una implantación y decidualización adecuadas, lo que resulta en una reducción de complicaciones durante el embarazo y una menor tasa de aborto espontáneo clínico en el grupo PGT-A+ERA (Kelleher et al., 2018a).

Asimismo, un ensayo controlado aleatorizado (ECA) llevado a cabo por Simon et al. evidenció mejoras estadísticamente significativas en las tasas de embarazo, implantación y nacimiento vivo acumulado con pET en comparación con los ET congelados y frescos (Simón et al., 2020a).

La comparación entre nuestro grupo de estudio y el grupo de control 2 reveló tasas de éxito equivalentes, con tasas de nacimientos vivos superiores en el grupo de edad materna avanzada (>35 años) PGT-A+ERA. A pesar de la correlación entre la edad materna avanzada y la receptividad endometrial, esto es multifacético, incluyendo aspectos hormonales, celulares y moleculares (Soares et al., 2005a).

Investigaciones recientes, incluida la nuestra, han intentado esclarecer estas complejidades, especialmente cómo el envejecimiento afecta la función endometrial y los resultados de fertilidad (Duster, Keefe, & Saketos, 2023).

Un análisis adicional de los resultados del ERA indicó que en pacientes de edad avanzada, el WOI se modificaba con mayor frecuencia en comparación con las pacientes más jóvenes, siendo la receptividad tardía más común en este grupo demográfico (Gruninger et al., 1998a). Estos hallazgos enfatizan la importancia clínica del ERA en la personalización de los procedimientos de ART de acuerdo con los perfiles endometriales individuales, optimizando así la probabilidad de embarazos exitosos. Asimismo, la comparación entre el grupo de estudio y el grupo de control 2, integrado por pacientes más jóvenes, no reveló una diferencia significativa en los resultados reproductivos, lo que indica que la pET guiada por ERA puede aumentar las tasas de éxito reproductivo de los pacientes de edad avanzada a niveles comparables a los de los pacientes más jóvenes. Los datos sugieren que el factor determinante de los resultados exitosos es la congruencia de la ET con el WOI del individuo, en lugar de la edad cronológica del paciente. No obstante, es importante destacar que el diseño del estudio comparable al nuestro requiere una descripción más exhaustiva en la literatura científica, y la generalización de estos hallazgos a otras poblaciones de estudio en diversos países aún debe ser investigada.

La pET asistida por ERA en pacientes de edad materna avanzada (>35 años) ofrece resultados equivalentes a los de las pacientes más jóvenes en el grupo de <35 años. Contrario a los informes que sugieren que el envejecimiento endometrial resulta en una reducción de la receptividad, nuestros hallazgos coinciden con los de Paulson et al. (Paulson, 2019a), indicando que la receptividad endometrial podría permanecer constante con la edad, especialmente con embriones de alta calidad.

Nuestros resultados revelaron tasas de receptividad desplazada del 23.8% en pacientes menores de 35 años y del 56.6% en aquellas mayores de 35 años, enfatizando la relevancia del ERA en la edad reproductiva avanzada. El ERA resultó fundamental para optimizar el momento de la transferencia y no exhibe diferencias significativas en los resultados clínicos entre estos grupos etarios.

De acuerdo con los hallazgos del estudio de Moreno et al., la comprensión contemporánea del envejecimiento endometrial enfatiza igualmente la función del microbioma en la salud reproductiva. Investigaciones recientes sugieren que un microbioma disbiótico en el endometrio se correlaciona con un endometrio pre-receptivo y podría perturbar la ventana de implantación y los resultados de fertilidad. This finding underscores the necessity of a holistic approach to address age-related infertility, taking into account both the microbiome and traditional hormonal and cellular markers (Moreno et al., 2016a). Nuestra investigación demostró una mejora significativa exclusivamente mediante el uso de ERA.

Asimismo, técnicas moleculares avanzadas como el ERA han demostrado ser prometedoras en la mejora de los resultados para pacientes con RIF. Estas herramientas permiten establecer con exactitud la WOI y sincronizar la ET con el estado receptivo del endometrio, aumentando las probabilidades de implantación exitosa y embarazo (Gajjar et al., 2024a).

Nuestra investigación evidencia que la PET dirigida por ERA optimiza de manera significativa los resultados de ART en pacientes de edad avanzada. La tasa de embarazo, la tasa de implantación y la tasa de nacimientos vivos fueron notablemente superiores en el grupo de estudio en comparación con el grupo de control 1, subrayando la efectividad del ERA en la mejora de los resultados reproductivos. The live birth rate in the study group was nearly double that of control group 1, indicating a significant improvement in outcomes with personalized approaches.

En síntesis, nuestro estudio subraya la función crucial de la pET orientada por ERA en la optimización de los resultados de la ART para pacientes de edad avanzada. Al sincronizar la transferencia de embriones (TE) con la única ventana de implantación (WOI) de la paciente, los clínicos pueden optimizar las tasas de éxito de la implantación, resultando en mayores tasas de embarazo y nacimiento vivo. La investigación futura debe enfocarse en perfeccionar estos enfoques personalizados y examinar factores adicionales que afectan la receptividad endometrial, como el microbioma, para optimizar los resultados reproductivos de todos los pacientes de ART (Tesarik & Mendoza-Tesarik, 2022a).

Una restricción de nuestro estudio podría ser el reclutamiento de participantes para los ensayos clínicos aleatorizados. El elevado costo de la ART, no cubierto por el seguro, plantea desafíos significativos para atraer a un número adecuado de participantes. Esta limitación financiera puede influir en la generalización y escalabilidad de nuestros resultados. Se requerirían más ensayos clínicos aleatorizados (RCT) para incrementar el número de pacientes en el grupo de edad menor de 35 años y para comparar de manera más exhaustiva los resultados de las transferencias de embriones (ET) evaluadas con PGT-A utilizando el enfoque convencional frente a la transferencia de embriones (pET) conforme al ERA. No obstante, desafíos como el retraso en la fertilidad debido a la edad reproductiva avanzada y la remisión tardía a especialistas en fertilidad continúan siendo obstáculos considerables.

Conclusiones

C1. En pacientes con antecedentes de RIF, la pET basada en la evaluación de la receptividad endometrial con embriones euploides mejora significativamente los resultados de la ART, como las tasas de embarazo, las tasas de implantación y las tasas de nacimientos vivos, y disminuye las tasas de complicaciones obstétricas, incluyendo las tasas de aborto espontáneo, las tasas de embarazo ectópico, el parto prematuro, la preeclampsia y el retraso del crecimiento intrauterino.

C2. En pacientes con antecedentes de RIF, la pET basada en la evaluación de la receptividad endometrial con embriones euploides disminuye significativamente las tasas de complicaciones obstétricas, incluyendo las tasas de aborto espontáneo, embarazo ectópico, parto prematuro, preeclampsia y restricción del crecimiento intrauterino.

C3. La frecuencia del desplazamiento del WOI endometrial en mujeres infértiles con RIF es significativamente mayor en aquellas de edad reproductiva avanzada (>35 años).

C4. En mujeres de edad avanzada, los desplazamientos del WOI endometrial se manifiestan con mayor frecuencia como receptividad tardía, mientras que en mujeres más jóvenes se presentan más comúnmente como cambios de receptividad temprana del WOI.

C5. Integrar diagnósticos moleculares como ERA en la práctica clínica y desarrollar recomendaciones para pET basadas en los datos obtenidos mejorará la efectividad de los programas de ART, asegurando tasas de éxito más altas y mejores resultados de salud reproductiva para pacientes de diversas edades.

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AFC	Antral Follicle Count
AMA	Advanced maternal age
AMH	Anti-Mullerian Hormone
AR	Androgen receptor
ART	Assisted reproductive technologies
ASRM	American Society for Reproductive Medicine
BMI	Body Mass Index
CDC	Centers for Disease Control and Prevention
COS	Controlled ovarian stimulation
CPR	Clinical pregnancy rates
DET	Double Embryo Transfer
DNA	Desoxyrribonucleic Acid
E2	Estradiol
EB	Endometrial biopsy
EC	Ethics Committee
EIM	The European IVF Monitoring Consortium
ER	Estradiol receptor
ERα	Estradiol receptor α
ERA	Endometrial receptivity analysis
ESHRE	European Society of Human Reproduction and Embryology
ET	Embryo transfer
EU	European Union
FET	Frozen Embryo Transfer
FSH	Follicle stimulating hormone
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GnRH	Gonadotropin-releasing hormone
HB-EGF	Heparin-binding EGF-like growth factor
hCG	Human chorionic gonadotropin
HMG	Human Menopausal Gonadotrophin
HRT	Hormonal replacement therapy
ICH	International Conference on Harmonisation

ICSI Intracytoplasmic sperm injection
IGFBP-1 Insulin-like growth factor binding protein 1
IL-6 Interleukine-6
IR Implantation rates
IRB International Review Board
IVF In vitro fertilization
LBR Live birth rates
LH Luteinizing hormone
LIF Leukemia inhibitory factor
MAPK Mitogen activated protein kinase
miRNA MicroRNA
MMP Matrix metalloproteinase
MRKH syndrome Mayer-Rokitansky-Küster-Hauser syndrome
MS Multiple sclerosis
NCR3 Natural cytotoxicity-triggering receptor 3
NGS Next-generation sequencing
NK Natural killer
OGP Ongoing Pregnancy Rate
PGS Preimplantation Genetic Screening
PGT-A Preimplantation genetic testing for chromosomal abnormalities
P4 Progesterone
P4R Progesterone receptor
PR Pregnancy Rate
PR-A Progesterone receptor A
PR-B Progesterone receptor B
PCOS Polycystic ovary syndrome
pET Personalized embryo transfer
PI Pulsatility index
RIF Recurrent Implantation Failure
SART Society for Assisted Reproductive Technology
SASP Senescence-associated secretory phenotype
SET Single embryo transfer
TNFα Tumor necrosis factor-α
Tregs Regulatory T cells
TVS Transvaginal sonography
VEGF Vascular endothelial growth factor
WOI Window of Implantation

INTRODUCTION

1.1 Infertility Prevalence

Addressing infertility is a crucial aspect of sexual and reproductive health and is imperative for enhancing the prevention, management, and treatment of infertility on a global scale (Starrs et al., 2018). Infertility is estimated to impact millions of individuals and couples worldwide, often leading to severe societal and health consequences, including social stigma, economic hardship, and diminished physical and mental well-being (Thoma et al., 2021). Given the global burden of infertility, there is an urgent need to intensify efforts to address it. Understanding the magnitude of infertility is essential for monitoring, assessing, and improving equitable access to quality fertility care services and addressing risk factors and consequences of infertility.

Even though determining the extent of infertility is crucial, there is a great deal of variation in how it is estimated at the population level. This diversity hinders comparisons among research, populations, and temporal contexts (Thonneau & Spira, 1990) (Schmidt et al., 1995) (Guzick & Swan, 2006) (Olive & Pritts, 2006) (Gurunath et al., 2011) (Stanford MSPH, 2013) (Thoma, 2015). The exact number of impacted individuals or couples remains uncertain, with estimates varying from 48.5 million couples worldwide (Mascarenhas et al., 2012) to 186 million ever-married women in developing nations alone (DHS Comparative Reports Infecundity, Infertility, and Childlessness in Developing Countries, n.d.). A detailed examination of infertility prevalence employed data from 277 reproductive health surveys and Bayesian hierarchical modeling to produce age-standardized estimates for 190 nations and territories, defining infertility as 5 years or more (Mascarenhas et al., n.d.). The investigation determined that 1.9% of women at risk of pregnancy faced primary infertility, characterized as the inability to achieve any live birth, while 10.5% encountered secondary infertility, defined as the inability to get a subsequent live delivery.

Assisted reproductive technologies (ART) should be accessible in all regions of the modern world. The availability of such high-tech methods is crucial for addressing infertility issues and ensuring that individuals and couples have the opportunity to conceive, regardless of their geographical location. This accessibility promotes reproductive health equity and supports the well-being of families and communities globally. (Adamson et al., 2023)

1.2 Assisted Reproduction

Over the last three decades, the number of assisted reproductive procedures has extremely increased. This trend is expected to persist as modern lifestyle changes have led to postponed parenthood. The 23rd European Society of Human Reproduction and Embryology (ESHRE) report highlights the escalating number of ART treatment cycles and resultant births, alongside a decline in twin deliveries due to decreased cases of multiple ETs; fresh in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) cycles exhibited higher delivery rates in Europe, more than a million IVF cycles are performed annually, yielding 203,665 live births, which constitutes approximately 1.5% of the total births in Europe (The European IVF Monitoring Consortium (EIM) for the ESHRE)(Smeenk et al., 2023a). Similarly, the annual number of ART cycles in the United States has increased 1.5-fold (Mardovich et al., 2021a).

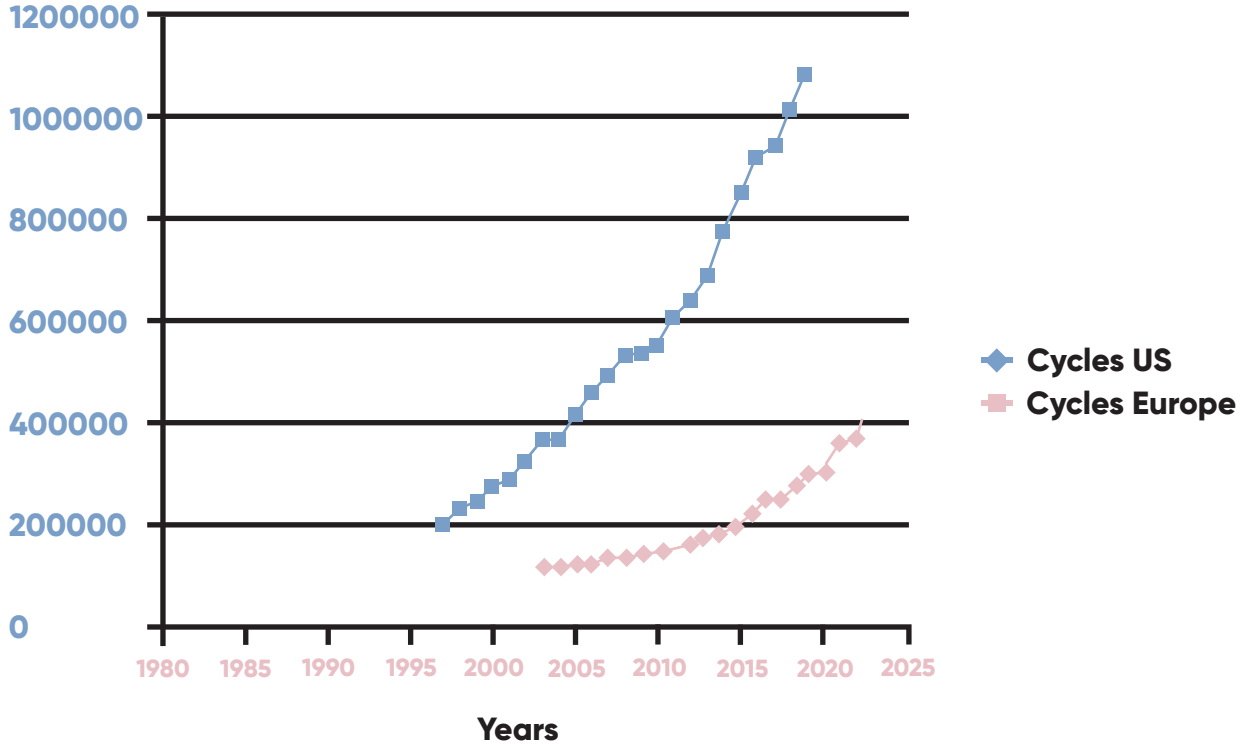


Chart 1. Number of assisted reproduction (ART) cycles in Europe (1997-2019) and the US (2003-2022).
Results were generated from the European IVF Monitoring Consortium (EIM) for the European registries by the European Society of Human Reproduction and Embryology (ESHRE) (Smeenk et al., 2023b) and Society for Assisted Reproductive Technology (SART) (SART: Society for Assisted Reproductive Technology, n.d.).

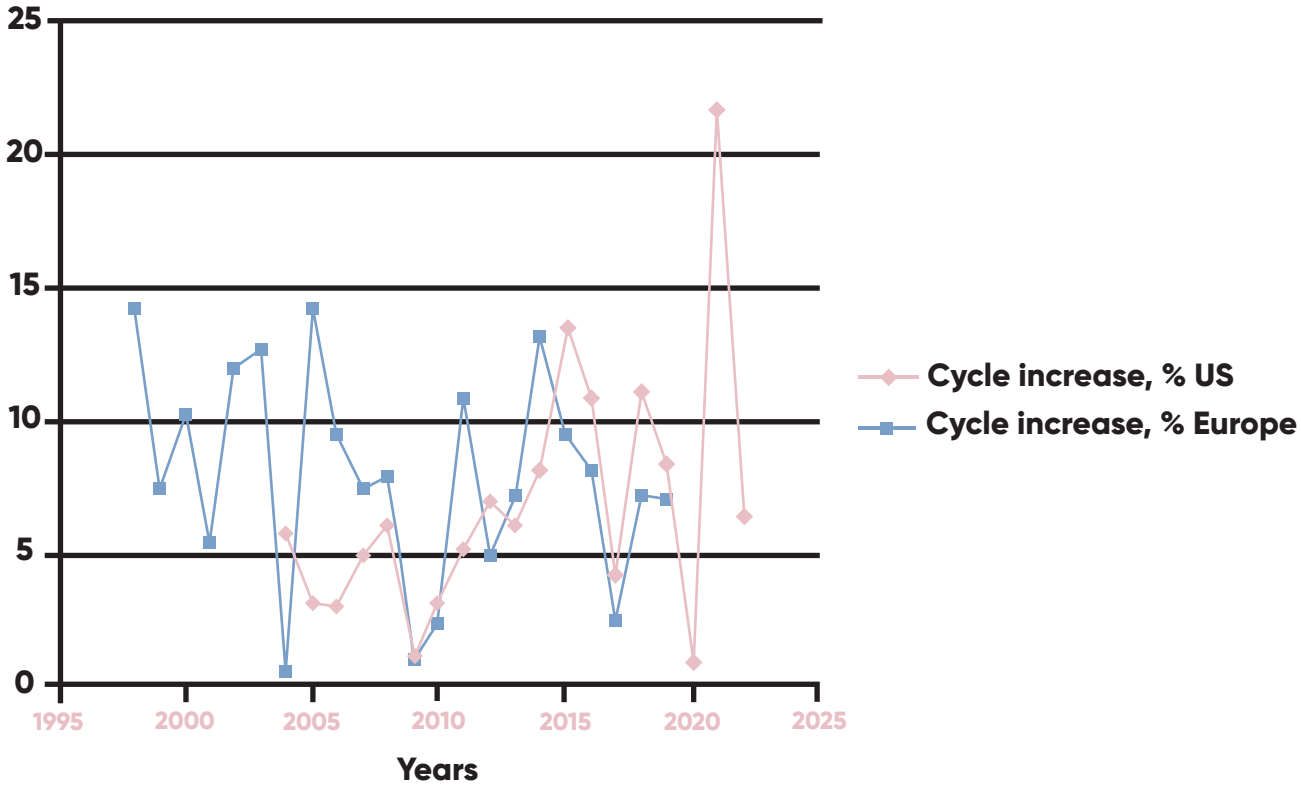


Chart 2. Percentages of assisted reproduction (ART) cycles in Europe (1997-2019) and the US (2003-2022).
Results were generated from the European IVF Monitoring Consortium (EIM) for the European registries by the European Society of Human Reproduction and Embryology (ESHRE) (Smeenk et al., 2023b) and Society for Assisted Reproductive Technology (SART) (SART: Society for Assisted Reproductive Technology, n.d.).

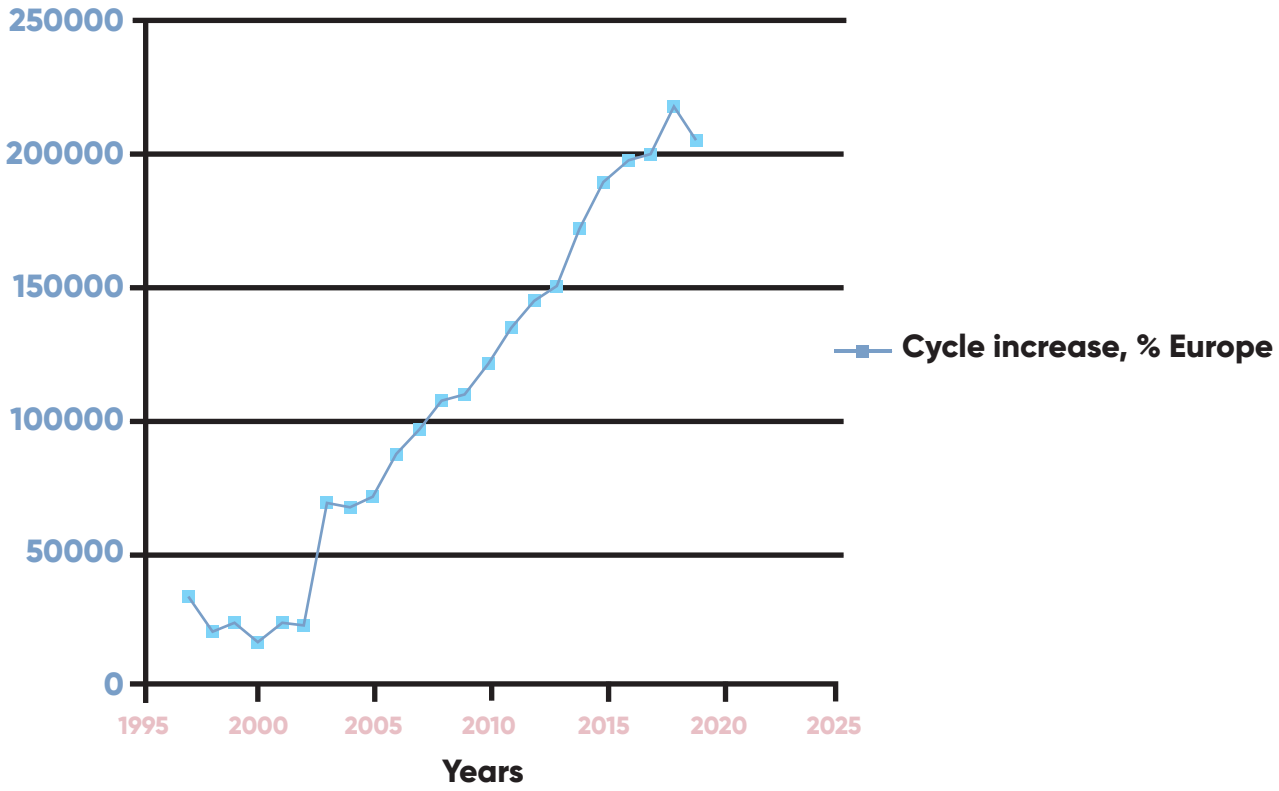


Chart 3. Number of infants born with assisted reproduction (ART) in Europe (1997-2019)
Results were generated from the European IVF Monitoring Consortium (EIM) for the European registries by the European Society of Human Reproduction and Embryology (ESHRE) (Smeenk et al., 2023b).

To elucidate the origins of the slow progress in IVF success rates, it is crucial to examine the demographics of the populations that require these techniques. An analysis of the age distribution within the United States ART population for 2021 reveals that 64.6% of patients were over 35, with 23.1% exceeding 40 years of age (Figure 1). Similar age distributions were observed in European countries during the same period (Chart 4,5,6), albeit with a slightly higher proportion of younger patients (<35 years) in the United States. The prevalence of women over 35 years old utilizing ART must be considered, as it is well-documented that the incidence of chromosomal abnormalities escalates with maternal age (Hassold & Hunt, 2001). Consequently, advanced maternal age (AMA) is associated with a higher number of aneuploid embryos (Demko et al., 2016) which in turn leads to elevated miscarriage rates and reduced live birth rates (Schoolcraft et al., 2009) (Rubio et al., 2013).

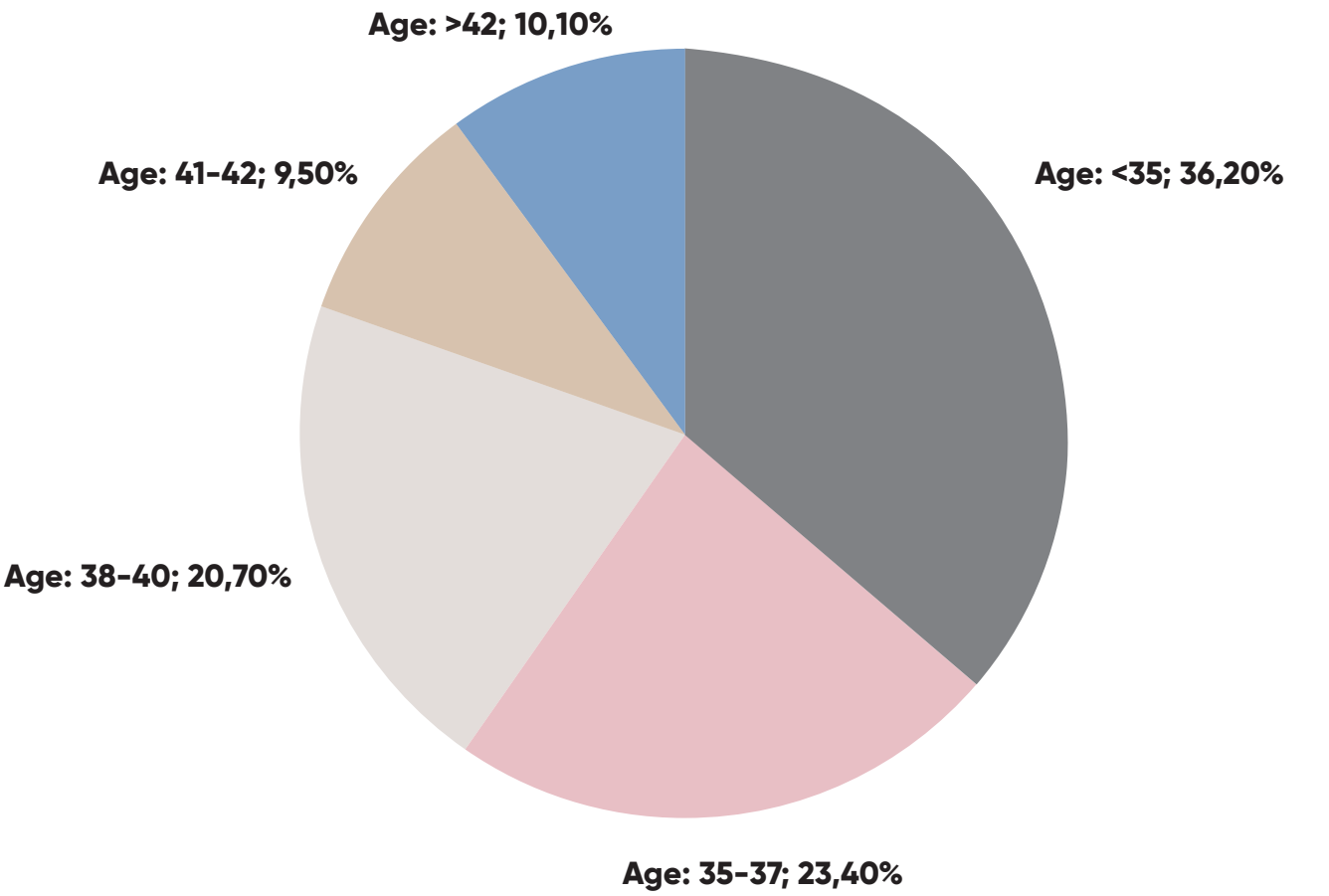


Figure 1. ART Use by Age Group, United States, 2021
Data was obtained from the Centers for Disease Control and Prevention (CDC) 2021 Assisted Reproductive Technology Fertility Clinic and National Summary Report (Mardovich et al., 2021b)

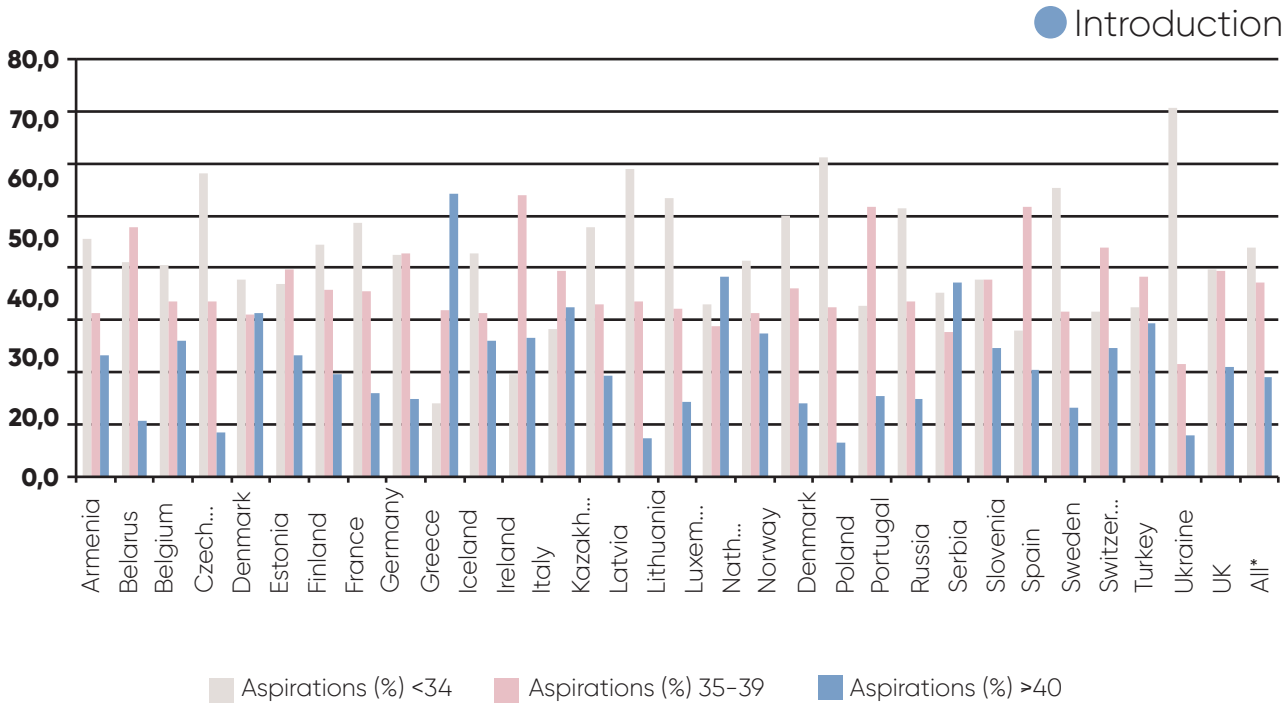


Chart 4. Aspiration rates by age distribution (years) of women treated with IVF in 2019. Results were generated from the European IVF Monitoring Consortium (EIM) for the European registries by the European Society of Human Reproduction and Embryology (ESHRE) (Smeenk et al., 2023b).

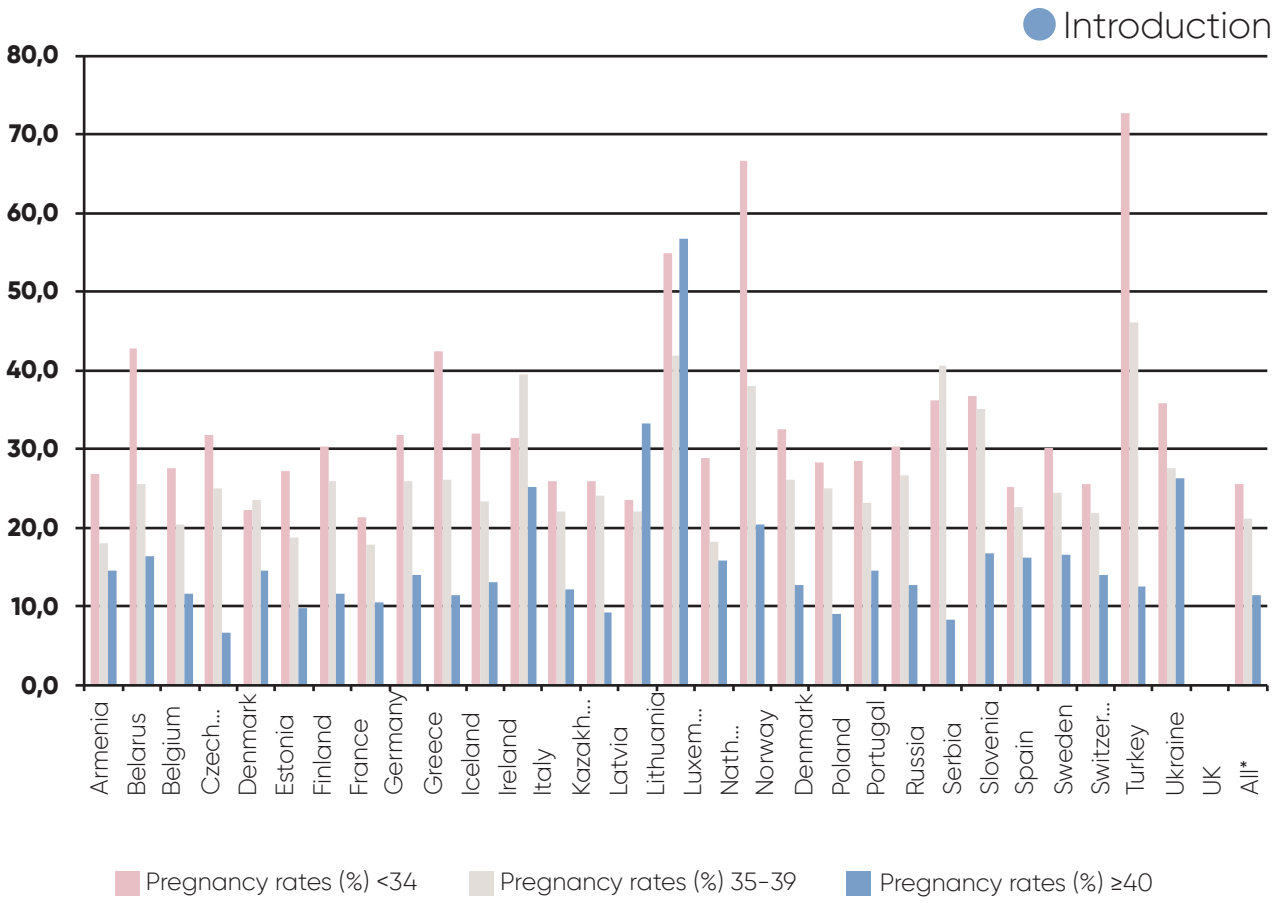


Chart 5. Pregnancy rates by age distribution (years) of women treated with IVF in 2019.

Results were generated from the European IVF Monitoring Consortium (EIM) for the European registries by the European Society of Human Reproduction and Embryology (ESHRE) (Smeenk et al., 2023b).

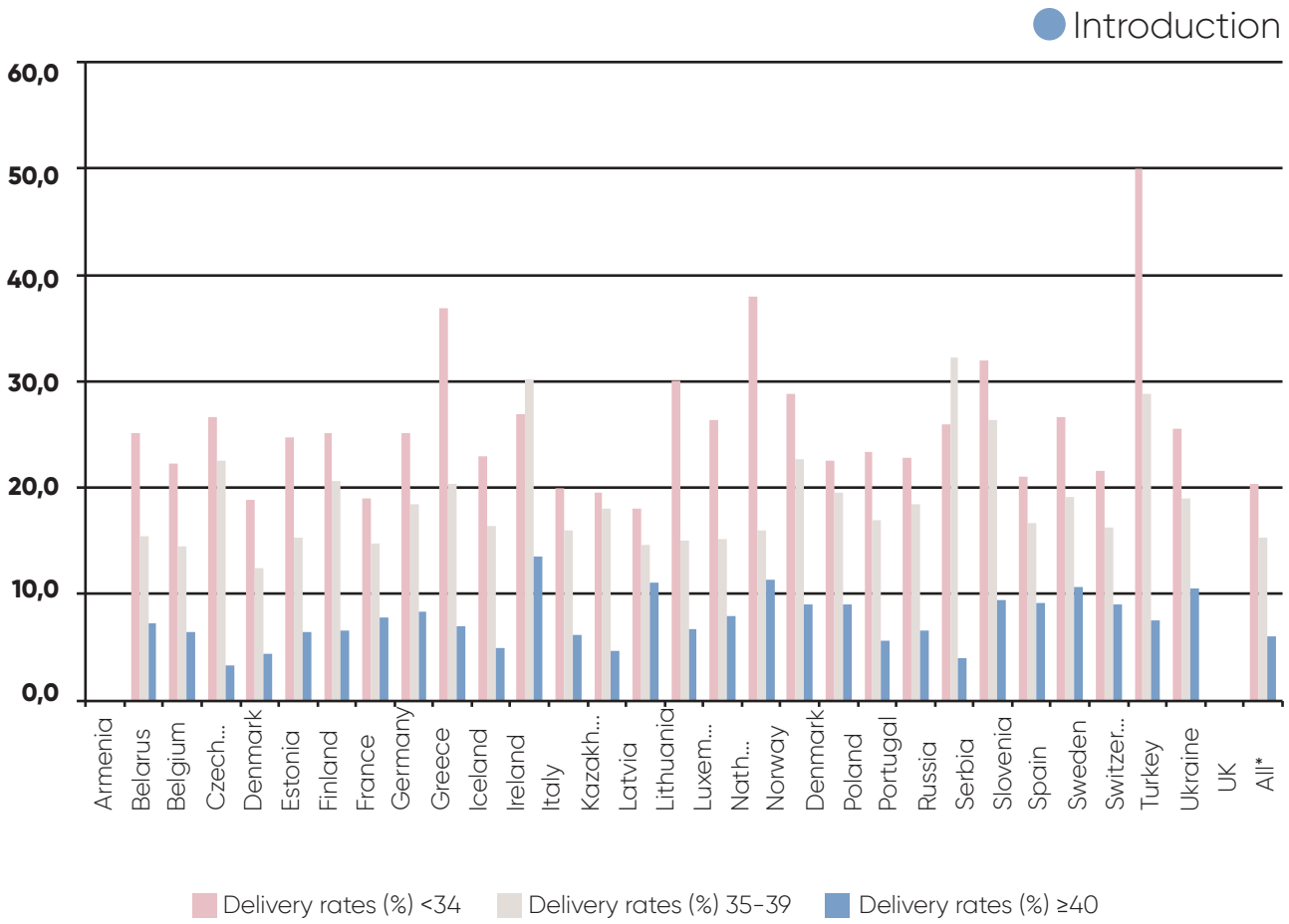


Chart 6. Pregnancy rates by age distribution (years) of women treated with IVF in 2019. Results were generated from the European IVF Monitoring Consortium (EIM) for the European registries by the European Society of Human Reproduction and Embryology (ESHRE) (Smeenk et al., 2023b).

Once the population requiring IVF treatments has been characterized, gaining a deeper understanding of human embryo biology becomes imperative. Specifically, it is essential to elucidate the impact of infertility on embryo development and the prevalence of aneuploidy. Despite the utilization of egg donation, the risk of aneuploid embryos remains substantial; in donor cycles, 68.8±22.2% of embryos were euploid, as reported by (Munné et al., 2017).

Considering our inability to alter the demographics of the IVF population or fundamentally improve embryo quality and endometrial changes associated with aging, the primary objective should be to enhance IVF techniques. This can be achieved by developing novel physiological stimulation protocols, optimizing culture systems, and employing more effective molecular biological methods. Such advancements, along with proper endometrial synchronization, would significantly improve ART success rates, ultimately resulting in the birth of healthy newborns.

1.3 Advanced maternal age as a critical risk factor for female infertility

The modern lifestyle has precipitated an increase in the age at which conception occurs, positioning AMA as a critical risk factor for female infertility. It is well-established that AMA correlates positively with a decline in oocyte quality and increased chromosomal abnormalities in both oocytes and embryos. However, the reduction in fertility with age is not solely due to ovarian factors (Pathare et al., 2023). Given the endometrium's crucial role in enabling embryo implantation and subsequent pregnancy, investigating the influence of age on endometrial assays, such as endometrial receptivity analysis (ERA), yields significant insights. This highlights a crucial aspect of age-related reproductive decrease that transcends ovarian factors (Duster, Keefe, & Saketos, 2023).

Advances in ART, such as oocyte donation and the selection of viable embryos through preimplantation genetic testing for chromosomal abnormalities (PGT-A), have addressed several ovarian-related diffi- culties associated with AMA. Nevertheless, other variables, particularly endometrial aging, significantly influence implantation rates (IR), clinical pregnancy rates (CPR), and live birth rates (LBR), as well as overall female fertility in women of advanced age (Pathare et al., 2023). Even so, the extant literature on this subject is sparse, indicating a significant gap in research. Enhancing our comprehension of endome- trial aging through biological (cellular, molecular, histological) and clinical lenses could substantially deepen our understanding of the risks associated with age-re- lated female infertility (Pathare et al., 2023).

Examining these effects within the IVF studies involving oocyte donation cycles offers valuable insights for clinicians and researchers in customizing fertility treatments. It underscores the necessity for a comprehensive approach to assisted reproduction, taking into account both ovarian and endometrial factors to optimize outcomes for women undergoing fertility treatments later in life (Duster, Keefe, & Saketos, 2023).

1.4 In vitro fertilization procedures

The natural reproduction process entails the ovulation of a single oocyte by the woman, which must be fertilized by a single sperm from the man's semen. Both gametes, the oocyte and the sperm are haploid, and their union results in the formation of a diploid cell that develops into a healthy newborn. In cases of infertility, IVF becomes necessary. This process begins with controlled ovarian stimulation (COS) and is followed by supervised embryo development to select the optimal embryo for transfer. The transfer must be precisely timed to ensure correct synchronization with the endometrium to maximize the chances of implantation and subsequent pregnancy. Each step in this process is critical to the overall success of the procedure; therefore, the reproductive medicine community should focus on the continuous improvement of each stage.

1.4.1 Egg donation

Human oocyte donation has become a very prevalent treatment that addresses many reproductive problems. In 2018, the CDC reported over 22,000 operations involving fresh or frozen embryos acquired through oocyte or embryo donation in the United States (Hacker et al., 2018). Oocyte donation is a recognized and very efficacious approach for addressing infertility in people undergoing physiological menopause or early ovarian insufficiency, facilitating pregnancy in individuals who have concluded their reproductive years.

In modern ART facilities, donor eggs are sometimes obtained from "egg banks," which preserve oocytes from young, healthy donors who get compensation for undergoing stimulation and oocyte retrieval. These oocytes are preserved for subsequent use or employed immediately for embryo formation on the day of retrieval. Recipients of donated oocytes demonstrate implantation and pregnancy rates akin to those seen in young women undergoing in vitro fertilization. It is crucial for physicians and patients to acknowledge that pregnancies resulting from oocyte donation are associated with a heightened risk of particular obstetrical and neonatal problems, such as pre-eclampsia, premature delivery, and low birth weight (Sauer, 2013).

1.4.2 Controlled ovarian stimulation

The human ovulation system typically results in the availability of only one oocyte per cycle, offering a singular opportunity for pregnancy approximately every 28 days. When coupled with infertility, the likelihood of successful conception diminishes significantly. This observation led to the establishment of COS as a procedure to circumvent the limited expectations of natural cycles in the context of infertility. COS facilitates the production of a higher number of mature follicles per cycle, thereby increasing the number of oocytes available for fertilization. However, recent studies indicate that lower doses of gonadotropins enhance embryo quality (Rubio et al., 2010).

Moreover, it has been observed that mild stimulation protocols result in aneuploidy rates comparable to those in natural cycles, with 40.6% and 34.8% aneuploid embryos, respectively, for an average female age of 25.4 ± 4 years (Labarta et al., 2012). The differences in aneuploidy rates between stimulation protocols may have a biological basis, as suggested by the events occurring in the oocyte during gonadotropin stimulation. In natural cycles, during follicle recruitment, meiosis I resumes with the extrusion of the first polar body and the selection of a single follicle, whereas, in COS cycles, this resumption and maturation occur simultaneously in multiple follicles.

1.4.3 In vitro fertilization process

Once high-quality mature oocytes are obtained, the focus shifts to their proper fertilization, a process facilitated through two primary techniques: conventional in IVF or ICSI. The selection between these techniques depends on the seminal and clinical background of the patients.

Conventional IVF is recommended for patients with good-quality sperm (Bhattacharya et al., 2001) and involves incubating the oocyte overnight with motile sperm according to the ESHRE Guideline Group on Good Practice in IVF, December 2015 (De Los Santos et al., 2016). The concentration of sperm is a crucial factor: if it is too low, it can reduce the chances of fertilization, while if it is too high, it can cause polyspermy and result in polyploid embryos (Fukuda & Chang, 1978). The recommended sperm concentration for conventional IVF ranges from 0.1 to 0.5 million/mL of motile sperm (De Los Santos et al., 2016).

Conversely, ICSI involves the hand insertion of a single spermatozoon into a mature egg and is specifically recommended for instances of severe male infertility. The choice of spermatozoon for injection is vital for subsequent embryonic development, as it has been linked to the incidence of aneuploidies in sperm (Rodrigo et al., 2011). It is essential to choose a motile spermatozoon with optimal morphology to reduce the danger of introducing aberrant chromosomal content (Collodel et al., 2007). The spermatozoon provides half of the embryonic chromosomal material and also supplies a crucial cellular organelle, the centrioles (Sathananthan et al., 1991). The centrioles and pericentriolar material constitute the centrosome, essential for cell division by coordinating the mitotic spindle. Moreover, ICSI requires scrupulous monitoring during the injection process to prevent harm to the oocyte's internal structures, including the spindle. To avert such harm, it is advisable to place the polar body opposite the injection site, as it functions as an indicator of the spindle's position (De Los Santos et al., 2016).

Regardless of the fertilization technique used, the introduction of the sperm into the oocyte initiates the resumption of meiosis II, triggering the extrusion of the second polar body. Correct fertilization is evaluated by observing the presence of two pronuclei, one maternal and one paternal, along with the first and second polar bodies. Abnormalities in the number of polar bodies and pronuclei can be attributed to errors in female meiosis or fertilization failures (Flaherty et al., 1998). This evaluation is recommended to be performed 17-20 hours post-insemination (De Los Santos et al., 2016).

1.4.4 Embryo culture

The preimplantation embryo development encompasses multiple mitotic divisions commencing from the zygote stage. The cell number increases exponentially, as each daughter cell undergoes mitosis in parallel, resulting in a doubling of the cell count. Hence, the typical mitotic progression of an embryo is from 1 to 2 cells, 2 to 4 cells, 4 to 8 cells, 8 to 16 cells, and so forth, reaching approximately 250 cells within five days of development (Niakan et al., 2012).

During this in vitro growth, a minimum of three stages can be distinguished based on cellular quantity and appearance. 1. The cleavage stage, occurring during the initial 3 days of development, involves the embryo comprising 2 to 8 distinct cells, referred to as blastomeres. 2. The morula stage transpires roughly 4 days after conception, during which all embryonic cells undergo compaction, merging their cytoplasm to create a syncytium. 3. The blastocyst stage, during which the morula accumulates fluid within the embryo through Na+/K+ channels, is referred to as cavitation, resulting in the formation of a cavity called the blastocoel. The blastocyst consists of two separate cell lineages: the trophectoderm, which creates a hollow spherical, and the inner cell mass, which forms a cluster among the trophoblastic cells.

Throughout the first two stages, cleavage and morula, the embryo divides without a significant change in size; However, during the blastocyst stage, various collapsing movements result in an increase in embryo size. Eventually, the zona pellucida develops a crack through which the blastocyst begins hatching, ultimately leaving the zona pellucida after multiple collapsing movements.

Occasionally, an embryo may be arrested at a particular stage of development and cease to progress, eventually leading to its demise. This phenomenon has been correlated with failures in embryo biology, such as transcriptomic alterations (Wong et al., 2010), metabolic issues (Leese et al., 2008), and an increased rate of chromosomal abnormalities (Munne et al., 1995).

The primary objective of in vitro embryo culture is to replicate the natural conditions of the endometrial cavity as closely as possible. Therefore, embryo culture must be conducted under meticulously monitored conditions (temperature, oxygen levels, humidity, etc.) using incubators and heated surfaces. The selection of the most appropriate culture media, based on the developmental stage and nutritional requirements of the embryo, is critical, whether for single embryo culture or co-culture of multiple embryos from the same patient. Each IVF laboratory develops distinct protocols for embryo culture to achieve optimal success rates, resulting in significant variations between centers, which pose challenges for comparative analyses.

1.5 Uterus and Endometrium

1.5.1 Ontogeny of the Uterus

The female reproductive tract is derived from the urogenital ridge, forming the fallopian tubes, uterus, and upper vagina by week 10 of gestation. The uterus matures structurally under the influence of placental hormones and regresses to an atrophic state post-delivery due to a fall in estrogen and progesterone levels.

The Wnt family and HOX genes play critical roles in the development and function of the reproductive tract. Mutations in these genes can cause developmental anomalies, such as Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome, affecting müllerian duct formation and function. Wnt signaling, including ligands WNT4, WNT5A, WNT7A, and transcriptional regulators HOX genes, is essential for müllerian duct elongation and differentiation. For instance, WNT4 absence leads to müllerian duct regression, while HOXA10 and HOXA11 are crucial for uterine morphology and implantation (Ghosh et al., 2020)(Boiani & Duncan, 2023).

The fallopian tubes, endometrium, myometrium, and uterine cervix function synergistically to receive gametes, facilitate fertilization, support embryo growth, and eventually orchestrate the expulsion of the mature fetus. The reproductive tract's preparation for these functions is programmed by ovarian steroid hormones, which act directly on their cognate receptors and indirectly through various steroid-regulated growth factors, cytokines, and intracellular signaling molecules.

The endometrium develops within the uterus from the urogenital ridge early in embryonic development. This mucosal lining, comprising epithelial and stromal cells, orchestrates cyclic menstruation and provides an immune privileged site for embryo implantation and nurtures the fetus during pregnancy. However, embryo implantation requires a series of complex endometrial changes essential for establishing receptivity.

1.5.2 Endometrium

The endometrium is a complex tissue that lines the interior of the uterine cavity. It is morphologically divided into functional and basal layers. The functional layer constitutes two-thirds of the endometrial thickness and comprises various cellular compartments: the luminal epithelium, the glandular epithelium, the stroma, and the vascular compartments. The epithelium consists of epithelial cells, which may be located on the surface or lining the epithelial glands. The stroma includes an extracellular matrix (ECM) and fibroblasts, which undergo differentiation during the decidualization process. Within the stroma, blood vessels (spiral arteries) are present, along with resident immune cells such as macrophages and natural killer (NK) cells (Beier & Beier-Hellwig, 1998).(Bulun, 2011).

Regarding their functions, the functional layer is responsible for the proliferation, secretion, and tissue degeneration, whereas the regenerative capacity of the endometrium resides in the basal layer (Hawkins & Matzuk, 2008) (Diedrich et al., 2007a). Ovarian steroids and paracrine-secreted molecules from neighboring cells regulate the gene expression of various endometrial cell types via specific receptors. Estradiol, through estradiol receptor (ER) α, promotes endometrial growth, while progesterone counteracts estrogen's effects by downregulating estrogen receptor expression and inducing enzymes that catabolize estradiol, ensuring proper endometrial preparation for implantation (Lessey & Young, 2019). Progesterone receptors (P4Rs), including isoforms PR-A and PR-B, mediate the anti-proliferative effects of progesterone in the glandular epithelium and stimulate stromal cell proliferation via the mitogen activated protein kinase (MAPK/AKT) pathway (Melton & Iv, 2019). Differential expression of these isoforms is crucial for normal endometrial function and pregnancy. The uterus is also a target for androgens, with androgen receptors (ARs) expressed in the endometrium and myometrium, especially in stromal cells during the proliferative phase and epithelial cells of the secretory phase. Estradiol increases endometrial stromal AR expression, and when combined with testosterone or progesterone, it enhances epithelial and myometrial AR levels (Simitsidellis et al., 2018).

It is noteworthy, that steroid hormones likewise regulate innate and adaptive immune functions, collectively defending the reproductive tract environment while modulating immune responses during pregnancy to accept the fetal semi-allograft (Hoffman et al., 2023). The endometrium balances immune tolerance and defense, crucial for accommodating semi-foreign placental cells while protecting against infections. Endometrial immune responses are influenced by cyclic sex steroid changes and human chorionic gonadotropin (hCG; Shih et al., 2022).

The vascular system nourishes the endometrium in the initial receptive phase and is remodeled by invading trophoblasts to establish the placental blood supply (Mendes et al., 2019). Without conception, the endometrium is shed through an inflammatory-like reaction involving matrix metalloproteinases (MMPs), cytokines, vasoactive substances, and uterine contractions, promoting remodeling, hemostasis, and expulsion of the spent endometrial lining. The endometrial cells' regenerative capacity ensures a new luminal surface for the next cycle (Arbeláez-Gómez et al., 2022).

During the endometrial cycle, distinct morphological and functional changes occur specific to each phase of the menstrual cycle. These changes result from the response of various endometrial cell types to steroid hormones and paracrine molecules. Since 1950, endometrial dating has been performed from a histological perspective. Subsequently, the need to comprehend the genetic mechanisms underlying the histological changes arose (August 1950 - Volume 5 - Issue 4: Obstetrical & Gynecological Survey, n.d.). Prior to the genomic era, researchers were constrained to studying molecular changes on a "gene-by-gene" basis to identify the alterations responsible for observed changes. However, in the genomic era, the prevailing trend has shifted toward global screening of all transcribed genes and their interactions (Sherwin et al., 2006). Thus, over the past decade, the transcriptional mechanisms underlying endometrial biology have been extensively investigated. Transcriptome profiling can detect abnormalities not identified by histology.

A classic approach in gene expression data analysis is the identification of gene clusters exhibiting similar expression patterns throughout the endometrial cycle. Based on data from samples taken at various cycle phases (early-proliferative, mid-proliferative, late-proliferative, early-secretory, mid-secretory, late-secretory, and menstrual), seven main groups of genes were identified exhibiting consistent expression patterns throughout the menstrual cycle, and with a peak of expression in all seven defined cycle phases, with a fold change greater than or equal to two (Ponnampalam et al., 2004). This finding aligns with those of (Talbi et al., 2006), who also identified groups of genes with peak expression in all studied phases. Díaz-Gimeno et al. (2011a) also identified distinct gene clusters between prereceptive and receptive samples.

These results support the existence of groups of genes that exhibit similar expression levels across all cycle phases (proliferative, early-secretory, mid-secretory, and late-secretory). This finding implies specific physiological processes characterizing each phase. However, establishing a functional association with the phases where these genes are overexpressed or underexpressed remains challenging.
The endometrium is a complex tissue with distinct cellular compartments. Generally, transcriptomic profiling of endometrial samples involves analyzing a combination of these compartments. Consequently, the reported gene expression levels represent a weighted average of the gene's expression in each cell compartment. This approach can obscure significant differences in gene expression between compartments, which has been proposed as a primary reason for the discrepancies reported in ostensibly similar studies (Carson et al., 2002).

To address this issue, laser capture microdissection has been utilized to separate different tissue compartments, tested in both mice (Niklaus & Pollard, 2006) and human endometrium (Gaide Chevronnay et al., 2010). Yanaihara et al. (Yanaihara et al., 2005) conducted a study during the proliferative phase (days 9–11), separating the stroma and epithelium. Their comparison revealed differentially upregulated genes, including enzymes, growth-related proteins, and intra/extracellular matrix proteins, predominantly present in the stroma with functions related to cell growth and remodeling. Another study conducted during the menstrual phase identified distinct gene expression profiles for stromal cells, glands, and various depths of the endometrium (Gaide Chevronnay et al., 2009).

However, this technique has its disadvantages, such as the requirement for prior manipulation using frozen sections, which extends processing time and can introduce errors due to the need for RNA amplification of the epithelial compartment (Horcajadas et al., 2007).

Menstrual Cycle

The onset of menstruation, marking day 1 of the cycle, typically lasts 3–5 days and The onset of menstruation, marking day 1 of the cycle, typically lasts 3–5 days and involves the shedding of the functional layer of the endometrium due to a decrease in estrogen levels (Hawkins & Matzuk, 2008). This phase is characterized by significant transcriptomic changes. Key processes include apoptosis and tissue breakdown. Various studies analyzing temporal gene expression patterns have identified a peak in the expression of genes associated with apoptosis, inflammation, signal transduction, transcription, and DNA repair (Ponnampalam et al., 2004). Notable genes include natural cytotoxicity-triggering receptor 3 (NCR3) (Punyadeera et al., 2005), Wnt family members (Wnt5a and Wnt7a), and the matrix metalloproteinase (MMP) family (Critchley et al., 2006).

Proliferative phase

The proliferative phase spans from the conclusion of the menstrual phase (days 3–5) until ovulation (days 12–14). During this phase, rising estrogen levels stimulate stromal and glandular proliferation, as well as the elongation of spiral arteries, leading to the formation of a new functional layer (Hawkins & Matzuk, 2008).
This phase encompasses several critical processes, including cellular proliferation and differentiation, extracellular matrix remodeling, angiogenesis, and vasculogenesis (Simmen & Simmen, 2006). Additionally, there is heightened expression of genes related to adhesion and ion channels (Talbi et al., 2006). Cellular proliferation during this stage is evidenced by mitotic activity, DNA synthesis, and an increase in tissue weight (Talbi et al., 2006).

Secretory Phase

The secretory phase occurs between ovulation (days 12–14) and the onset of menstruation in the next cycle (days 28–30). It is subdivided into early, mid, and late secretory phases, corresponding to the luteal phase of the ovary(Hawkins & Matzuk, 2008). This phase has been the most extensively studied because the endometrium becomes receptive during the mid-secretory subphase, allowing for embryo implantation.
During the secretory phase, the endometrium is influenced by increasing levels of estrogen and progesterone. The rise in progesterone leads to the secretion of glycogen and mucus. In the late secretory phase, estrogen and progesterone levels drop in the absence of implantation, signaling the preparation for the menstrual phase (Hawkins & Matzuk, 2008).

Early Secretory Phase

The early-secretory phase is characterized by a predominance of processes related to cell metabolism (including fatty acids, lipids, eicosanoids, and amino alcohols), transport (featuring a significant number of transporters for biological molecules involved in these metabolic processes), germ cell migration (potentially facilitating sperm transport and maintaining an aseptic environment), and negative regulation of cell proliferation. The increase in metabolic activity aligns with the phase's high biosynthetic activity, likely in preparation for embryo implantation, while the inhibition of mitosis is supported by the down-regulation of numerous growth factors (Talbi et al., 2006).

Mid-Secretory Phase

The mid-secretory phase is characterized by high metabolic activity, secretory functions, and a non-proliferative state, with significant emphasis on the innate immune response, stress response, and wound healing (Simmen & Simmen, 2006). This phase sees the most substantial gene expression changes, driven by the peak in progesterone levels. These changes coincide with the period when the endometrium becomes receptive. Genes that show expression changes during the transitions from early- to mid-secretory phases and from mid- to late-secretory phases are likely regulated by progesterone (Talbi et al., 2006).

Ponnampalam et al. (Ponnampalam et al., 2004) identified a cluster of genes exhibiting temporal regulation patterns throughout the endometrial cycle that mirror the increase in circulating progesterone. These genes are involved in critical biological processes during implantation, including signaling, growth, differentiation, and cell adhesion. Notably, there are no significant changes associated with the peak in estrogen levels.

Late Secretory Phase

During the late secretory phase, estrogen and progesterone levels decrease, and key processes such as extracellular matrix degradation, inflammatory response, and apoptosis predominate (Simmen & Simmen, 2006).

The transition from the mid-secretory to the late secretory phase involves significant changes in the extracellular matrix and cytoskeleton, with processes such as vasoconstriction, smooth muscle contraction, hemostasis, and a shift in the immune response towards inflammation being favored (Tseng et al., 2010). Genes regulated during this transition are primarily associated with the innate immune system, humoral and cellular immune responses (Talbi et al., 2006), hemostasis, blood coagulation, steroid biosynthesis, and prostaglandin metabolism (Critchley et al., 2006), aligning with previous findings.

The processes characteristic for the late secretory stage matrix degradation, inflammatory response, and cell apoptosis do not support implantation. Consequently, the transition from the mid-to the late secretory phase marks the closure of the window of implantation (WOI) and a return to the non-receptive endometrial phenotype (Talbi et al., 2006).

1.5.3. Endometrial Receptivity Diagnosis

Infertility remains a significant issue for many couples undergoing fertility treatments. Despite the success of these treatments, embryo implantation failures still occur. The primary causes of implantation failure include low endometrial receptivity, embryonic defects, and other multifactorial effects, such as diseases or disorders of the endometrium (Margalioth et al., 2006). It is currently estimated that two-thirds of these implantation failures result from poor endometrial receptivity or defects in the embryo endometrial dialogue (Haouzi et al., 2009) . For many years, the histological criteria established by Noyes (August 1950 - Volume 5 - Issue 4: Obstetrical & Gynecological Survey, n.d.) were considered the gold standard for endometrial dating to determine the receptive state (Talbi et al., 2006). However, this method has faced criticism in recent years because it cannot distinguish between fertility and infertility. While histological endometrial morphology may indicate a mid-secretory phase, this does not necessarily imply receptivity (Sharkey & Smith, 2003).

Endometrial receptivity has long been studied from morphological, biochemical, molecular, and cellular perspectives to identify potential markers of receptivity. However, none of the proposed markers have become reliable diagnostic tools due to their low predictive value (Aghajanova et al., 2008). Some authors suggest that "poor receptivity" could be due to altered gene expression (Sharkey & Smith, 2003), indicating that the search for receptivity markers should focus on analyzing and understanding these genes as potential underlying causes of infertility (Kao et al., 2003).

Several studies have linked gene expression with implantation failure by comparing endometrial samples from the pre-receptive versus receptive phases in healthy women and women with implantation failure. These studies concluded that implantation failure can partly be attributed to the endometrium's inability to enter the receptive phase (Sharkey & Smith, 2003). Many studies seeking endometrial markers have focused on single molecules or specific families of molecules, such as integrins, mucins , cytokines , monoamine oxidase A (Kao et al., 2003), C4BPA, CRABP2, and OLFM1 (Lee et al., 2007).

Despite research linking deficient expression of individual genes or small groups of genes with implantation failure, these molecules have not proven successful as clinical markers, as demonstrated by leukemia inhibitory factor (LIF; Brinsden et al., 2009). The consensus is that a single molecule cannot adequately describe the complex phenomenon of receptivity. Recognizing this complexity, transcriptomic profiles offer a promising approach to classify different endometrial cycle phases, including receptivity (Ruiz-Alonso et al., 2012a).

Ultrasonography and Hysteroscopy in the Assessment of Uterine and Endometrial Abnormalities

Abnormalities in uterine structure, whether from congenital defects, neoplasia, or synechiae, can significantly impair fertility. Diagnostic imaging methods are crucial for evaluating these uterine defects.

Transvaginal Sonography

Transvaginal sonography (TVS) is a widely accepted, noninvasive, convenient, and safe technology that provides high-resolution imaging of the female internal reproductive organs, offering rapid diagnoses that are highly correlated with pathology.

TVS is primarily used to monitor follicular development and endometrial thickness during exogenous hormone treatments in infertile patients. This method is advantageous in diagnosing various endometrial conditions, including polyps, submucosal and intramural leiomyomas, hyperplasia, and carcinoma. It is also valuable in assessing early pregnancy.

Endometrial growth is easily measured with ultrasound. During treatment with human menopausal gonadotropins, endometrial thickness and texture are commonly assessed. In the early proliferative phase, immediately post-menses, the endometrium is typically thin. In response to estrogen, it thickens and adopts a trilaminar appearance, growing between 0.1 and 0.5 mm daily. Post-ovulation, the endometrium becomes hyperechoic due to secretory changes. Studies suggest that an endometrial thickness of 8 mm or greater with a trilaminar appearance is conducive to implantation during IVF cycles, although beyond a certain threshold, there is no further correlation with IR.

The cyclic changes in the endometrium, induced by varying estrogen and progesterone levels, result in predictable sonographic changes, particularly in blood flow and vessel density. Both endometrial thickness and echogenic patterns have been studied as potential markers of uterine receptivity and predictors of successful embryo implantation. Transvaginal pulsed Doppler ultrasound measures uterine artery blood flow, expressed as the pulsatility index (PI), which varies across the menstrual cycle and may predict implantation success post-assisted reproductive techniques. Increased PI has been linked to elevated markers of pregnancy loss, such as anticardiolipin antibodies. However, studies using Doppler flow ultrasound to correlate pregnancy rates have been inconclusive.

Hysteroscopy

Hysteroscopy is an essential instrument for detecting and rectifying lesions shown by hysterosalpingography or sonohysterography. This treatment facilitates direct visualization of the endometrial cavity, which is advantageous for examining uterine cavities in women with abnormal bleeding, infertility, and recurrent pregnancy loss (RPL). It allows the operator to resect or biopsy detected lesions and is frequently employed to excise uterine septa in Müllerian fusion anomalies.

Transcriptomics of the human endometrium

In recent years, groundbreaking research has shown the pivotal nature of high throughout "omics" (including genomics, transcriptomics, epigenomics, proteomics, and metabolomics) in the realm of endometrial research (Altmäe et al., 2014).

Microarray-based gene expression technology, which simultaneously monitors the expression of thousands of genes (Altmäe et al., 2014), represented the first transcriptomic technique widely used in endometrial research. This approach has aided:

- The study of the healthy endometrium and embryo implantation (Aghajanova et al., 2008; Giudice, 2006; Horcajadas et al., 2007; Koot et al., 2012; Ruiz-Alonso et al., 2012; Toth et al., 2011).

- Exploring diseases such as endometriosis or endometrial cancer (Doll et al., 2008; Matsuzaki, 2011).

- The characterization of cellular compartments by physical separation (G. E. Evans et al., 2012; Spitzer et al., 2012; Yanaihara et al., 2005).

Even given this early success, disadvantages associated with microarrays and the contemporaneous progress in transcriptomic technology led to a shift towards applying another more advanced technique - bulk RNA sequencing (RNA-seq).

Endometrial characterization with bulk RNA sequencing

Bulk RNA-seq employs next-generation sequencing (NGS) to uncover the presence and abundance of RNA within a pooled cell population, tissue section, or biopsy and assays the whole transcriptome. This method represents a straightforward and economical approach to studying mRNA and provides gene expression profiles from the studied sample or pool of samples (X. Li & Wang, 2021).

With the arrival of the genomics revolution, endometrial biology became deeply scrutinized. Four independent groups simultaneously reported on transcriptomic profiling of the secretory phase of the human endometrium in natural cycles, searching for the WOI (Kao et al., 2002; Carson et al., 2002; Riesewijk et al., 2003; Mirkin et al., 2005). Two other groups extended this transcriptomic characterization across the menstrual cycle (Borthwick et al., 2003; Ponnampalam et al., 2004). Subsequent studies were extended to COS cycles (Mirkin et al., 2004; Horcajadas et al., 2005; Simon et al., 2005), and even refractory cycles in patients with inert intrauterine devices (IUD) (Horcajadas et al., 2006) (for review see Horcajadas et al., 2007). Since 2005, myriad papers further described the transcriptomic profile across the menstrual cycle (Haouzi et al., 2009; Punyadeera et al., 2005; Revel et al., 2011; Van Vaerenbergh et al., 2010; Yanaihara et al., 2005; Mirkin et al., 2005; Simon et al., 2005; Talbi et al., 2006; Critchley et al., 2006; Horcajadas et al., 2008; Kuokkanen et al., 2010; Tseng et al., 2010). The next step was a comparison of endometrial profiles between fertile patients and those with pathologies such as recurrent implantation failure (Altmäe et al., 2010; Koler et al., 2009; Tapia et al., 2008; Macklon et al., 2017), endometrial cancer (Habermann et al., 2011), endometriosis (Matsuzaki et al., 2011; Garcia-Velasco., 2015), and obesity (Comstock et al., 2017). This progress thereby facilitated the transition from anatomical to molecular medicine of the endometrial factor and ultimately paved the way for its clinical application.

While transcriptomic studies vary in their findings, they unanimously acknowledged the presence of specific transcriptomic profiles throughout the menstrual cycle, mainly in the WOI (Wang & Yu, 2018). As the masking of expression profiles of rare cell populations by the average profile of all analyzed cells represents a significant drawback of RNA-seq, a separate analysis of endometrial compartments - offering a deeper understanding of endometrial physiology and elucidating interactions between cell types and their regulatory mechanisms had remained a pending task. The emergence of single-cell RNA-seq (scRNA-seq) has recently helped to overcome this significant biological challenge (X. Li & Wang, 2021).

Endometrial characterization with scRNA sequencing

scRNA-seq technology allows gene expression profiling in a heterogeneous sample at the single-cell level. Gene expression profiles correspond to individual cells and not to an average of the expression of all cells within a sample; therefore, the individual characteristics of each cell are represented. Since 2017, scRNA-seq has emerged as a widely embraced genomic tool for analyzing transcriptome diversity, such as in the identification of rare cell types and states within healthy donor samples or diverse tumor samples (X. Li & Wang, 2021). scRNA-seq can support the characterization of the transcriptome of every human cell type, encompassing those located in the reproductive organs (Wang et al., 2020) and involving the emergence of intriguing insights into endometrial function during menstruation and implantation (Vento-Tormo et al., 2018).

Endometrial tissue characterization at a single-cell level began before the popularization of scRNA-seq; the first single-cell technique employed fluorescence-activated cell sorting (FACS), a procedure that isolates cells based on antibodies against typical cell markers (Krjutškov et al., 2016). This FACS-based research focused on endometrial stromal cells and revealed transcriptomic differences between freshly isolated cells and cultured cells, observing upregulated genes (e.g., FOS and SERPINF1) and downregulated genes (e.g., TMSB10 and MYLI2A). Although this approach provided valuable information, the difficulty of using independent reactions in wells limited its applicability; in addition, FACS results only covered known, previously isolated populations requiring specific cell markers. Of note, microfluidic techniques later coupled to these protocols allowed for the more advanced characterization of cell populations in a heterogeneous sample.

A recent study utilized modern single-cell microfluidics, C1 Fluidigm, and a 10x genomics system to map cellular and molecular changes in the endometrium throughout the menstrual cycle, characterizing diverse cell populations (Wang et al., 2020). The employed endometrial biopsies covering the entire menstrual cycle, analyzed the transcriptomic information of more than 73,000 cells, and clustered them into seven distinct groups based on the expression profiles. Four cell types were easily identified based on gene expression differences and canonical markers (endothelial cells, stromal fibroblasts, lymphocytes, and macrophages) and a small population as smooth muscle expressing PDGFRB, MCAM, and SUSD2, which suggested that the population might include cells previously classified as mesenchymal progenitor cells (Masuda et al., 2012; Schwab & Gargett, 2007). Finally, two remaining cell types expressed markers associated with epithelial cells, with gene expression profiles allowing the distinction between ciliated and unciliated epithelial cells. Employing RNA and protein co-staining, the spatial arrangement of cells in situ was observed and validated four genes (C20orf85, FAM183A, C11 or f88, and CDHR3) as highly indicative markers of ciliated epithelial cells, which displayed the consistent co-expression of FOXJ1 (a canonical regulator of cilia function) (Wang et al., 2020). The investigation of transcriptomic changes across the menstrual cycle established four main phases based on the transcriptome analysis of unciliated epithelial cells and stromal cells as leading players in endometrial transformation.

A discontinuity in unciliated epithelia trajectory dynamics led to the identification of an abrupt activation of several genes at the early beginning of phase 4 (e.g., PAEP, GPX3, and CXCL14) that consistently displayed overexpression in whole-tissue transcriptomic datasets during the WOI (Figure x). Thus, we linked the beginning of the WOI to the entrance into phase 4, starting with an abrupt transcriptional activation in the unciliated epithelium but with gradual and discrete dynamic changes in stromal cells. The changes in gene expression profiles in stromal cells included the upregulation of DKK1, CRYAB, FOXO1, and IL15, with the latter demonstrating the onset of decidualization prior to the opening of the WOI. In contrast, the closure of the WOI associated with gradual transitional transcriptional dynamics in both epithelium and stroma. We integrated the four phases with traditional histologically determined phases by studying cellular mitosis as a differential characteristic between the proliferative and secretory phases. We determined the transition between the proliferative and secretory phase between transcriptomic phases 2 and 3 so that phase 3 corresponds to an early secretory phase and phases 1 and 2 correspond to early and late proliferative phases **(Figure 2).** This new classification at the transcriptomic level allowed the establishment of new histologically unrecognizable subphases, representing a paradigm shift in the transition of the endometrium during the menstrual cycle.

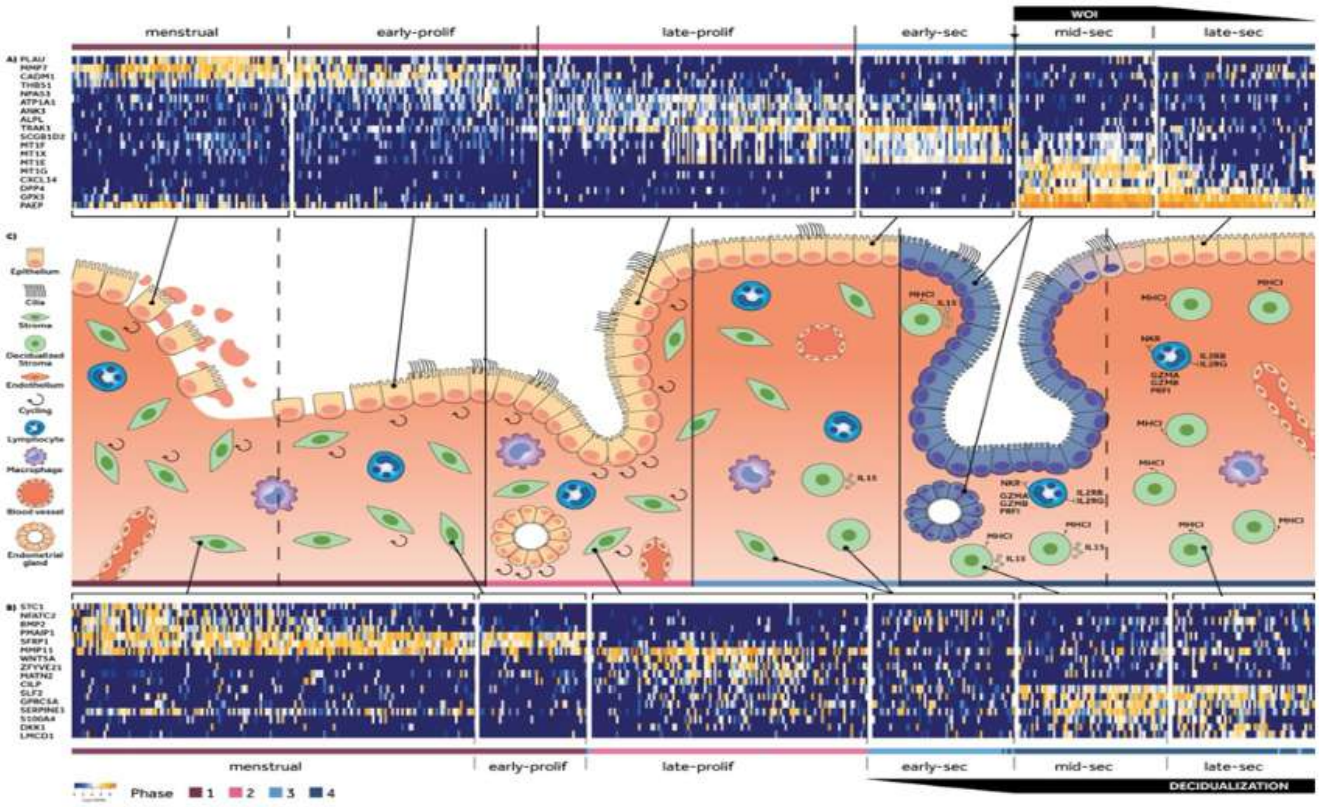


Figure 2| Fluctuating gene expression in the endometrium throughout the human menstrual cycle. A-C, Phase and sub-phase defining genes, and the relationship between transcriptomically defined and histologically defined (canonical) endometrial phases for (a) unciliated epithelium (epi) and (b) stromal fibroblasts (str) across (c) a human menstrual cycle. Genes shown are differentially expressed (-log10(p.adj of a Wilcoxon's rank sum test)>10, log2(FC)>1) in a phase or sub-phase and are not differentially expressed between luminal and glandular cells in the phase where the gene peaked. Genes were further filtered for their potential to be deconvoluted between unciliated epithelial and stromal fibroblasts in bulk data to obtain those that are temporally in synchrony between the two cell types or those with negligible expression in one cell type across the cycle but significant phase-specific dynamics in another. (Cells (column) were ordered by pseudotime. Dashed lines: within-phase transition; Solid lines: boundaries between the four phases; WOI: window of implantation; pro: proliferative; sec: secretory. Figure reproduced with permission from Wang et al. (2020).

Collectively, single-cell transcriptomic "atlases" (Garcia-Alonso et al., 2021; Wang et al., 2020) provide a comprehensive depiction of the normal human endometrium; however, other research groups have also contributed to the characterization of the endometrium. Queckbörner et al. reported ten different stromal cell types and two subsets of pericytes depending on cell markers expression (e.g., ISG15, THY1, ACTA2, and BMP7), indicating the presence of distinct stromal niches capable of regulating inflammation and ECM composition (Queckbörner et al., 2021). Diniz-da-Costa et al. characterized a novel discrete population of highly proliferative mesenchymal cells during the WOI-expressing genes related to vascular transmigration and chemotaxis (Diniz-da-Costa et al., 2021). Finally, Kirkwood et al. employed in silico trajectories to indicate that stromal cell populations (PDGFRb+) expressing genes typically associated with epithelial cells were stromal fibroblasts undergoing MET (Kirkwood et al., 2022).

1.6 Mechanisms of Aging and Their Impact on Endometrial Receptivity

Investigating the influence of advanced maternal age on endometrial receptivity is critical, given the rising trend of delayed childbearing and its known adverse effects on fertility. Understanding the mechanisms underlying the hormonal, cellular, molecular, epigenetic, and immunological changes that contribute to the decreased receptivity of the endometrium in older women is essential for developing targeted interventions to improve reproductive outcomes.

With advancing age, the levels of key reproductive hormones, estradiol (E2) and progesterone (P4), exhibit significant fluctuations. These hormonal alterations lead to a reduction in endometrial proliferation and overall impair the endometrial environment necessary for embryo implantation. Notably, the downregulation of estrogen and progesterone receptors in the aged endometrium results in decreased hormonal responsiveness, further compromising endometrial function (Cakmak & Taylor, 2011).

Cellular senescence is a pivotal factor in the aging endometrium. Senescent cells, which accumulate with age, adopt a senescence associated secretory phenotype (SASP). This phenotype is characterized by the secretion of pro-inflammatory cytokines, chemokines, and proteases that create a detrimental pro-inflammatory environment, impairing tissue function and endometrial receptivity (Jabbour et al., 2009). Cellular senescence disrupts the normal cyclical changes of the endometrium, leading to impaired stromal cell function and compromised implantation potential.
Aging leads to significant molecular changes in the endometrium, including the downregulation of genes critical for decidualization and receptivity. For instance, reduced expression of HOXA10 and insulin-like growth factor binding protein 1 (IGFBP-1), which are essential for stromal cell differentiation and embryo implantation, has been observed in aged endometrium (Gellersen & Brosens, 2014). These molecular alterations disrupt the signaling pathways necessary for preparing the endometrium for implantation, leading to decreased fertility in older women.

The epigenetic clock is essential for comprehending the biological aging of the endometrium. Age-associated alterations in DNA methylation patterns and histone modifications significantly influence gene expression profiles, thus impacting endometrial function (Biron-Shental et al., 2015).

Epigenetic modifications can impair endometrial receptivity by disrupting the expression of genes essential for implantation, thereby contributing to infertility in women of advanced age (Chemerinski et al., 2024).

Furthermore, aging induces significant changes in the immune landscape of the endometrium. The immune cells, such as NK cells, macrophages, and T cells, play crucial roles in the maintenance of endometrial receptivity and embryo implantation. In older women, notable alterations in the number and function of these immune cells have been noted. The proportion of uterine NK cells, which are essential for vascular remodeling and creating a receptive endometrial environment, decreases with age (Moffett & Loke, 2006). Additionally, aging is associated with a shift in macrophage polarization towards a pro-inflammatory phenotype, exacerbating the pro-inflammatory environment in the endometrium (Zhang et al., 2022).

The dysregulation of cytokine and chemokine secretion further contributes to an unfavorable immune milieu. For example, elevated levels of pro-inflammatory cytokines such as interleukine-6 (IL-6) and tumor necrosis factor-α (TNF-α) in the aged endometrium can impair embryo implantation and endometrial receptivity (Elhamouly et al., 2018). Moreover, the decreased presence of regulatory T cells (Tregs), which are essential for maternal-fetal immune tolerance, can lead to increased immune rejection of the embryo (Erlebacher, 2013). These immunological changes, coupled with hormonal and cellular senescence, create a hostile environment for embryo implantation in older women.

The clinical implications of endometrial aging are underscored by studies involving oocyte donation cycles. These studies provide contradictory findings, highlighting the complexity of the issue and the necessity for more comprehensive research to elucidate the exact mechanisms and effects of advanced maternal age on endometrial receptivity (Paulson et al., 2002). Understanding these mechanisms is vital for developing therapeutic strategies, such as the use of senolytic agents, which selectively eliminate senescent cells to restore endometrial function and improve reproductive outcomes in older women (Deryabin et al., 2020).

Overall, the clinical importance of understanding the mechanisms of endometrial aging and their impact on fertility calls for more robust research to develop targeted interventions that can enhance pregnancy outcomes for women of advanced age. By elucidating the hormonal, cellular, molecular, epigenetic, and immunological changes associated with endometrial aging, the field of reproductive medicine offers promising directions for future research and clinical applications.

1.7 Implantation

Successful embryo implantation depends on three factors: embryo quality, the endometrium state of receptivity, and synchronized dialogue between the maternal tissue and blastocyst (Ruiz-Alonso et al., 2012b). After obtaining a high-quality blastocyst, successful implantation is anticipated. Embryo implantation in the maternal uterus represents a critical event in mammalian reproduction. The stages of implantation encompass apposition, adhesion, and the subsequent invasion of the blastocyst trophectoderm through the uterine epithelium into the stroma. Successful implantation necessitates both uterine receptivity and blastocyst activation (Cha & Simón, 2013).

However, at this stage, the endometrium becomes a critical factor. For implantation to occur, the endometrium must be receptive, which is possible only during a limited period known as the WOI. This period varies among patients, typically occurring between days 19 to 23 of the natural cycle or from progesterone+3 to progesterone+7 in hormonal replacement cycles (Díaz-Gimeno et al., 2011b). This variability contrasts with the traditionally established window, highlighting why standard ET often fails. Consequently, a pET approach is necessary to accommodate individual differences in the WOI among women.

The process of implantation is intricately synchronized with ovarian events, initiated by the rise in estrogen associated with follicular development, leading to endometrial proliferation. Following ovulation, the increase in progesterone from the corpus luteum transforms the endometrium into a secretory structure, making it receptive to the newly fertilized ovum. This hormonal environment facilitates the embryo's interaction with the surface epithelium and invasion into the underlying stroma, thereby initiating gestation (Díaz-Gimeno et al., 2011b).

Implantation transpires at specific stages, each meticulously synchronized with steroid-induced alterations in the endometrium. The process of embryo implantation can be succinctly summarized as follows: Following fertilization in the fallopian tube, the embryo, at the morula stage, migrates to the uterine cavity and continues to divide, developing into the blastocyst within a precise timeline of 72 to 96 hours post-fertilization. Upon entering the uterine cavity, a communication is initiated between the blastocyst and the receptive endometrium, facilitated by hormones and growth factors. This contact guarantees the proper orientation of the blastocyst (apposition), which is essential and potentially the rate-limiting factor in the implantation process. Thereafter, the blastocyst contacts the endometrial epithelium and attaches to its surface (adhesion), subsequently penetrating the endometrium (invasion) (Lessey, 2000) (Diedrich et al., 2007b). To achieve this stage, the endometrium must exhibit appropriate, coordinated gene expression regulated over the whole endometrial cycle.

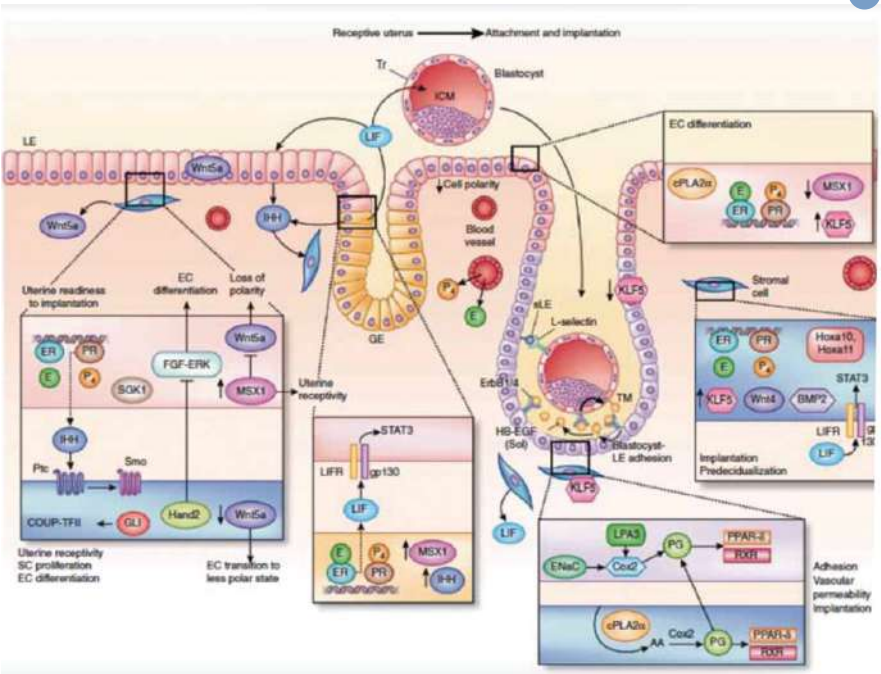


Figure 3| Signaling network for uterine receptivity and implantation. Hybrid cartoon, based on mouse and human studies, portraying compartment and cell-type specific expression of molecules and their potential functions critical for uterine receptivity, implantation, and decidualization. Interplay of ovarian P4 /estrogen-dependent and independent factors in the pregnant uterus in specific compartments contributes to the success of implantation in a juxtacrine/paracrine/autocrine manner. During attachment, interactions between the blastocyst and luminal epithelium (LE) involve ErbB1/4 (blue) and both transmembrane (TM) and soluble (Sol) forms of HB-EGF, as well as L-selectin ligands expressed by the luminal epithelium to L-selectin receptors on the blastocyst. The other critical signaling pathways for uterine receptivity and implantation are also shown. AA, arachidonic acid; COUP-TFII, chicken ovalbumin upstream promoter transcription factor-2; E, estrogens; EC, epithelial cell (luminal and glandular epithelia); ENaC, epithelial sodium channel; ErbB1/4, epidermal growth factor receptor 1/4; GE, glandular epithelium; Hand2, Heart- and neural crest derivatives expressed protein 2; HB-EGF, heparin-binding EGF-like growth factor; ICM, inner cell mass; KLF5, Kruppel-like factor 5; LPA3, lysophosphatidic acid receptor 3; MSX1, muscle segment homeobox 1; PPARδ, peroxisome proliferator-activating receptor δ; Ptc, Patched; RXR, retinoid X receptor; SC, stromal cell; SGK1, serum and glucocorticoid inducible kinase 1; Smo, Smoothened; Tr, trophectoderm. Compartment colors: blue, stroma; pink, luminal epithelium; orange, glandular epithelium; purple, epithelium at the attachment site (Cha & Simón, 2013).

1.7.1 Embryo-Endometrial Synchrony

The synchronized development of a viable embryo and a receptive endometrium is paramount for successful implantation in IVF. Implantation necessitates effective bidirectional communication between the receptive endometrium and an implantation-competent blastocyst. The WOI is transient, yet crucial for achieving optimal pregnancy outcomes. The implantation process commences with the blastocyst's attachment to the uterine luminal epithelium, which subsequently triggers localized stromal cell proliferation and differentiation into specialized decidual cells at the implantation site a process known as decidualization.

Proper decidualization is essential for placentation, whereby the maternal circulation interfaces with the embryonic vascular system, thus establishing a functional placenta. This placenta facilitates the transfer of maternal resources, nourishing and protecting the fetus. Implantation represents the first significant interaction between the mother and embryo and is pivotal for a successful pregnancy (Cha & Simón, 2013) Conventionally, the initial attachment and invasion of the blastocyst into the endometrium, is WOI, however, this term can be misleading, as it suggests a singular critical period for successful implantation. In reality, embryo maturation and endometrial development are continuous and independent processes. Implantation transpires when these two tissues achieve a state of fusion, resulting in a pregnancy.

A crucial concept in this context is developmental 'synchrony,' which occurs when the early embryo and uterus develop at coordinated rates, enabling them to initiate and sustain implantation concurrently. Various factors, including the controlled ovarian hyperstimulation commonly used in IVF, may disrupt this synchrony. Evidence indicates that implantation rates in humans significantly decrease when the asynchrony between embryo and endometrial development exceeds 3 days (±1.5 days). Overall, embryo-endometrial synchrony is essential for successful implantation. There is a pressing need for enhanced precision in assessing endometrial development to better synchronize the embryo and endometrium before implantation (Teh et al., 2016).

1.7.2 Endometrial Receptivity

Endometrial receptivity is defined as "the period of endometrial maturation during which the trophectoderm of the blastocyst can attach to the endometrial epithelial cells and subsequently invade the endometrial stroma and vasculature." Attainment of endometrial receptivity is neither a binary event nor adequately described by the metaphor of a window. This simplistic analogy fails to capture the complexity of endometrium related subfertility observed in clinical practice. Instead, various degrees and types of abnormal receptivity contribute to a spectrum of reproductive issues, ranging from complete implantation failure (infertility) to severely deficient implantation (miscarriage), and mildly abnormal implantation and invasion (e.g., pre-eclampsia).

Numerous factors that disrupt receptivity may not yet be fully identified, but recognized causes include endocrine imbalances, inflammatory events, thin endometrium, fibroids, polyps, septa, and immunologically mediated disturbances (Lessey & Young, 2019).

1.7.3 Timing of Implantation

Historically, luteal phase hysterectomy samples suggested that embryos attach to the endometrium around day 20 of a 28-day cycle. Studies with donor oocyte-derived embryos transferred to hormonally prepared recipients indicate that implantation occurs between cycle days 20 and 24 (Bakos et al., 1993). These findings underscore that estrogen and progesterone alone can prepare the endometrium for implantation, and the duration of progesterone exposure determines the window of receptivity.

More recent research showed that implantation typically occurs between 7 and 10 days post-ovulation (days 21 to 24)(Sunder & Lenton, 2000). Implantation beyond the normal window is associated with a higher miscarriage rate, likely due to a loss of synchrony between the endometrium and embryo, which may explain some cases of unexplained infertility and RPL (Dimitriadis et al., 2020).

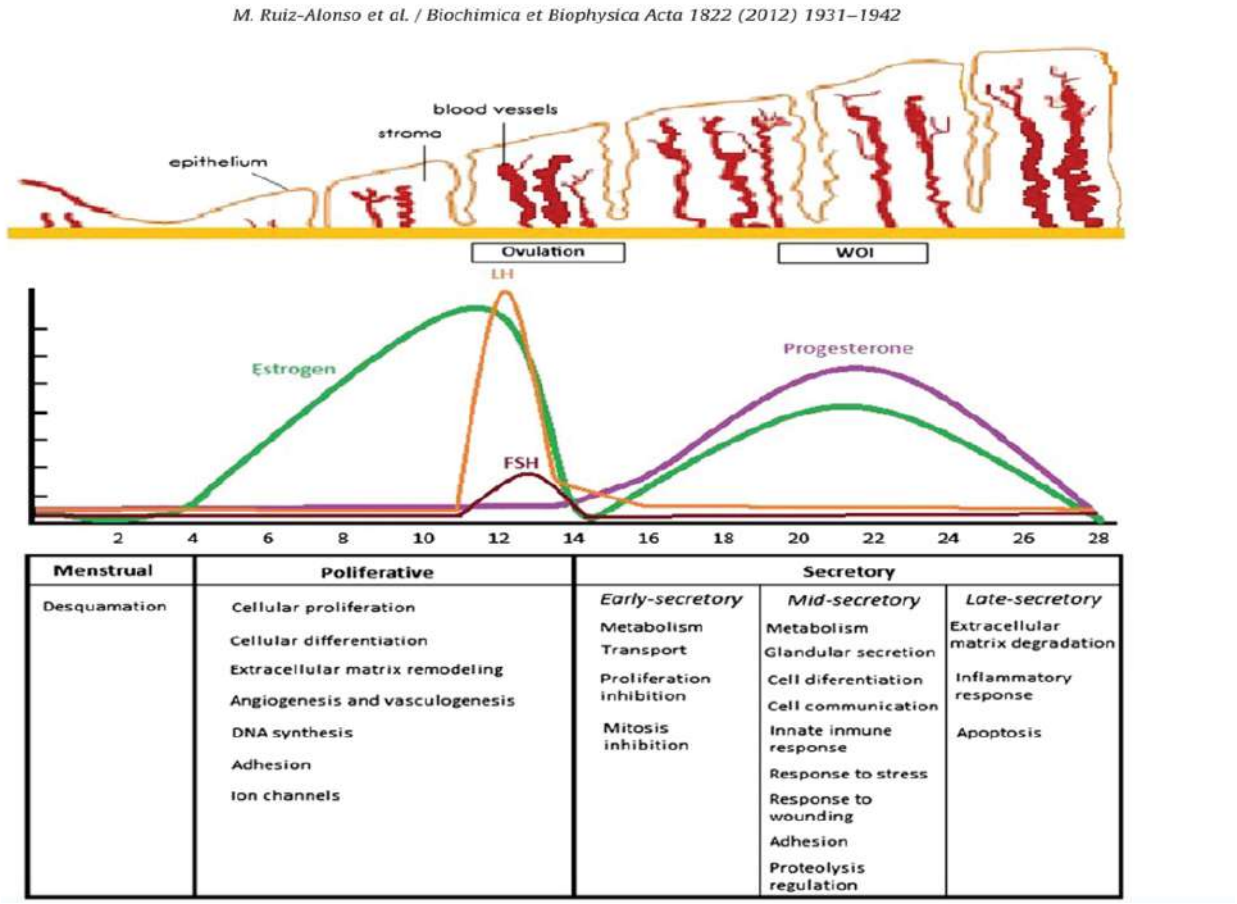


Figure 4| Biological processes along the endometrial cycle. Cartoon depicting the endometrium tissue evolution along the menstrual cycle. Underneath are represented the relative levels of the main regulatory hormones affecting the endometrium. Principal biological processes are indicated at the cycle phase when they are more significantly regulated (Ruiz-Alonso et al., 2012b).

Implantation is a complex interaction between the maternal endometrium and the embryo, involving multiple soluble and membrane-bound factors that facilitate embryo growth, differentiation, attachment, invasion, and immunologic evasion. The maternal immune system permits intrusion while limiting excessive embryonic invasion. The embryo uses strategies like mimicking maternal antigens to avoid immune rejection (Petroff et al., 2022). Upon fertilization, hCG from the embryo rescues the corpus luteum, maintaining progesterone secretion and endometrial integrity. This synchrony is essential for pregnancy success, as delays in implantation beyond the normal window increase the risk of miscarriage.

The evaluation of hysterectomy specimens reveals early pregnancy changes in the endometrium, such as glandular secretory activity, edema, and decidual reaction. Increased blood flow and vascular endothelial growth factor (VEGF) production contribute to these changes. Endometrial biopsies during conception cycles show stromal edema and vascular congestion as early persistent features of pregnancy.
Within weeks, the endometrium undergoes further changes, with epithelial cells of the glands becoming distended and hyperchromatic, known as the Arias-Stella reaction. These changes support early embryo nutrition before the establishment of placental blood flow.

The decidualized stroma, which develops under continued progesterone exposure, regulates trophoblast invasion and placentation. It creates a permissive yet restrictive barrier, crucial for placental morphogenesis and establishing uteroplacental circulation. The decidual cells exhibit secretory characteristics, producing extracellular matrix components and cytokines that modulate cell behavior.

For successful implantation, the blastocyst must hatch from the zona pellucida to interact with the endometrium. The syncytial trophoblast layer initiates interaction and invasion into the endometrial epithelium. By day 12 post-ovulation, the embryo is almost entirely embedded in the endometrium, with the surrounding stroma displaying decidual reaction and edema.

Receptor-mediated paradigms suggest that various adhesion molecules facilitate attachment. Integrins, CD44, L-selectins, and Heparin-binding EGF-like growth factor (HB-EGF) play pivotal roles in the attachment and invasion processes. These interactions may suppress the maternal immune response to protect the embryo during initial attachment and invasión.

Pinopodes (or uterodomes) on the endometrial surface appear to play a role in embryo attachment as well. Their formation correlates with the window of implantation, potentially providing a platform for surface adhesion receptors. However, their utility as markers of uterine receptivity is debated, as their presence does not always correlate with the actual WOI (Yen & Jaffe's Reproductive Endocrinology - 8th Edition | Elsevier Shop, n.d.).

To conclude, implantation is a critical and tightly regulated process that involves a complex interplay of hormonal, cellular, molecular, and immunological changes. A thorough understanding of these mechanisms provides valuable insights for improving fertility treatments and addressing implantation-related infertility issues. The interplay between maternal and embryonic factors is essential for successful implantation and subsequent pregnancy.

1.8 Molecular Biological Techniques as Tools for Improvement of ART Outcomes

Molecular biological techniques have profoundly revolutionized the field of assisted reproduction. A substantial number of couples grappling with infertility have achieved the successful birth of healthy infants due to the implementation of two pivotal molecular biology methodologies: PGT-A (Rubio et al., 2017) and ERA (Díaz-Gimeno et al., 2013). These advanced techniques have markedly enhanced the precision and efficacy of reproductive interventions, thereby improving clinical outcomes and patient satisfaction rates.

1.8.1 PGT-A

Chromosomal abnormalities in the embryo and insufficient uterine receptivity are the primary causes of implantation failure. For patients experiencing implantation failure, a reproductive specialist should consider employing PGT-A to identify and select euploid embryos, as well as assess the endometrium to determine its receptivity at the time of ET.

PGT-A is a critical genetic test developed to select embryos during IVF treatment, thereby preventing embryonic chromosomal abnormalities that can lead to miscarriage or the birth of infants with chromosomal diseases. Embryonic aneuploidy, often associated with maternal age, commonly results in implantation failure, miscarriage, or the birth of infants with multiple congenital anomalies. The selective transfer of euploid embryos, identified through PGT-A, has been shown to improve implantation rates significantly (Rubio et al., 2017).

From a morphological perspective, the majority of defective embryos are indistinguishable from normal embryos when examined microscopically. Consequently, morphological examination should not be the exclusive method for choosing embryos for transfer. This DNA analysis, conducted on material acquired from an embryo biopsy before transfer, facilitates the identification of chromosomally normal embryos. Aneuploidy screening by next-generation sequencing (NGS) offers an extensive examination of all 23 chromosomal pairs: 22 autosomes and 1 sex chromosome. The principal advantages of PGT-A encompass enhanced implantation rates, diminished miscarriage rates, and an elevated probability of delivering a healthy infant (Rubio et al., 2017).

1.8.2 ERA

However, despite the transfer of euploid embryos, ERA results are not always optimal. Conversely, the endometrial gene expression signature facilitates the diagnosis of endometrial receptivity status. This diagnostic process is conducted using a molecular tool developed and patented by Igenomix (PCT/ES2009/00386), known as the ERA. The ERA test enables molecular diagnosis through transcriptomic analysis of 238 genes associated with endometrial receptivity, coupled with a bioinformatic predictor that identifies the peak receptivity period, thereby determining the optimal timing for pET.

To perform the ERA test, an endometrial biopsy sample is collected at a specific point in the woman's cycle, when maximum receptivity is anticipated. A diagnosis of "Receptive" indicates that the implantation window aligns with the biopsy timing. A "Pre-Receptive" diagnosis suggests that the optimal implantation window occurs after the biopsy, while a "Post-Receptive" diagnosis indicates that the optimal window preceded the biopsy. Once the ERA test results pinpoint the period of greatest receptivity, a pET is conducted.

The present study aims to validate them through a prospective, randomized, and controlled trial to substantiate the significance of the endometrial factor and its impact on improving outcomes in patients indicated for egg donation and PGT-A. Should this hypothesis be confirmed, the ERA test may be recommended not only for patients undergoing PGT-A but for all ART patients, thereby demonstrating the diagnostic value of the implantation window and its influence on enhancing reproductive outcomes.

The efficacy of endometrial receptivity analysis has been demonstrated in patients with recurrent implantation failure (RIF), defined as two or more failed ETs (Ruiz et al., 2013). Approximately 30% of patients with RIF exhibit a displaced implantation window. PET, guided by ERA test results, leads to implantation rates comparable to those of patients without RIF. In the broader population of infertile patients without RIF, 12-20% also exhibit implantation window displacement. However, the effectiveness of the ERA test followed by the transfer of euploid embryos has yet to be thoroughly evaluated, which constitutes the primary objective of this study.

The clinical significance of the ERA lies in its capacity to direct pET by correlating the ETs with the patient's specific WOI. This synchronization is especially advantageous for patients experiencing implantation failure attributed to endometrial issues. By utilizing ERA, doctors can adjust the timing of ET to align with the individual's endometrial receptivity profile, so maximizing the likelihood of successful implantation through a tailored strategy and ultimately improving overall reproductive outcomes. In elderly individuals, attaining outcomes similar to those shown in younger reproductive-age patients is achievable (Ruiz-Alonso et al., 2021a).

Nevertheless, the scarcity of published research on this subject indicates that the field need further investigation. Augmenting our comprehension of techniques that enhance ART in AMA. The project seeks to enhance ART outcomes using pET based on ERA in advanced-age individuals with complex reproductive histories and RIF by employing donor oocytes and PGT-A for embryo assessment.

1.8.3 Social Impact

Our research underscores the pivotal function of pET, directed by ERA, in enhancing ART results for elderly patients. By synchronizing ET with the patient's specific WOI, physicians can improve implantation success rates, resulting in increased pregnancy and live birth rates. Future research must concentrate on enhancing these individualized strategies and investigating supplementary factors affecting endometrial receptivity, including the microbiota, to enhance reproductive outcomes for all ART patients (Tesarik & Mendoza-Tesarik, 2022b).

1.9 Hypothesis

Based on preliminary results, our hypothesis is that combining the utilization of egg donation, PGT-A, and ERA might improve reproductive success in patients with RIF, especially those of advanced age undergoing assisted reproductive technology (ART). Therefore, our study addresses the following question: Does the evaluation of the endometrium through the ERA test and personalized embryo transfer provide additional value to egg donation and PGT-A in RIF patients with advanced-age?

OBJECTIVES

Objectives

O1. To evaluate the clinical benefit of personalized embryo transfer (pET) guided by ERA in advanced-age patients with recurrent implantation failure (RIF) using egg donation and PGT-A with euploid embryos, compared to control group 1, consisting of patients with the same indication undergoing non-ERA guided frozen embryo transfer (FET). This objective is assessed by comparing the implantation rates, clinical pregnancy rates, biochemical pregnancy rates, miscarriage rates, ectopic pregnancy rates, obstetric complications, and live birth rates between the study group and control group 1.

O2. To determine whether ERA-guided pET achieves reproductive success rates in advanced-age patients with RIF that are comparable to those of younger patients with RIF. This involves a comparative analysis of reproductive outcomes between the study group and control group 2, composed of younger RIF patients, to assess whether there are significant differences in the clinical outcomes indicated above.

O3. To analyze the types and frequencies of endometrial window of implantation (WOI) displacement in infertile women of various reproductive ages with RIF and conduct a comparative analysis across different age groups, particularly:
- Between women younger than 35 years old and those older than 35 years old.
- To identify the types of WOI displacement in relation to age.

This analysis aims to elucidate the impact of age on endometrial receptivity and assess the potential benefits of personalized treatment approaches.

O4. To develop recommendations for ERA-guided pET based on the obtained data regarding implantation window displacement in advanced-age patients with RIF using egg donation and PGT-A with euploid embryos, and to determine its impact on reproductive outcomes.

MATERIALS AND METHODS

3.1 Study design

A prospective, randomized, observational follow-up study was performed from March 2020 to September 2023 at the Georgian-American Center for Reproductive Medicine ReproArt.

Patients were assigned to the research and control groups using randomization and consistent application. The center was tasked with facilitating patient enrollment and gathering samples for PGT-A and ERA.

Patients qualified for trial enrollment if they possessed a minimum of one frozen euploid blastocyst (assessed via PGT-A) and satisfied all selection criteria. Upon signing the Informed Consent form, participants were deemed eligible for randomization into the study group, control group 1, or control group 2.

Study group: PGT-A and test ERA; age group 35-45; Control group 1: PGT-A without ERA; age group 35-45; Control group 2: PGT-A and ERA test; age group 28-34; Participants in the study group and control group 2 underwent the ERA test to evaluate the status of endometrial receptivity under HRT cycle during the standard implantation window (day 5 of progesterone supplementation: P+5/120h). ET was scheduled during the period confirmed as "Receptive" by the ERA analysis (pET).

For participants randomized into control group 1, embryo transfer was performed under a hormonal replacement (HRT) cycle during the standard implantation window (day 5 of progesterone supplementation: P+5/120h). The IR, PR, and LBR were recorded and compared across all groups. Participants had the ability to withdraw from the study at any time.

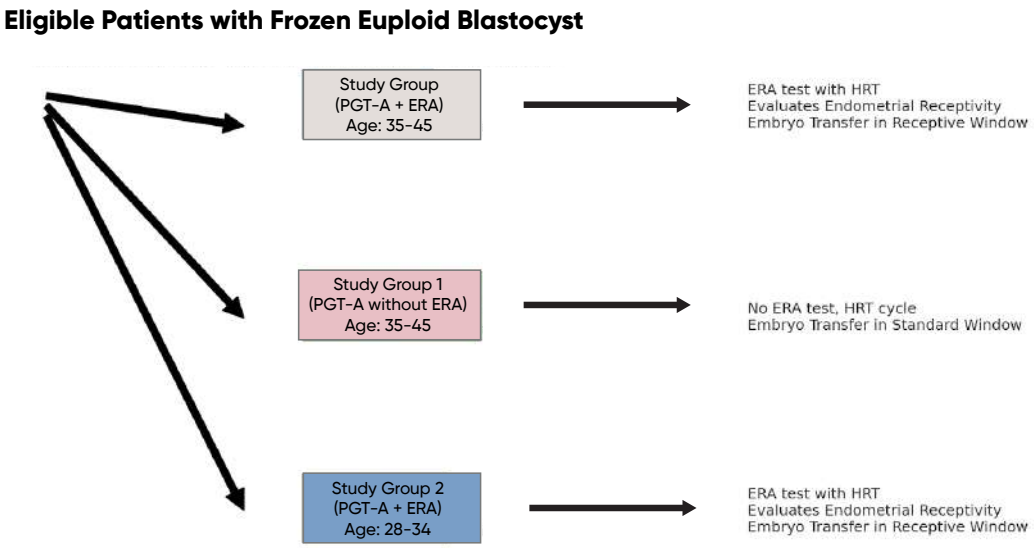


Figure 5| Study Experimental Design

3.2 Study population

The study population consisted of reproductive-age patients who had frozen euploid blastocysts (day 5/6 development) obtained from donor oocytes in the study group and control group 1, and from their own oocytes in control group 2. The oocytes were fertilized via ICSI and analyzed by PGT-A, with an expected ET of one or two embryos in HRT cycle.

3.3 Recruitment materials and procedures

The procedures for recruiting patients at the center included:

- Patient acquisition through the office of the principal investigator
- The principal investigator conducted a review and periodic evaluation of clinical histories
- Contacting the patients who could meet the requirements of the study directly

3.3.1 Ethical considerations

I confirm that no additional materials were used for the recruitment of subjects in the study, ensuring adherence to the essential principles established in the Declaration of Helsinki, the Good Clinical Practices of the International Conference on Harmonization (ICH-GCP), the European Council concerning Human Rights and Biomedicine, and the Universal Declaration of UNESCO on the Human Genome and Human Rights, as well as in compliance with the requirements established in the biomedical research field, personal data protection, and local legislation of bioethics. Patients were required to sign the informed consent granted by the Research Ethics Committee (CEI) to participate in the study.

All procedures performed in the study were according to the ethical standards of the institutional and national research committee, along with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study protocol and a draft consent agreement for participation in the study were approved by the Ethics Committee of the Institution Review Board of the Georgian-American Center for Reproductive Medicine, ReproArt (#2-20/287; February 7, 2020). Informed consent was obtained from all individual participants included in the study.

3.4 Selection criteria

The inclusion criteria were as follows:

The age range for the study group and control group 1 was 35-45, while for control group 2, it was 28-34.Patients must have had frozen euploid blastocysts (developed to day 5/6) analyzed by PGT-A.
In the study group and control group 1, patients had embryos obtained from donor oocytes fertilized via IVF/ ICSI. In contrast, in control group 2, patients had embryos obtained from their own oocytes fertilized via IVF/ICSI.
The expected ET involved one (single embryo transfer - SET) or two embryos (double embryo transfer - DET) in a hormonal replacement therapy (HRT)cycle. Patients' Body Mass Index (BMI) ranged from 18.5 to 30 kg/m^2.

The exclusion criteria for the study included:

The presence of uterine cavity pathologies or malformations, such as polyps, intramural myomas of 4 cm or larger, submucosal myomas, septum, or hydrosalpinx, was identified during the patient's participation in the study. However, patients diagnosed with these conditions before or after inclusion were permitted to participate if the pathology was corrected before any study procedures.
Additionally, any illness or medical condition deemed unstable or that, according to medical judgment, could compromise the patient's safety and compliance with the study was grounds for exclusion.

After the patients had at least one euploid embryo, they were suitable for randomization into the study group and control group 1 and for selection in the control group 2.

3.5 Sample size

The primary objective of this study was to compare the live birth rate between the two study groups. This parameter was calculated in cycles where the ET was performed.

The sample size calculation was based on the unilateral hypothesis contrast of detecting a 10% difference in LBR between the two groups studied. The one-sided statistical significance analysis, with an alpha level of 0.05 and a power of 80%, determined that at least 95 patients are needed in each group. We have used SPSS 22.0 to estimate the independent sample size.

Considering an anticipated dropout rate of approximately 30% due to possible treatment failures, or monitoring issues, or the absence of normal embryos to transfer, it was estimated that 120 patients are required for each group, and a total of 240 participants should be included in the study.

3.6 Study design and description of interventions

Investigators recruited subjects who met the selection criteria and invited them to participate in the study. For the inclusion of patients in the study, the signature of the informed consent approved by the Ethics Committee / International Review Board was required. Only self-reliant subjects who voluntarily agreed to participate and signed the consent form were included in the study.

Upon signing the informed consent, each patient was assigned a unique identification number by the investigator, according to the chronological order of their inclusion in the study (the first identification number was 001, the next 002...). This number was exclusive to each patient and could not be duplicated or reassigned to another patient.

Study Group and Control Group 1 were randomized groups to compare PGT-A + ERA and PGT-A outcomes in the same age groups.

Study Group: PGT-A and ERA test

Control Group 1: PGT-A without ERA

Control Group 2 was specially selected to compare PGT-A and ERA outcomes in different age groups.

Control Group 2: PGT-A and test ERA

Therefore, each patient included in the study received an identification number or code, and the investigator later assigned a randomization number and designated the study group. The patient code or the randomization/group number could not be reassigned.

3.6.1 Ovarian Stimulation and Egg Retrieval

Egg donors and patients underwent ultrasonographic and hormonal check-ups. Following the routine basal ultrasound on days 2-3 of menstruation, controlled ovarian stimulation was initiated using 375 IU recombinant follicle-stimulating hormone (FSH), with 75 IU HMG for the first two days, followed by 150 IU recombinant FSH and 75 IU HMG from the third day of ovarian stimulation. As for the trigger, gonadotropin-releasing hormone (GnRH) agonist 0.2mg/2ml and hCG1500 IU were used.
Monitoring included transvaginal ultrasound examinations and serum E2, FSH, and luteinizing hormone (LH) determinations, which were initiated from the first day of ovarian stimulation and repeated every 48 hours. The average duration of ovarian stimulation was 10-12 days. Oocyte retrieval was performed 36 hours after the trigger injection.

3.6.2 IVF/ICSI, Embryo Culture and Embryo Grading

ICSI was performed, and fertilization was assessed 17-20 hours post-microinjection. According to the IVF laboratory protocol, embryos were cultured in a SAGE 1-Step continuous culture medium. Embryo quality was evaluated based on Gardner's criteria (Gardner & Balaban, 2016b). At the blastocyst stage, day 5/6 of embryo development, several cells (typically 5-10) were removed from the trophectoderm, which is the outer layer that eventually forms the placenta, leaving the inner cell mass, which will develop into the fetus, intact.

Although lasers are occasionally used to assist in cell removal, this is rare; most biopsies at this stage are performed mechanically with precision tools to gently remove cells from the trophectoderm through a pre-made opening in the zona pellucida. During the biopsy process, specialized tubing was used to control the flow and aspiration of cells precisely. This tubing connects the micro-pipettes to the suction device, containing a micro drop of PBS (phosphate-buffered saline) + PVP (polyvinylpyrrolidone) buffer. This buffer solution helps to maintain cell integrity and prevent cell adhesion to the tubing during aspiration.

According to the IVF laboratory protocol, Kitazato vitrification media was used for embryo vitrification. Moreover, biopsied samples were sent for genetic testing for aneuploidy.

3.6.3 Screening for aneuploidies or PGT-A

Genetic laboratories utilized the massive sequencing technique NGS to carry out the PGT-A to detect aneuploidies. NGS is a last-generation technology that allows high accuracy and resolution tests, which means only a few cells or even a single cell is required to carry out the pre-implantation genetic test. Test validations should be performed as usual in the laboratories to optimize DNA quality and amplification, homogenize the results, and avoid other external biases. The PGT-A tests with NGS have an accuracy of 98-99%.

3.6.4 Hormone replacement cycle for ET (Study Group and Control Groups 1 and 2) and endometrial biopsy collection for ERA test (Study Group and Control Group 2)

Prior to commencing study procedures, all patients underwent office hysteroscopy to evaluate the uterine cavity.

All cycles, including the one in which the biopsy for the ERA was taken, were performed using the HRT therapy, according to medical criteria and following the clinic's usual practice. Ideally, participants initiated Estradiol on the first or second day of their menstrual cycle. In the study and the control group 2, the patient underwent one or two endometrial biopsies. The ET was done in an HRT cycle at the timing indicated by the ERA test. The endometrium was prepared for the ERA, pET, and frozen embryo transfer (FET) cycles using HRT. On the second or third day of menstruation, vaginal ultrasound and measurements of E2 and P4 levels were conducted.

Patients received 6 mg of oral oestradiol and 3 grams of transdermal oestradiol daily. Sonographic evaluations and E2 and P4 assessments were performed between 7 and 12 days after endometrial preparation, with a mean endometrial thickness of 8.7 mm [standard deviation (SD) - 0.92]. On the day of progesterone introduction, P4 levels were <1 ng/mL. For luteal phase support, patients received 600 mg of vaginal progesterone and 20 mg of dydrogesterone per day.

In control group 1, ET was performed approximately 120 hours after progesterone introduction. For the study group and control group 2, embryo transfer was conducted according to the ERA-recommended period. High-quality euploid embryos were transferred in all three groups, with either SET or DET performed. To avoid bias, the quantity of transferred embryos did not differ significantly.

3.6.5 Test ERA (Study Group and Control Group 2)

The molecular diagnostic tool ERA uses a massive sequencing technique (NGS) and a customized bioinformatic system that analyzes the expression levels of 238 genes and relates them to the endometrium receptivity status. Genetic expression profiles are evaluated, and the Endometrium is classified as Receptive or Non-receptive. In this late case, it can be Pre-receptive or Post-receptive. The endometrial receptivity analysis (ERA®) has been designed, developed, and patented by Igenomix (PCT/ES2009/000386).

According to the bioinformatic predictor of the ERA test, for those cases in which patients show a Receptive profile or have the implantation window slightly displaced, requiring 24h or less of additional Progesterone administration (Pre-Receptive on day 1), the ET must be carried out in the same kind of cycle and accordance with the recommendations indicated by the ERA test report. For those patients with a Post-Receptive or Pre-Receptive on day 2 (Pre-Receptive requiring more than 24h of additional administration of Progesterone) profiles, a second biopsy should be taken according to the ERA test results to confirm the diagnosis of receptivity during the displaced implantation window (estimated for less than 10% cases out of the total). On rare occasions, a third biopsy may be necessary.

If the ERA test result is “Non-informative” (due to bad amplification or poor sample quality), another biopsy is taken for a new ERA test. These cases are estimated to be less than 5.5%.

The ET in group 2 coincided with the protocol of the cycle in which the endometrial biopsy had been taken and whose result had been receptive (pET). The embryos were devitrified on the same day of the transfer, so the blastocysts coincided on the day the biopsy was taken with the "Receptive" result. The ET was done following the usual clinical practice according to the ERA test recommendation for the endometrial biopsy.

3.6.6 Reproductive results

The fertility clinic systematically followed up on and compiled participants' reproductive outcomes. Data regarding pregnancy, implantation, miscarriage, and live births were collected. Obstetrical and neonatal outcomes were meticulously documented, with pregnancy outcomes data sourced from a secure electronic national registry.

Participants' pregnancies were monitored until their discharge from the clinic. Once the patients have left the clinic, reproductive data were obtained by direct contact with participants.

3.6.7 Collection of biological samples

Samples of endometrial tissue obtained via biopsy were collected from patients in the study group and control group 2 and used for the ERA test.

3.6.8 Purpose of sample collection

The purpose of collecting endometrial biopsy samples in patients from the study group and control group 2 was to assess the endometrial factor, determining the optimal receptivity window for the ET, guided by the ERA test. Subsequently, these receptivity assessments were correlated with IVF treatment success between the study groups.

3.6.9 Sample collection

The endometrial biopsy involves obtaining a small sample of the uterine endometrium. The biopsy was performed by inserting a very thin catheter (Pipelle et al. (CCD Laboratories)) through the vagina into the uterus or by office hysteroscopy. The catheter had transverse and beveled holes in the tip, which were used to scrape the walls of the uterine cavity to extract a small sample for biopsy. Once the sample (approximately 30 mg of tissue) was obtained, it was transferred to a cryotube containing 1.5 mL of RNA later (Qiagen) and shaken vigorously for a few seconds. Then it was stored at 4°C for 4 hours and subsequently sent to Igenomix at room temperature. At Igenomix, it was processed for the diagnosis of endometrial receptivity by ERA.

The endometrial biopsy for this study was taken without anesthesia. The risks and complications associated with this technique are rare, with the most frequent being a slight pain after the introduction of the cannula or a scanty spotting after the biopsy. Nonetheless, in several cases, the following complications have been described: uterine or cervical perforation, genital infection, or prolonged bleeding. This procedure is usually part of the common procedures of clinical practice.

3.6.10 Sample identification

After extracting, the samples were appropriately labeled with the code/title of the study, the patient's code, and the date of the sample. This code allowed medical staff and researchers to maintain traceability throughout the process and to monitor the clinical outcomes after the transfer. This permanent code consisted of 3 digits as indicated below:

Number of patients included in the study according to chronological order of entry into the study (001, 002,...).

Sample number from the same patient (there was more than one sample per patient only in case the result of ERA for the first biopsy had been Post-Receptive or Pre-Receptive on day 2).

Example: 1-001-EB1 corresponded to the first sample of the first patient recruited, and 001-EB2 was for the second sample of the same patient.

Confidentiality was guaranteed in compliance with the provisions of the Law on Data Protection specific to each country and the new Regulation UE 2016/679 of April 27, 2016, the English General Data Protection Regulation (GDPR), related to the protection of physical persons regarding the processing of personal data and the free circulation of these data.

3.6.11 Sample storage

The samples were conserved and transported to Igenomix according to standard conditions. Upon arrival at Igenomix, they were stored in a secure location with restricted access. Access was limited to authorized personnel involved in the investigation during a limit- ed period, proportional to the time necessary to accomplish the established objectives. The samples were preserved for their molecular diagnosis at the facilities of Igenomix, ERA Department (Valencia et al., 11 Plot B, Europark Building, 46980 Pater- na-Valencia.). The person responsible for the custody was Dr. María Ruiz. It is important to note that the samples were fully utilized during the molecular study, eliminating the need for their further storage or destruction.

The patients were required to provide consent to obtain, send, preserve, store, and analyze the sample according to the study's objectives. They also were informed about the specifics of where the samples would be stored, the duration of storage, and the purpose of their use.

3.6.12 Processing of samples

The usual protocol for diagnosis of endometrial receptivity, ERA, commercialized by the company Igenomix, was followed. Briefly, the RNA from the endometrial biopsy samples was obtained using the commercial kit RNeasy mini kit (Qiagen). Next, the complementary DNA was obtained by a retro-transcription reaction. The endometrial receptivity diagnosis was made by sequencing a customized panel of specific amplicons designed for the ERA tool (ThermoFisher). Through this process, the bioinformatic predictor classified the endometrial biopsy samples as Receptive (R) or Non-Receptive (NR), and the report specified if a second biopsy was needed or the most appropriate time to perform the pET.

3.7 Data analysis

3.7.1 Database

A database was designed with the variables detailed below. The results were drawn from the medical records database, along with the different analyses performed by ERA (endometrial receptivity). Qualitative and quantitative data were generated and stored in the database designed for this study.

The data exported from the clinical histories and source documents were duly codified in compliance with legislation to protect the clinical and personal information of the patients and safeguard patient confidentiality. This information was exported to a Clinical Report Form (CRF) following the guidelines of this protocol. No visits or procedures out of the protocol were observed. The study database was transferred and underwent meticulous review to ensure its quality, correcting any potential errors or inconsistencies, and later, statistical analysis was performed using SPSS.

3.7.2 Study variables

The following variables are sorted according to their role in the study:

- *The main variables to solve the objectives of the study in the following order:*

Live birth YES/NO
ERA diagnosed Endometrial receptivity in an endometrial biopsy (Receptive / Non-Receptive each possibility).
Pregnancy YES/NO
Implantation YES/NO

- *Control Variables: They were used to control the study variables and mitigate possible biases by seeing their distribution in each of the study groups or identifying factors that may affect them:*

Age (numerical)
Race / Ethnicity (list)
BMI (numerical)
ART indication (list)
Number of previous implementation failures (numerical) or Previous miscarriages (numerical)
Basal FSH (numerical)
Basal AMH (numerical)
Sperm concentration (numerical)
Type of treatment (FIV/ICSI)
Cycle type (fresh/vitrified oocytes)
Details of the HRT for collecting the biopsy for ERA: Days of Estradiol, Progesterone concentration (P) on the day of beginning of supplementation with P, time of onset of P, time of taking the biopsy.
Day of samples (biopsy) collection (numerical)
AFC (Antral Follicles Count) (numerical)
Number of vitrified embryos (numerical)
Days elapsed from ovarian puncture to ET (numerical)
Number of Oocytes MII (numerical)
Number of fertilized oocytes (Fertilization rate) (numerical)
Details of HRT for ET: Days of Estradiol, Progesterone concentration (P) on the day of initiation of P supplementation, time of onset of P, and time of ET.
ET day (numerical)
Number of transferred embryos (numerical)
Embryo quality assessed by the morphology of transferred embryos (AA, AB, AC, AD, BA, BB, BC, etc)
Number of implanted sacks (numerical)
Miscarriage (yes/no)

- Group variables:

Study group control group 1/2
ERA results (Receptive/Non-Receptive-every possibility)
Pregnancy YES/NO
Implantation YES/NO
Miscarriage YES/NO

- Result variables:

Endometrial receptivity was diagnosed by ERA in an endometrial biopsy (EB) (Receptive/Non-Receptive)
Implantation rates, pregnancy rate, transfer cancellation rate, and miscarriage rate, the latter as a secondary outcome variable (descriptive).

Normal implantation evolution per pick-up rate. Karyotypes of miscarriages, amniocentesis, and LB in cases where it is possible.

3.7.3 Outcome measures

The primary outcome was the LBR after the first ET. Secondary outcomes were LBR, pregnancy rate, and implantation rate at the first ET. Percentages of biochemical pregnancy, clinical miscarriage, and ectopic pregnancy from the total number of beta-HCG-positive patients, as well as obstetric, delivery, and neonatal outcomes, were also reported.

The International Glossary on Infertility and Fertility Care (Zegers-Hochschild et al., 2017) considered the following standardized definitions.

Pregnancy rate

The pregnancy rate is the number of patients with a positive serum level of beta-HCG (≥25 mIU/ml) per ET.

Live birth rate

The LBR is the number of deliveries that resulted in at least one live birth per ET. Live birth is defined as the complete expulsion or extraction from a woman of a product of conception after 22 weeks of gestation, which, after such separation, breathes or shows any other evidence of life, such as heartbeat, umbilical cord pulsation or definite movement of voluntary muscles, irrespective of whether the umbilical cord has been cut or the placenta is attached.

Implantation rate

The implantation rate is the number of gestational sacs observed by vaginal ultrasound at the fifth gestational week divided by the number of embryos transferred.

Clinical miscarriage rate

The clinical miscarriage rate is the number of spontaneous pregnancy losses in which a gestational sac or sacs was previously observed per number of pregnancies.

Biochemical pregnancy rate

The biochemical pregnancy rate is the number of pregnancies diagnosed only by beta-HCG detection without a gestational sac visualized by vaginal ultrasound at the fifth week of pregnancy per number of pregnancies.

Ectopic pregnancy rate

The ectopic pregnancy rate is the number of pregnancies outside the uterine cavity, diagnosed by ultrasound, surgical visualization, or histopathology, per number of pregnancies. For cumulative outcomes, we considered the clinical results obtained from all the ETs performed in the same arm of the study up to 12 months follow-up.

3.7.4 Statistical analysis of data

As indicated above, a database was constructed comprising all the variables previously listed by patients included in the study. The fertility clinic managed all medical information, test results, and reproductive data. All information was meticulously collected, recorded, and managed by the professionals in adherence to protocol guidelines and regulatory standards.

Descriptive and analytical statistics:

- The averages, ratios, and confidence intervals of the variables that describe the patient population included in the study were calculated and compared
- Normal distribution was assumed, given the sample size used, so there should be no need to test for normality.
- Implantation, miscarriage, and pregnancy rates were compared between the two study groups.

The reproductive outcomes at the initial embryo transfer over a one-year follow-up were evaluated in both the study and control groups using intention-to-treat analysis. The study's data were analyzed statistically using SPSS 22.0 software. Continuous variables were represented as mean Â± standard deviation (SD). The independent t-test was utilized to compare these parameters between groups. Categorical variables were represented as percentages. The comparison of these attributes among groups was conducted using the Chi-square test. The parameters were compared between groups using odds ratio (OR) and 95% confidence intervals (95% CI). P-values less than 0.05 were deemed indicative of statistical significance.

RESULTS

4.1 Study period and participant enrolment

From March 2020 to September 2023, 328 patients were included after fulfilling the inclusion criteria and giving informed permission. The research group comprised 138 patients, control group 1 included 140 patients, and control group 2 contained 50 individuals. After the follow-up period, 16 patients were eliminated from the study group due to an endometrial thickness of less than 6 mm or progesterone levels over 1 ng/ml; likewise, 8 patients were excluded from control group 1 for identical reasons. Furthermore, 6 patients from control group 2 achieved spontaneous pregnancies before to the commencement of the cycle for embryo transfer. Consequently, 122 patients in the study group, 132 in control group 1, and 44 in control group 2 completed all procedures outlined in the study protocol **(Figure 6).**

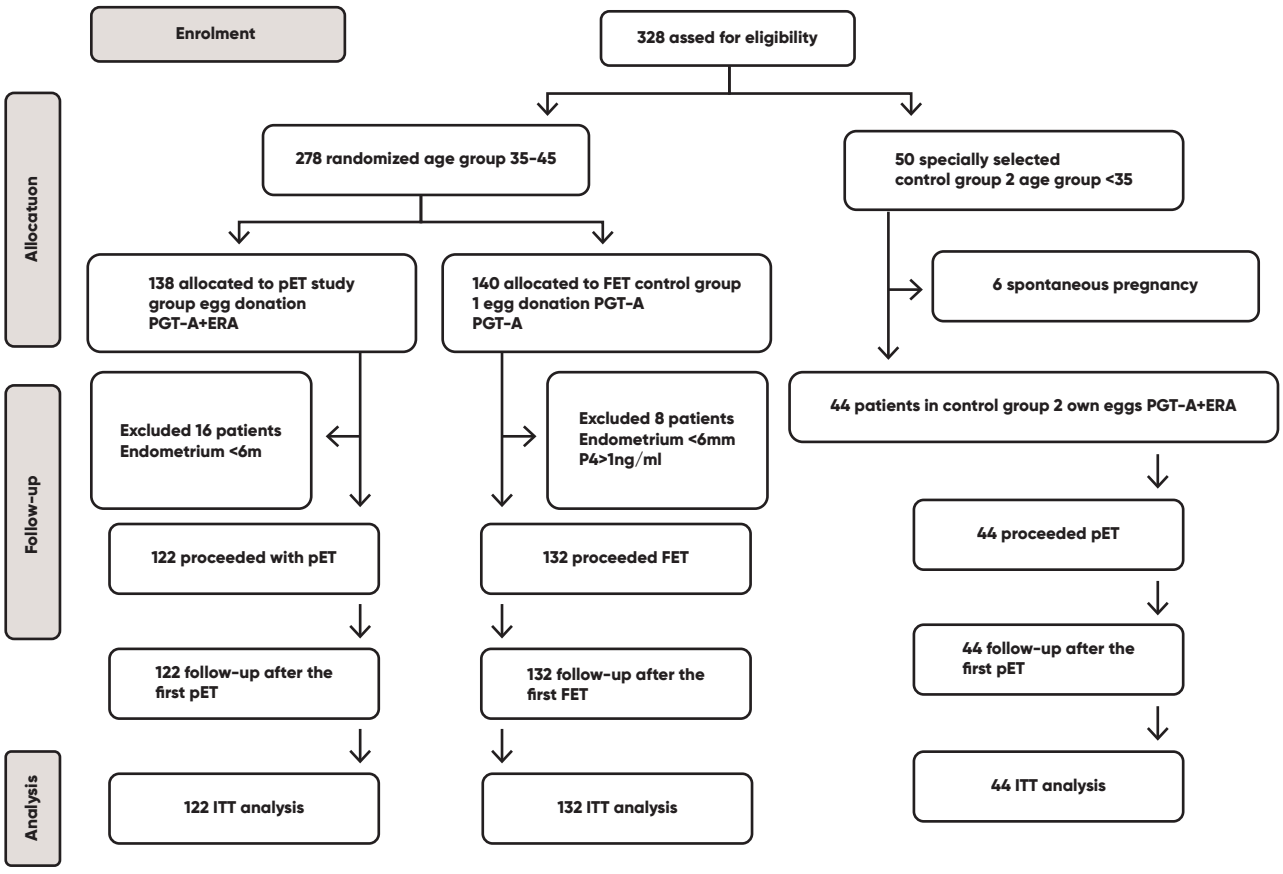


Figure 6| CONSORT flow diagram: PGT-A, preimplantation genetic testing, ERA, endometrial receptivity analysis, FET, frozen embryo transfer; pET, personalized embryo transfer.

4.2 The comparative analysis of PGT-A+ERA vs. PGT-A age group 35-45 utilizing egg donation

In this age group, we compare the patients in an age range of 35 to 45 years old, utilizing egg donation, PGT-A, and in the study group additional test is ERA. A comparison of these groups showed how the ERA improves the ART outcome even with high-quality euploid ET in advanced-age patients.

4.2.1 Baseline demographic and clinical characteristics

The baseline demographics, family history, personal history, detrimental behaviors, prior surgeries, and clinical characteristics were similar among the groups. The FSH and anti-Müllerian hormone (AMH) levels in egg donors were comparable between the study group and control group 1. In control group 2, the FSH and AMH readings exhibited minor fluctuations in comparison to the egg donor group. Cycle features and embryological data were largely uniform throughout the groups, with no significant differences detected.

A comparison of the mean reproductive parameters between the study group and control group 1 revealed no statistically significant differences.

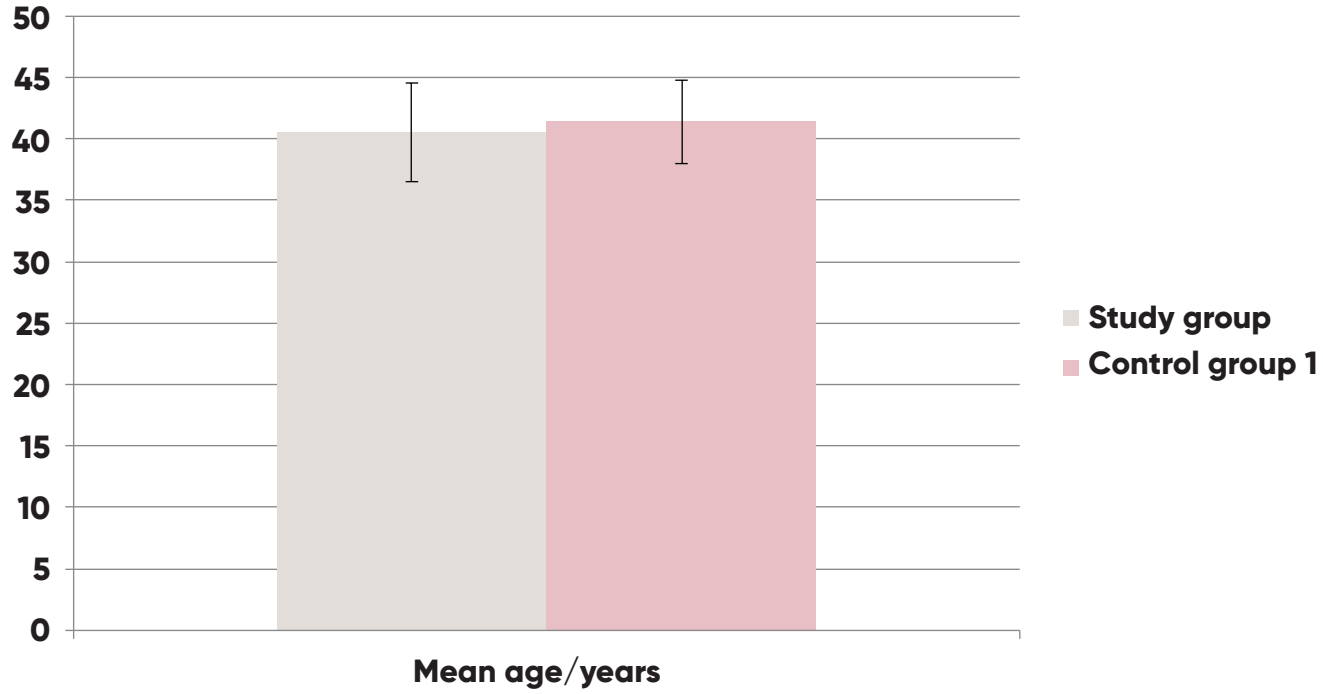
Table 1. Reproductive Parameters:
Comparison of means in the study and control Groups

Parameter	PGT-A+ERA (n=122)	PGT-A (n=132)	PGT-A+ERA vs. PGT-A	
	Mean ±SD	Mean ±SD	Independent t-test	P-value
Egg Donor Age	23.4±3.7	23.4±3.7	1.850	0.066
Egg Donor Basal FSH	6.7 ± 1.3	6.1 ±3.3	1.878	0.061
Egg Donor AMH	5.8± 2.0	5.3± 2.3	1.842	0.066
Sperm concentration	82.3± 51.7	75.1± 45.2	1.184	0.238
Egg Donor AFC	26.6 ± 8.4	24.8 ± 9.0	1.644	0.101
Egg Donor Metaphase II M II	16.7 ± 5.9	15.9 ± 5.8	1.634	0.104
Fertilization rate - fertilized oocytes/M II	0.89 ± 0.10	0.90± 0.22	0.460	0.646
Blastocyst formation rate - Vitrified embryos/fertilized oocytes	0.62± 0.19	0.59 ± 0.19	1.257	0.210

FSH - Follicle-stimulating hormone; AMH - Anti-Müllerian hormone; AFC - Antral follicle count; MII - Metaphase II; PGT-A - Preimplantation genetic testing for aneuploidy; ERA - Endometrial receptivity analysis; Standard deviation (SD)

The values of the mean ages of patients in the study group and control group 1 are given in Chart 7.

Chart 7. The values of the mean ages of patients in the groups

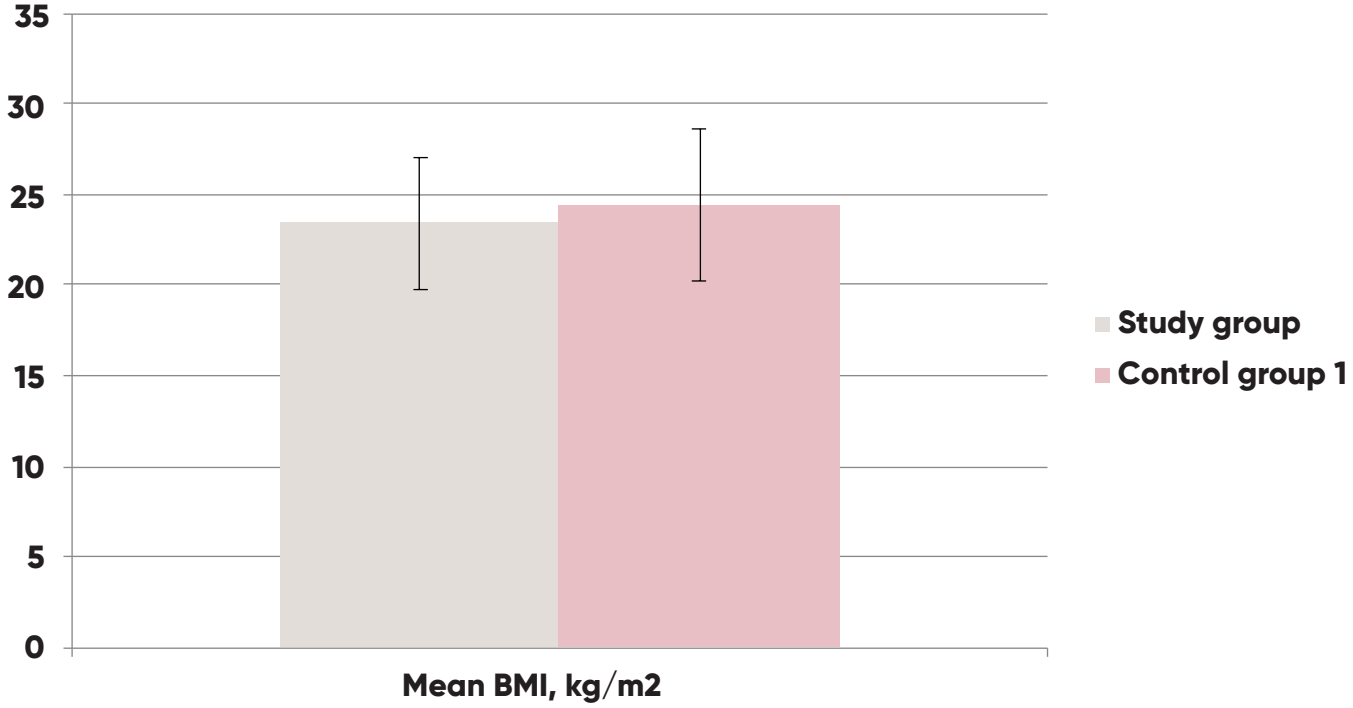


	Study group	Control group 1
Mean Age (SD), years	40.5 (4.0)	41.4 (3.4)
Levene's Test for Equality of Variances		
F-test=, p=	12.44, <0.001	
t-test for Equality of Means		
independent t-test=, p=	1.85, 0.066	

There was no statistically significant difference in age between the study group and control group 1.

The values of mean BMIs of patients in the study group and control group 1 are given in Chart 8.

Chart 8. The values of mean BMIs of patients in the groups



	Study group	Control group 1
Mean BMI (SD), kg/m^2	23.4 (3.7)	24.4 (4.2)
Levene's Test for Equality of Variances		
F-test=, p=	6.79, 0.010	
t-test for Equality of Means		
independent t-test=, p=	1.92, 0.056	

There was no statistically significant difference in age between the study group and control group 1.

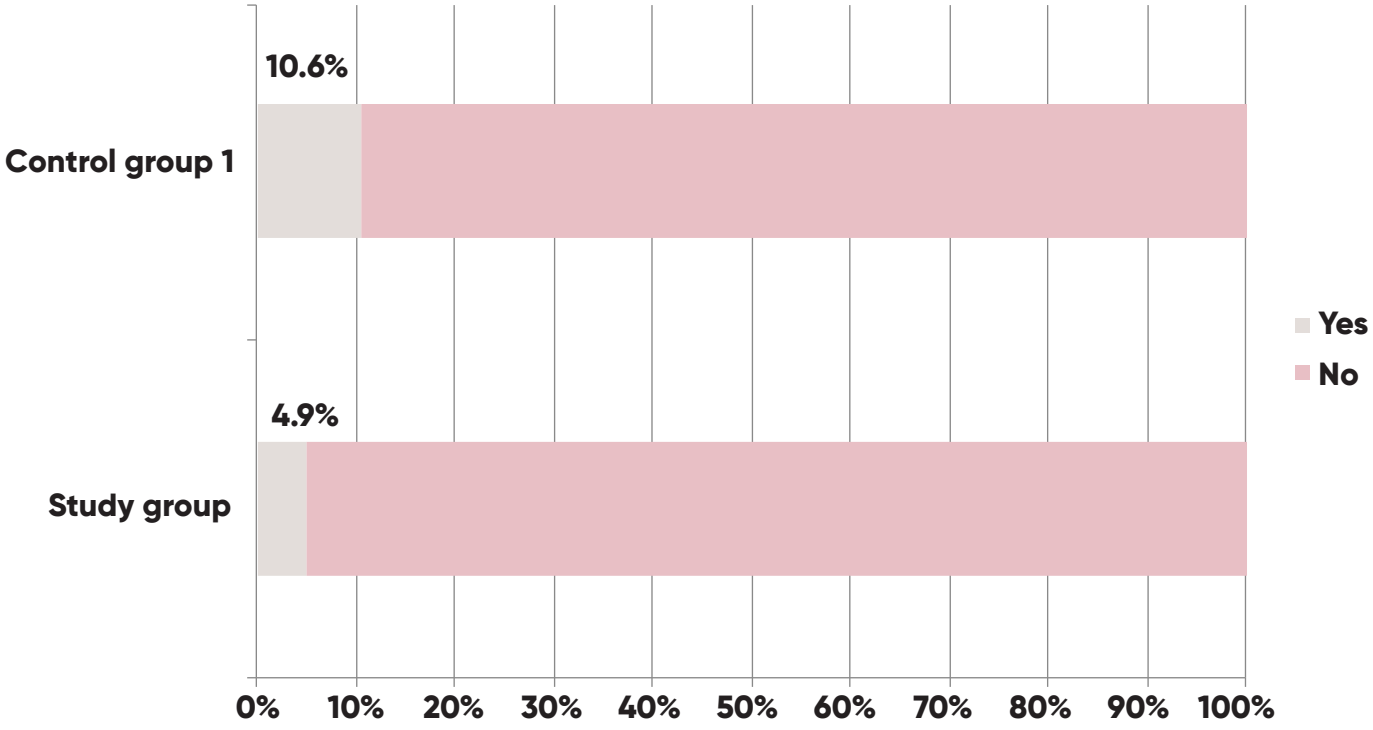
4.2.2 Personal history

Table 2. The percentage values of the distribution of patients by personal history data in the groups.

#	Personal history data	Study group, n(%)	Control group 1, n(%)	Chi-square test, (P-value)
1	HTA	0 (0.0%)	0 (0.0%)	N/A
2	Diabetes	0 (0.0%)	0 (0.0%)	N/A
3	Hypercholesterinemia	0 (0.0%)	0 (0.0%)	N/A
4	Cardiovascular	0 (0.0%)	2 (1.5%)	N/A
5	Neurologic	0 (0.0%)	2 (1.5%)	N/A
6	Immunologic	0 (0.0%)	2 (1.5%)	N/A
7	Oncologic	0 (0.0%)	6 (4.5%)	N/A
8	Alcohol	0 (0.0%)	4 (3.0%)	N/A
9	Drugs	0 (0.0%)	2 (1.5%)	N/A
10	Psychiatric	0 (0.0%)	0 (0.0%)	N/A
11	Low Ovarian reserve	100 (100.0%)	100 (100.0%)	N/A

HTA, Health Technology Assessment

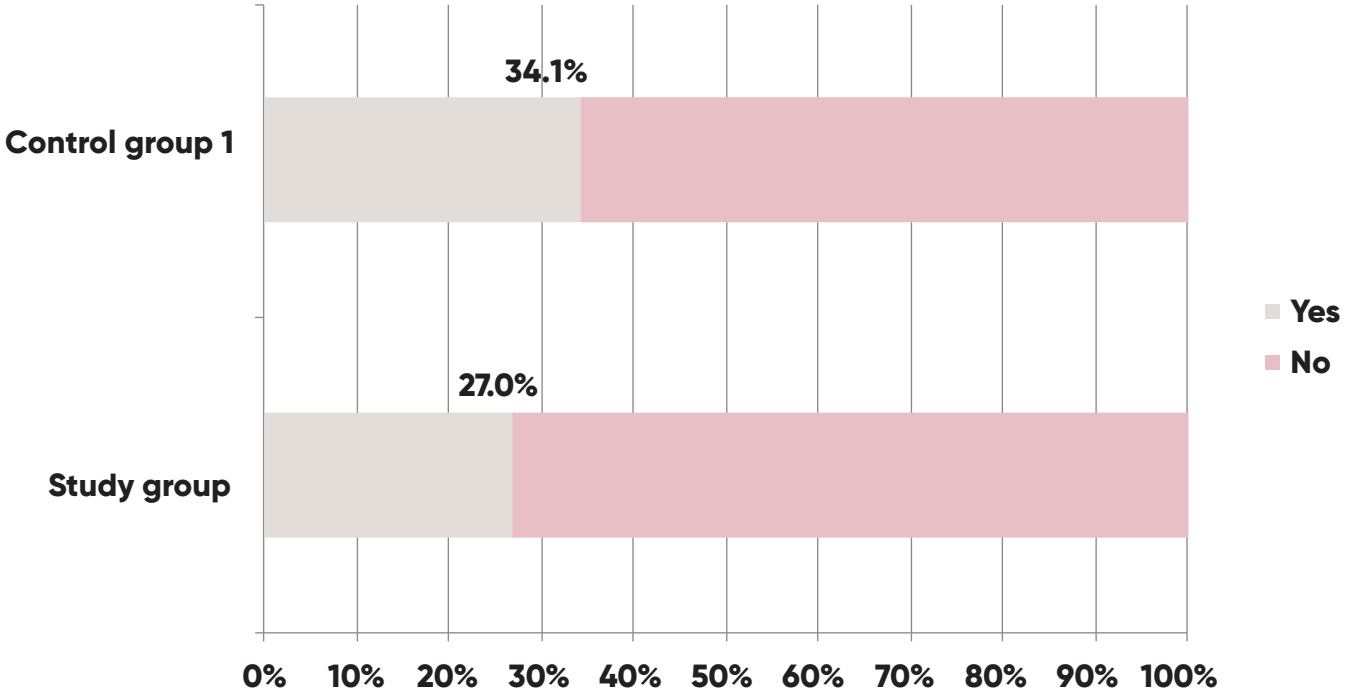
Chart 9. The percentage values of the distribution of patients by the presence of allergy in the groups.



Allergy	Study group	Control group 1
Yes, n(%)	6 (4.9%)	116 (95.1%)
No, n(%)	14 (10.6%)	118 (89.4%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	2.83	1	0.093	0.107
Likelihood Ratio	2.91	1	0.088	
Fisher's Exact Test Continuity Correction	2.10	1	0.147	

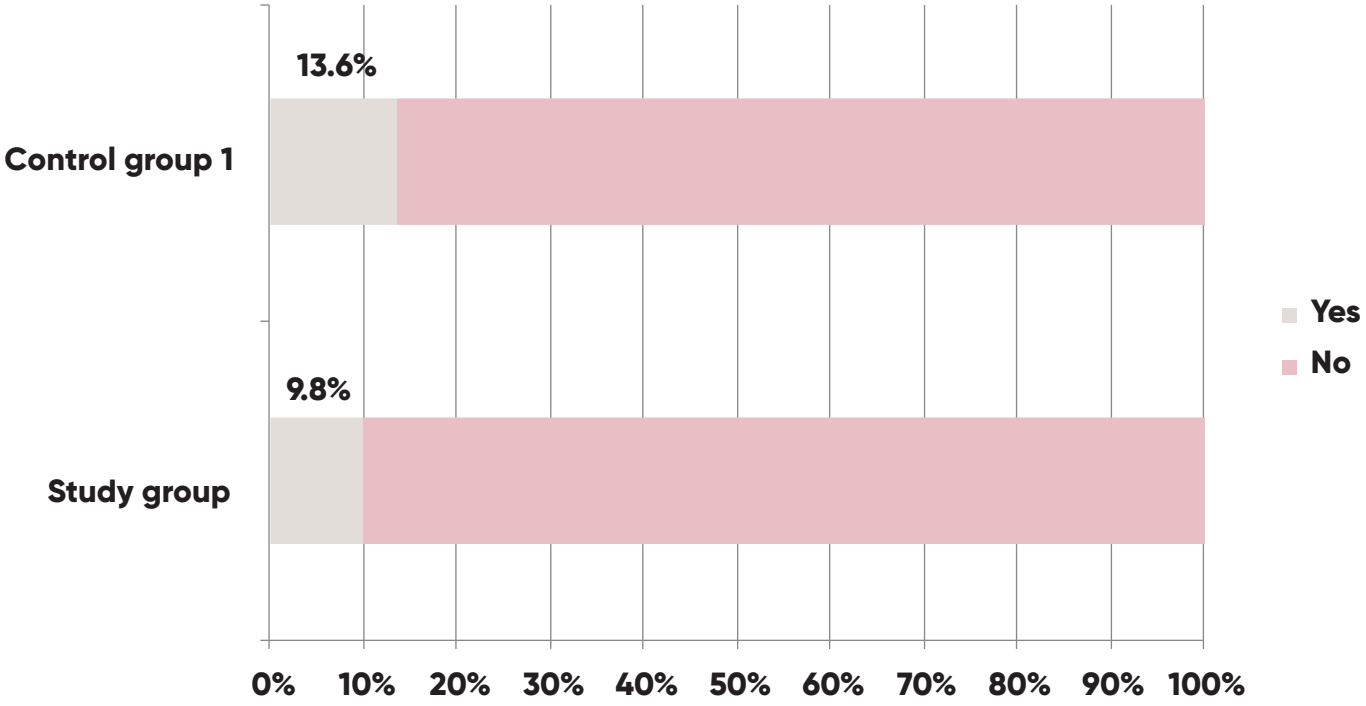
Chart 10. The percentage values of the distribution of patients by the presence of surgery in the groups.



Surgery	Study group	Control group 1
Yes, n(%)	33 (27.0%)	45 (34.1%)
No, n(%)	89 (73.0%)	87 (65.9%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	11.47	1	0.225	0.225
Likelihood Ratio	11.51	1	0.223	
Fisher's Exact Test Continuity Correction	11.45	1	0.225	

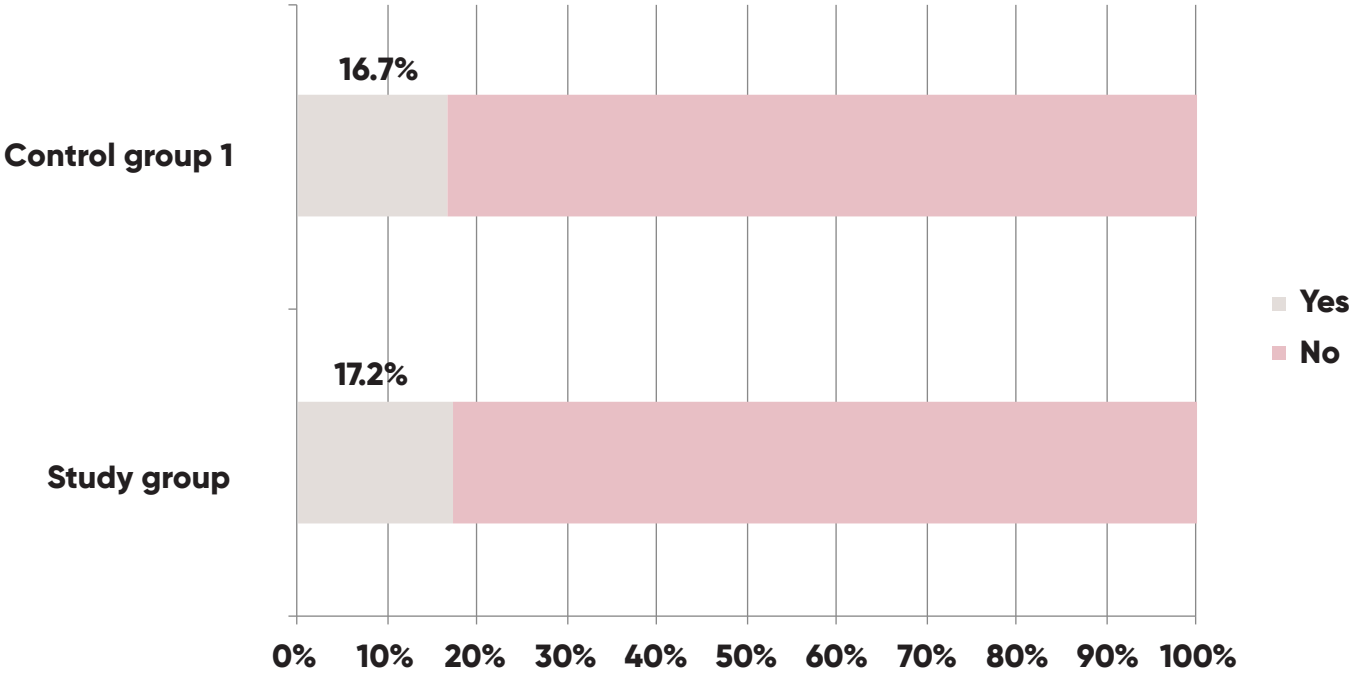
Chart 11. The percentage values of the distribution of patients by the presence of Tobacco users in the groups.



Tobacco user	Study group	Control group 1
Yes, n(%)	12 (9.8%)	18 (13.6%)
No, n(%)	110 (90.2%)	114 (86.4%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.88	1	0.348	0.437
Likelihood Ratio	0.89	1	0.347	
Fisher's Exact Test Continuity Correction	0.55	1	0.457	

Chart 12. The percentage values of the distribution of patients by the presence of Exercise in the groups.



Exercise	Study group	Control group 1
Yes, n(%)	21 (17.2%)	22 (16.7%)
No, n(%)	101 (82.8%)	110 (83.3%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.01	1	0.908	0.909
Likelihood Ratio	0.02	1	0.905	
Fisher's Exact Test Continuity Correction	0.01	1	0.910	

4.2.3 Family History

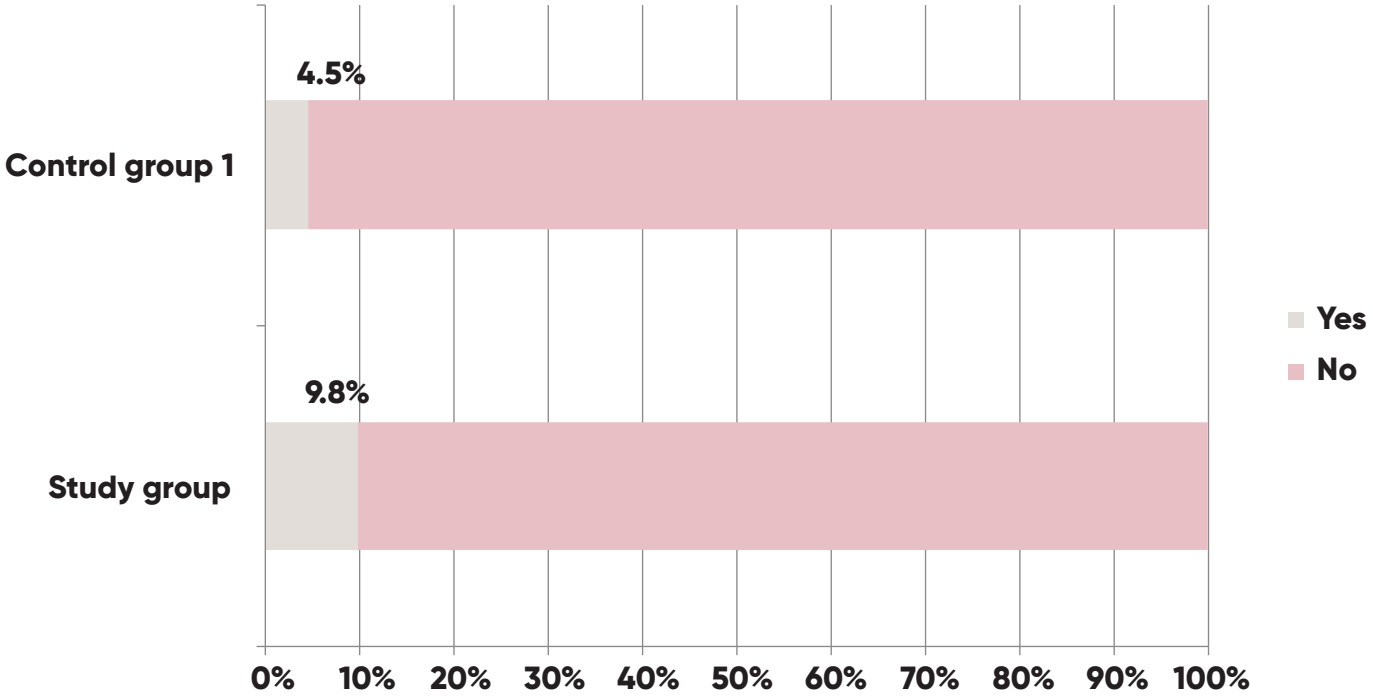
The percentage values of the distribution of patients by family history data in the study group and control group 1 are given in Table 3(statistically non-significant data) and in Chart 13-16. Statistically significant differences were revealed in the data on the presence of Oncologic diseases in family history (p<0.001).

Table 3. The percentage values of the distribution of patients by family history data in the groups.

#	Family history data	Study group, n(%)	Control group 1, n(%)	Chi-square test, (P-value)
1	HTA	0 (0.0%)	4 (3.0%)	N/A
2	Fertility problems	3 (2.5%)	0 (0.0%)	N/A
3	Genetics	0 (0.0%)	2 (1.5%)	N/A
4	Immunologic	0 (0.0%)	0 (0.0%)	N/A
5	Twins in Family	0 (0.0%)	2 (1.5%)	N/A
6	Azoospermia	0 (0.0%)	2 (1.5%)	N/A

HTA - Health Technology Assessment

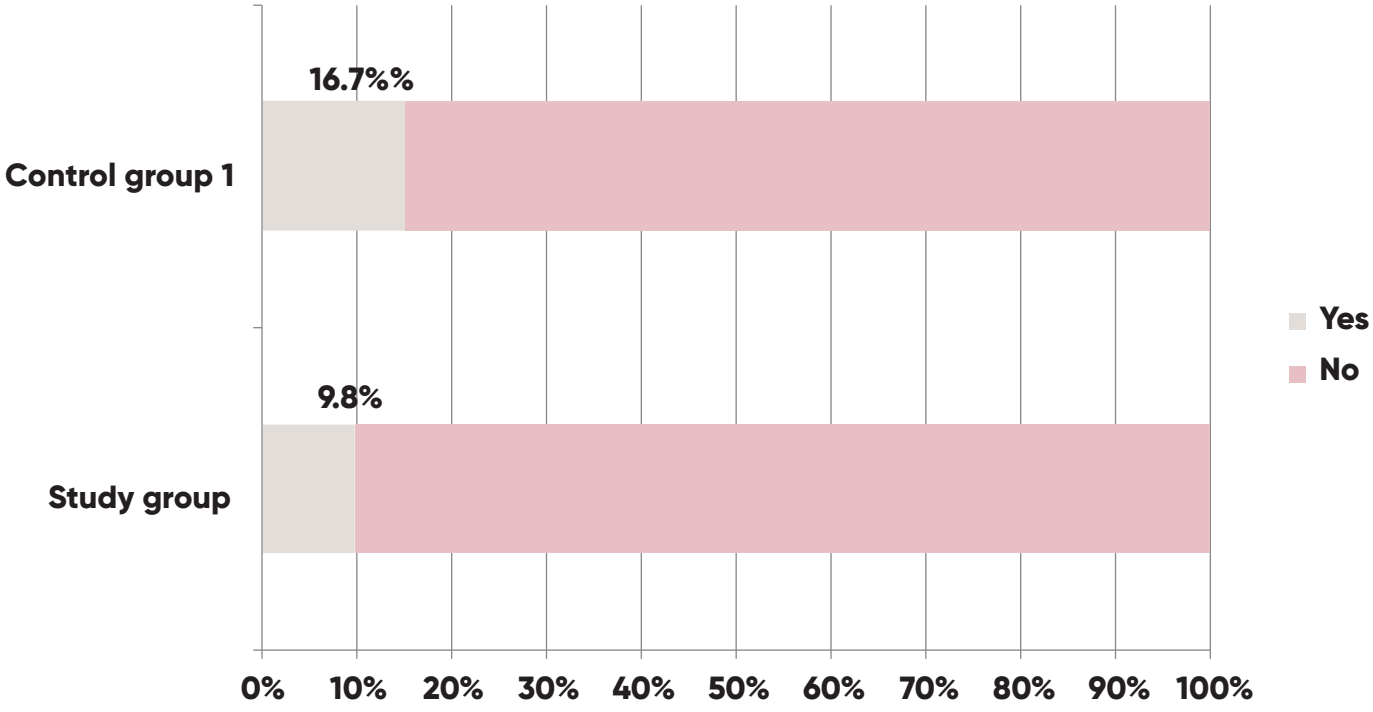
Chart 13. The percentage values of the distribution of patients by the presence of diabetes in family history in the groups.



Diabetes in family history	Study group	Control group 1
Yes, n(%)	12 (9.8%)	6 (4.5%)
No, n(%)	110 (90.2%)	119 (95.5%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	2.70	1	0.101	0.141
Likelihood Ratio	2.73	1	0.098	
Fisher's Exact Test Continuity Correction	1.95	1	0.162	

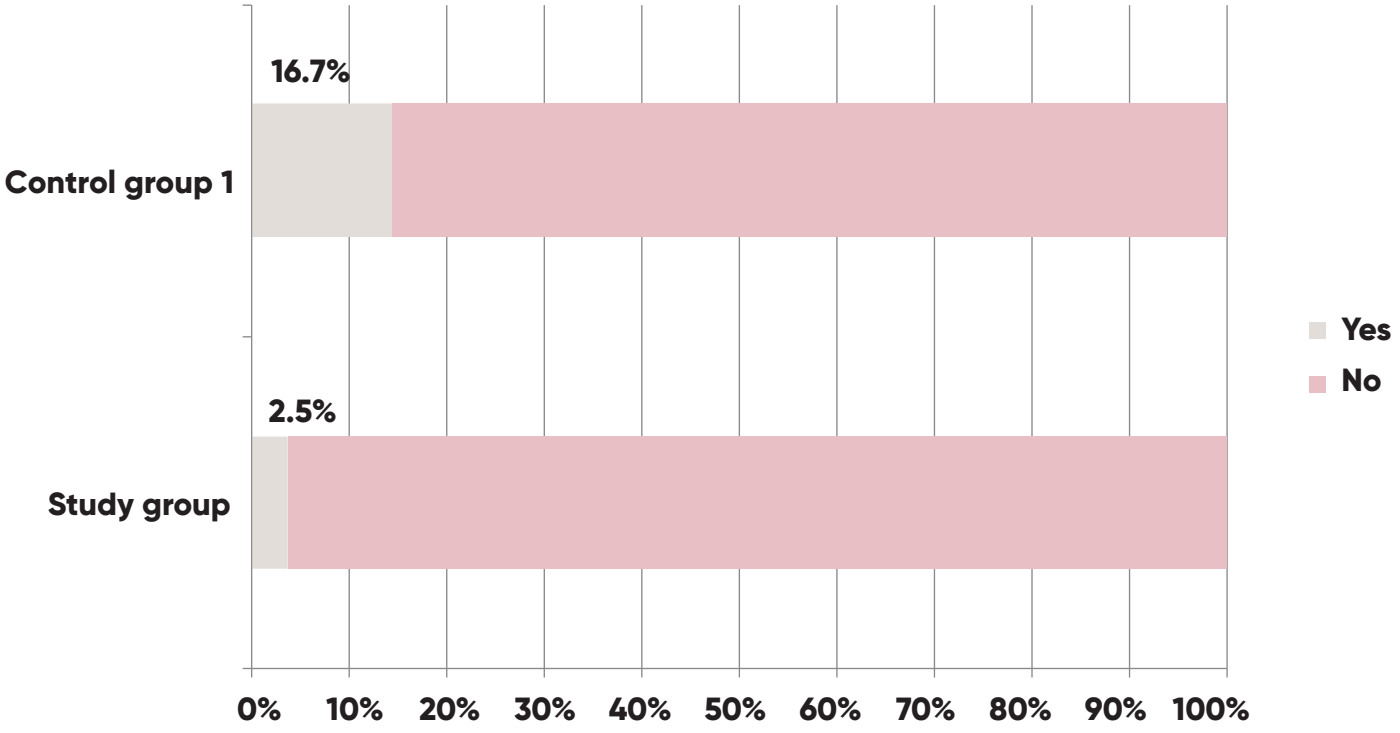
Chart 14. The percentage values of the distribution of patients by the presence of cardiovascular diseases in family history in the groups



Cardiovascular diseases in family history	Study group	Control group 1
Yes, n(%)	12 (9.8%)	22 (16.7%)
No, n(%)	110 (90.2%)	119 (95.5%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	2.55	1	0.110	0.140
Likelihood Ratio	2.59	1	0.107	
Fisher's Exact Test Continuity Correction	2.00	1	0.158	

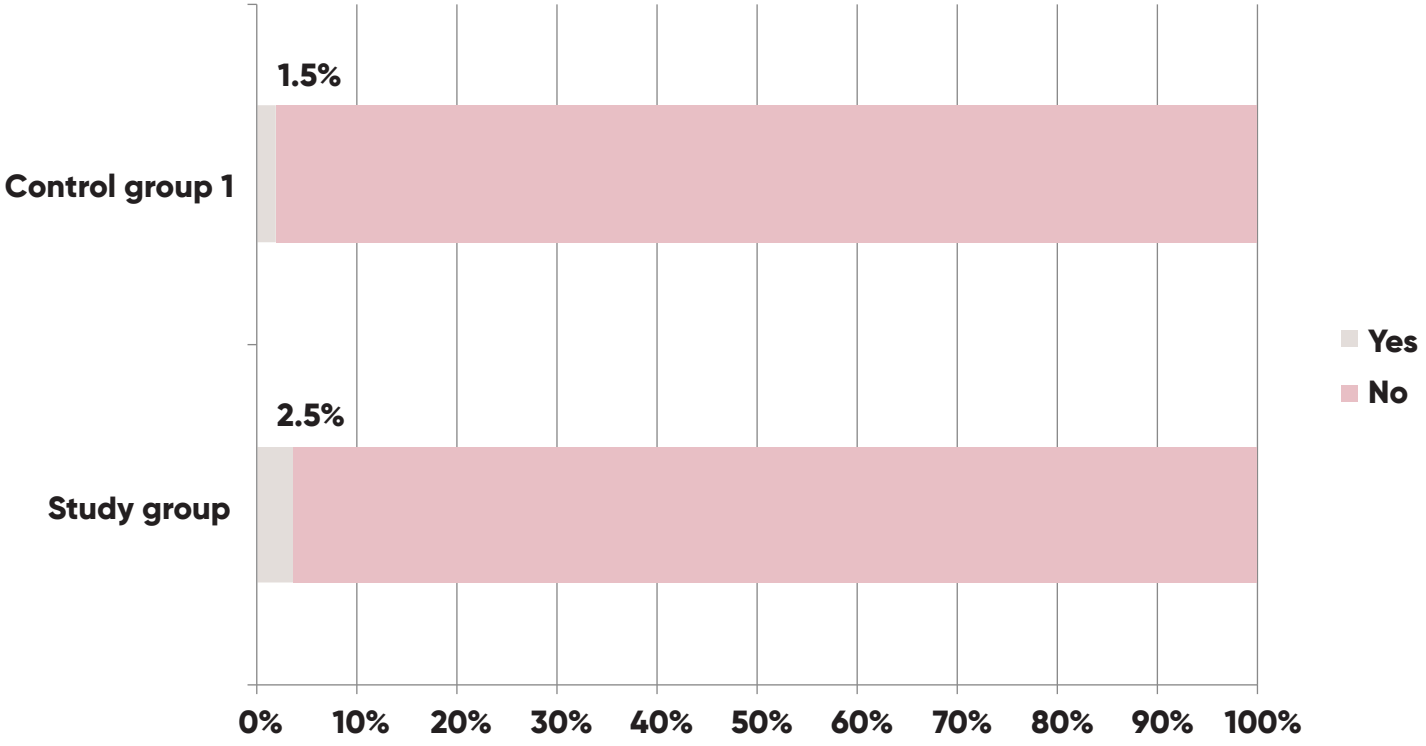
Chart 15. The percentage values of the distribution of patients by the presence of oncologic diseases in family history in the groups.



Oncologic diseases in family history	Study group	Control group 1
Yes, n(%)	3 (2.5%)	22 (16.7%)
No, n(%)	119 (97.5%)	119 (95.5%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	14.42	1	<0.001	<0.001
Likelihood Ratio	16.27	1	<0.001	
Fisher's Exact Test Continuity Correction	12.87	1	<0.001	

Chart 16. The percentage values of the distribution of patients by the presence of male infertility in family history in the groups.



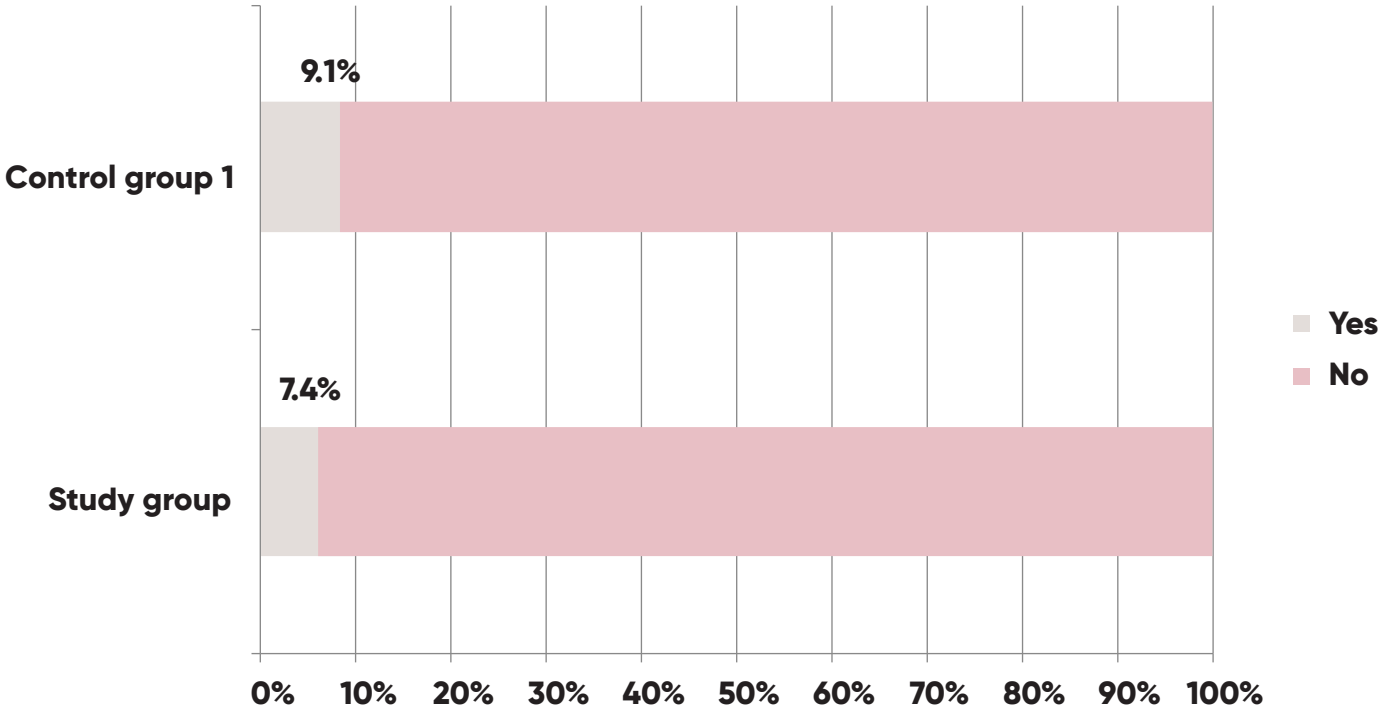
Male infertility in family history	Study group	Control group 1
Yes, n(%)	3 (2.5%)	2 (1.5%)
No, n(%)	119 (97.5%)	130 (98.5%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.29	1	0.588	0.673
Likelihood Ratio	0.29	1	0.588	
Fisher's Exact Test Continuity Correction	0.01	1	0.929	

4.2.4 Gynecology and Obstetrical Anamnesis

Gynecology anamnesis

Chart 17. The percentage values of the distribution of patients by regularity of menstrual cycle in the groups.



Regularity of menstrual cycle	Study group	Control group 1
Yes, n(%)	9 (7.4%)	12 (9.1%)
No, n(%)	113 (92.6%)	120 (90.9%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.24	1	0.621	0.631
Likelihood Ratio	0.26	1	0.619	
Fisher's Exact Test Continuity Correction	0.19	1	0.641	

The prevalence of regular and irregular menstrual cycles did not significantly differ between the study and control group 1.

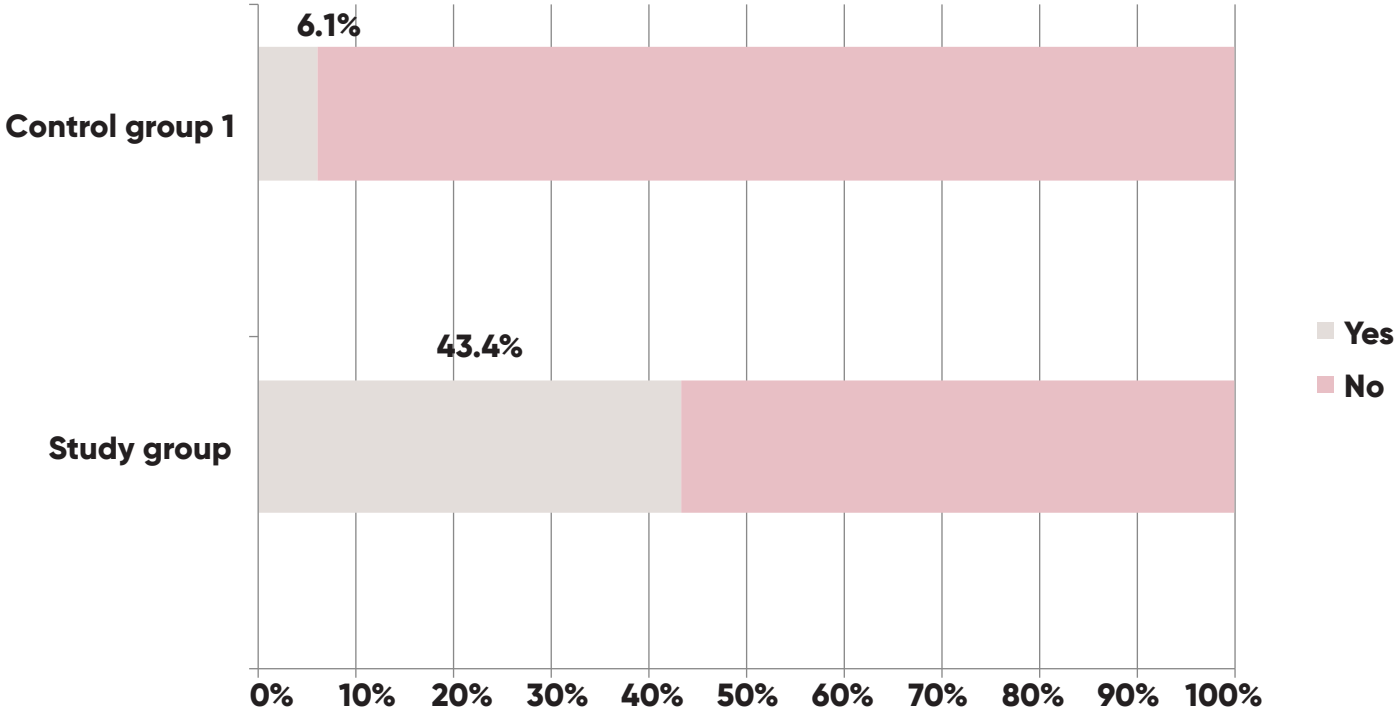
Uterine pathologies

The percentage values of the distribution of patients by uterine pathologies in the study group and control group 1 are given in Table 4 (statistically non-significant data) and in Chart 18-19. Statistically significant differences were revealed in the data on the presence of adenomyosis (p<0.001).

Table 4. The percentage values of the distribution of patients by gynecology history data in the groups.

#	Personal history data	Study group, n(%)	Control group 1, n(%)	Chi-square test, (P-value)
1	Fibroids	0 (0.0%)	2 (1.5%)	N/A
2	Mulerian	0 (0.0%)	0 (0.0%)	N/A
3	Polyps	0 (0.0%)	4 (3.0%)	N/A
4	Endometritis	0 (0.0%)	2 (1.5%)	N/A
5	Other	3 (2.5%)	0 (0.0%)	N/A

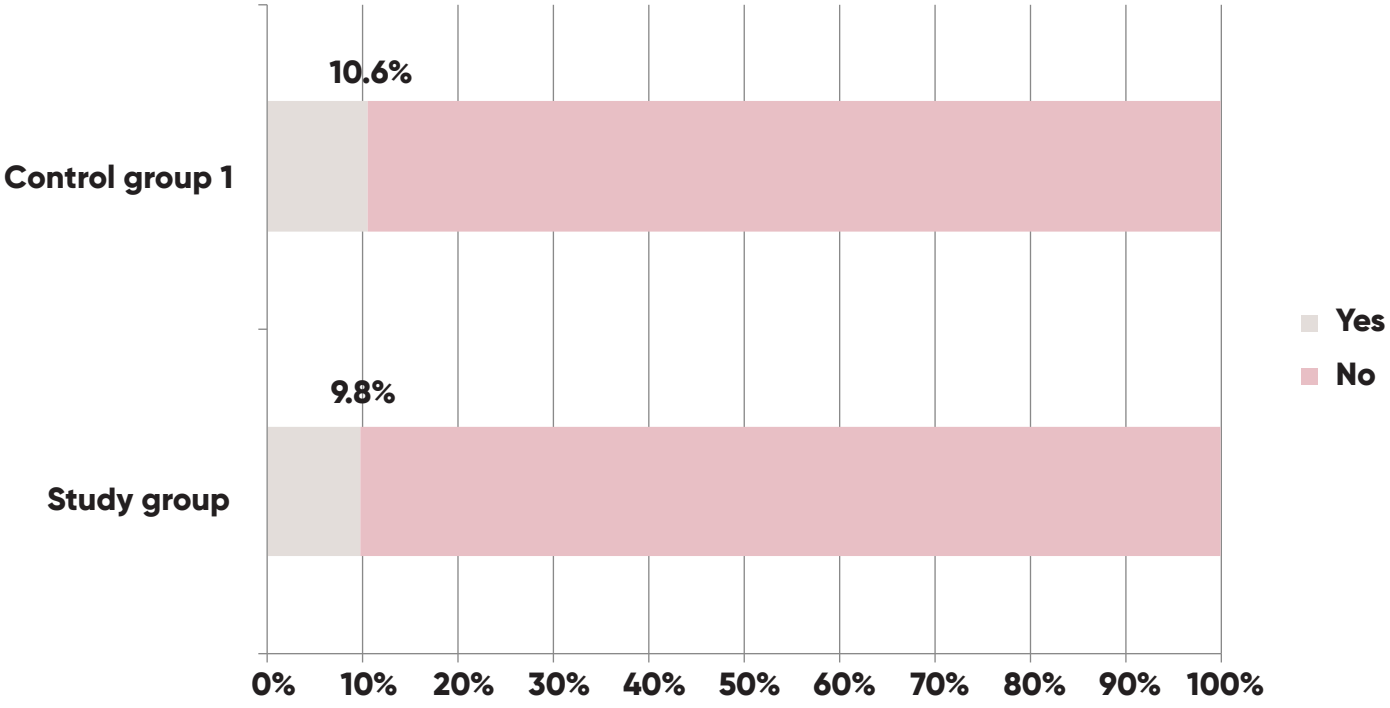
Chart 18. The percentage values of the distribution of patients by adenomyosis in the groups.



Uterine pathologies - adenomyosis	Study group	Control group 1
Yes, n(%)	53 (43.4%)	8 (6.1%)
No, n(%)	69 (56.6%)	124 (93.9%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	48.55	1	<0.001	<0.001
Likelihood Ratio	52.66	1	<0.001	
Fisher's Exact Test Continuity Correction	46.52	1	<0.001	

Chart 19. The percentage values of the distribution of patients by myomas in the groups.



Uterine pathologies - myoma	Study group	Control group 1
Yes, n (%)	12 (9.8%)	14 (10.6%)
No, n (%)	110 (90.2%)	118 (89.4%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.04	1	0.840	1.000
Likelihood Ratio	0.04	1	0.840	
Fisher's Exact Test Continuity Correction	0.00	1	1.000	

Previous pregnancies and obstetric history

The percentage values of the distribution of patients by the data of previous pregnancies and obstetric history in the study group and control group 1 are given in Table 5 and 6. The comparative analysis revealed that the groups were homogenous according to these data.

Table 5. The percentage values of the distribution of patients by data from previous pregnancies

#	Previous pregnancies	Study group, n(%)	Control group 1, n(%)	Chi-square test, (P-value)
1	**Previous Pregnancies**	**75 (61.5%)**	**32 (24.2%)**	**37.16 (<0.001)**
	1	72 (59.0%)	32 (24.2%)	
	2	0 (0.0%)	0 (0.0%)	
	3	3 (2.5%)	0 (0.0%)	
2	**Previous Term Pregnancies**	**18 (14.8%)**	**6 (4.5%)**	**13.10 (0.004)**
	1	15 (12.3%)	4 (3.0%)	
	2	0 (0.0%)	2 (1.5%)	
	3	3 (2.5%)	0 (0.0%)	
3	**Previous preterm Pregnancies**	0 (0.0%)	2 (1.5%)	N/A
	1	0 (0.0%)	2 (1.5%)	
4	**Previous miscarriages**	**48 (39.3%)**	**28 (21.2%)**	**13.48 (0.004)**
	1	39 (32.0%)	26 (19.7%)	
	2	3 (2.5%)	2 (1.5%)	
	3	6 (4.9%)	0 (0.0%)	
5	**Previous stillbirths**	0 (0.0%)	2 (1.5%)	N/A
	1	0 (0.0%)	2 (1.5%)	
6	**Previuous live births**	**18 (14.8%)**	**6 (4.5%)**	**13.10 (0.004)**
	1	15 (12.3%)	4 (3.0%)	
	2	0 (0.0%)	2 (1.5%)	
	3	3 (2.5%)	0 (0.0%)	
7	**Previous curettages**	**33 (27.0%)**	**14 (10.6%)**	**12.51 (0.002)**
	1	30 (24.6%)	14 (10.6%)	
	2	0 (0.0%)	0 (0.0%)	
	3	3 (2.5%)	0 (0.0%)	

Table 6. The percentage values of the distribution of patients by data of previous pregnancies and obstetric history in the groups.

#	Previous pregnancies and obstetric history	Study group, n(%)	Control group 1, n(%)	Chi-square test, (P-value)
1	Previous chromosomal abnormalities	0 (0.0%)	2 (1.5%)	N/A
2	Fetal Malformations	0 (0.0%)	2 (1.5%)	N/A
3	Preeclampsia	6 (4.9%)	0 (0.0%)	N/A
4	Preterm birth	0 (0.0%)	2 (1.5%)	N/A
5	Twins	0 (0.0%)	0 (0.0%)	N/A
6	Molar pregnancy	0 (0.0%)	0 (0.0%)	N/A
7	Anembryonic pregnancy	6 (4.9%)	0 (0.0%)	N/A
8	Prematurity	0 (0.0%)	2 (1.5%)	N/A

4.2.5 ART history

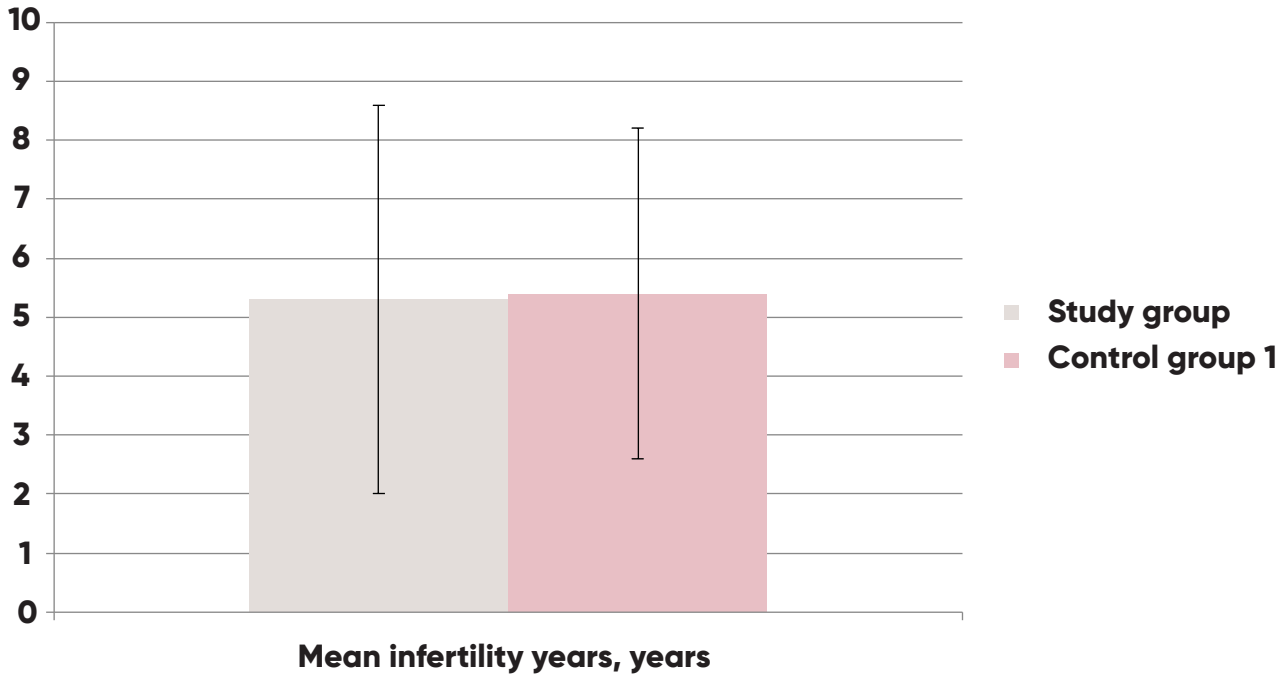
The percentage values of the distribution of patients by the data of previous ART history in the study group and control group 1 are given in Table 7. The comparative analysis revealed that the groups were homogenous according to these data.

Table 7. The percentage values of the distribution of patients by data of previous ART history in the groups.

#	Previous ART history	Study group, n(%)	Control group 1, n(%)	Chi-square test, (P-value)
1	**Previous implantation failure**	**116 (95.1%)**	**132 (100.0%)**	**61.88 (<0.001)**
	1	0 (0.0%)	0 (0.0%)	
	2	47 (38.5%)	6 (4.5%)	
	3	48 (39.3%)	84 (63.6%)	
	4	15 (12.3%)	36 (27.3%)	
	5	6 (4.9%)	2 (1.5%)	
	6	0 (0.0%)	2 (1.5%)	
	7	0 (0.0%)	0 (0.0%)	
	8	0 (0.0%)	2 (1.5%)	
2	**Previous implantation failure with PGT-A**	**119 (97.5%)**	**132 (100.0%)**	**18.18 (<0.001)**
	1	0 (0.0%)	0 (0.0%)	
	2	98 (80.3%)	128 (97.0%)	
	3	21 (17.2%)	4 (3.0%)	

Mean values of infertility years in the study and control group 1 are given in chart 20.

Chart 20. The values of infertility years of patients in the groups

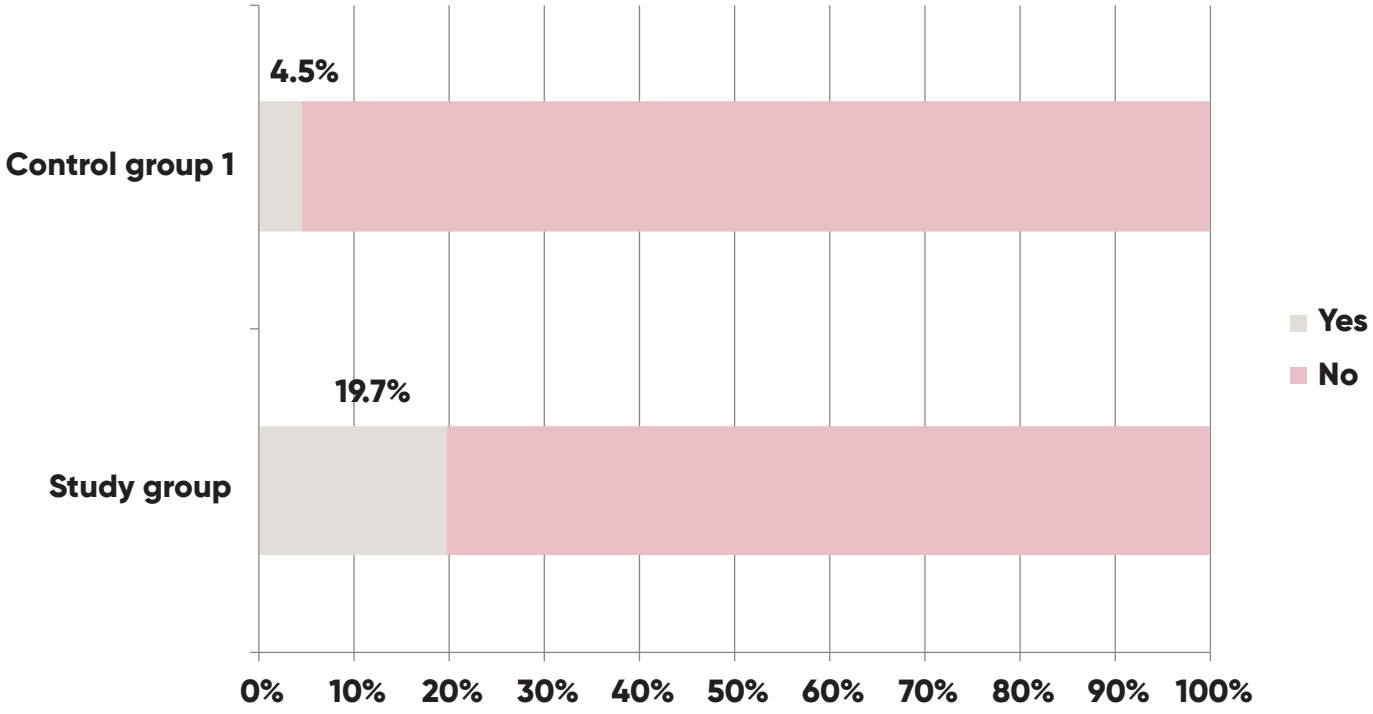


	Study group	Control group 1
Mean infertility years (SD), years	5.3 (3.3)	5.4 (2.8)
Levene's Test for Equality of Variances		
F-test=, p=	13.30, <0.001	
t-test for Equality of Means		
independent t-test=, p=	0.32, 0.753	

ART indication

The percentage values of the distribution of patients by ART indication for male factor in the study group and control group 1 are given in Chart 21. The difference between data was significant (p<0.001).

Chart 21. The percentage values of the distribution of patients by ART indicated for male factor in the groups.

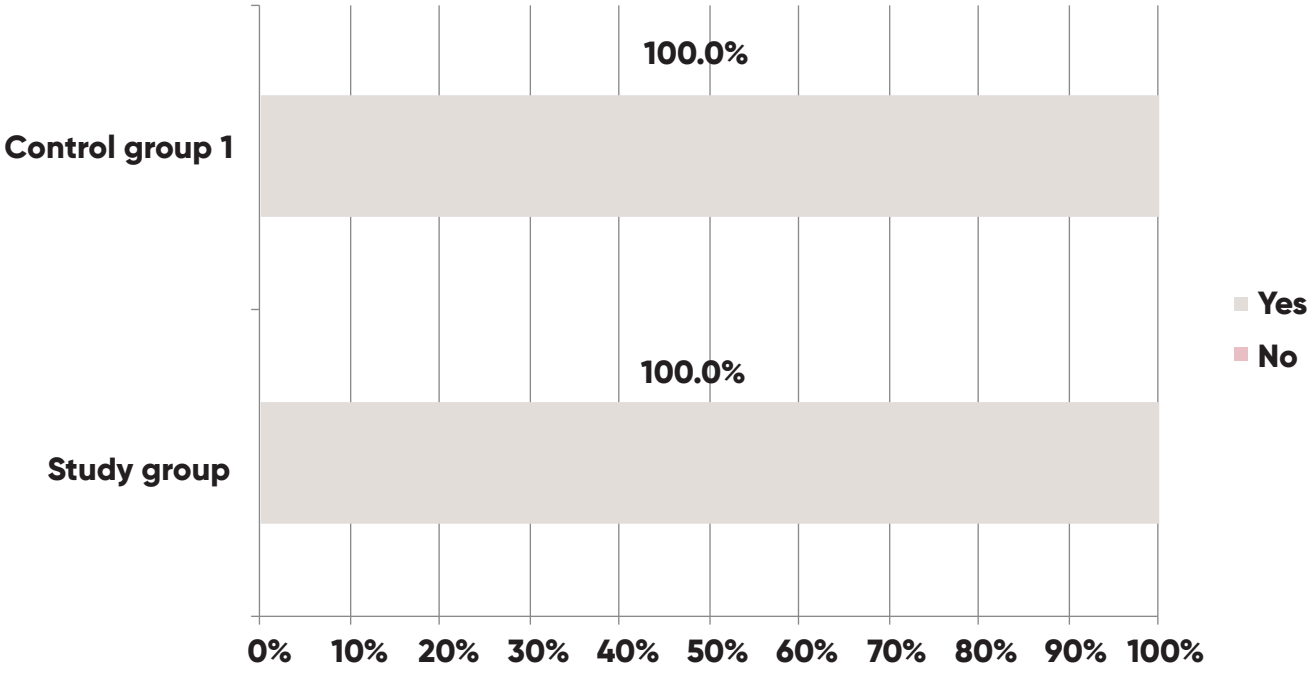


ART indication - male factor	Study group	Control group 1
Yes, n(%)	24 (19.7%)	6 (4.5%)
No, n(%)	113 (80.3%)	132 (95.5%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	13.93	1	<0.001	<0.001
Likelihood Ratio	14.68	1	<0.001	
Fisher's Exact Test Continuity Correction	12.51	1	<0.001	

The percentage values of the distribution of patients by ART indication for advanced maternal age in the study group and control group 1 are given in Chart 22. The difference between data was significant (p<0.001).

Chart 22. The percentage values of the distribution of patients by ART indicated advanced maternal age in the groups.

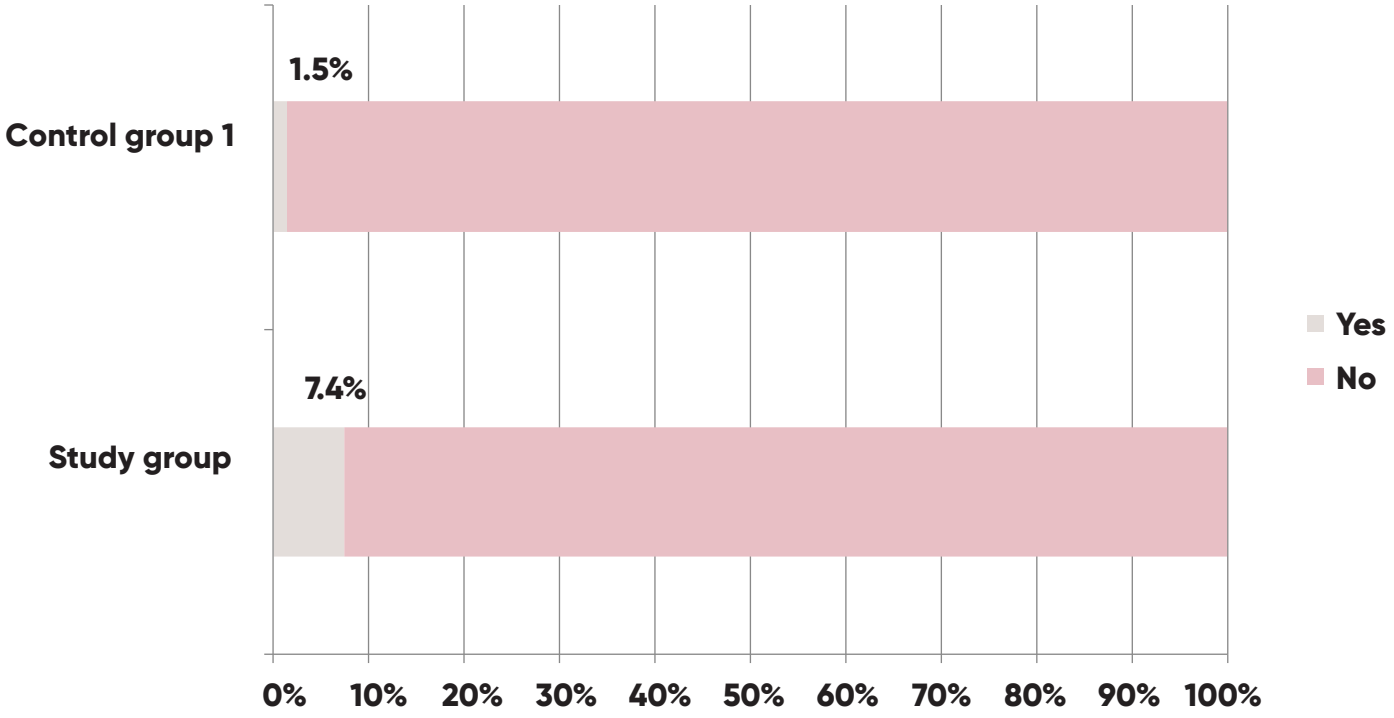


ART indication - advanced maternal age	Study group	Control group 1
Yes, n(%)	122 (100.0%)	132 (100.0%)
No, n(%)	0 (0.0%)	0 (0.0%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.00	1	1.000	1.000
Likelihood Ratio	0.00	1	1.000	
Fisher's Exact Test Continuity Correction	0.00	1	1.000	

The percentage values of the distribution of patients by ART indication for endometriosis in the study group and control group 1 are given in Chart 23. The difference between data was significant (p=0.029).

Chart 23. The percentage values of the distribution of patients by ART indication for endometriosis in the groups.

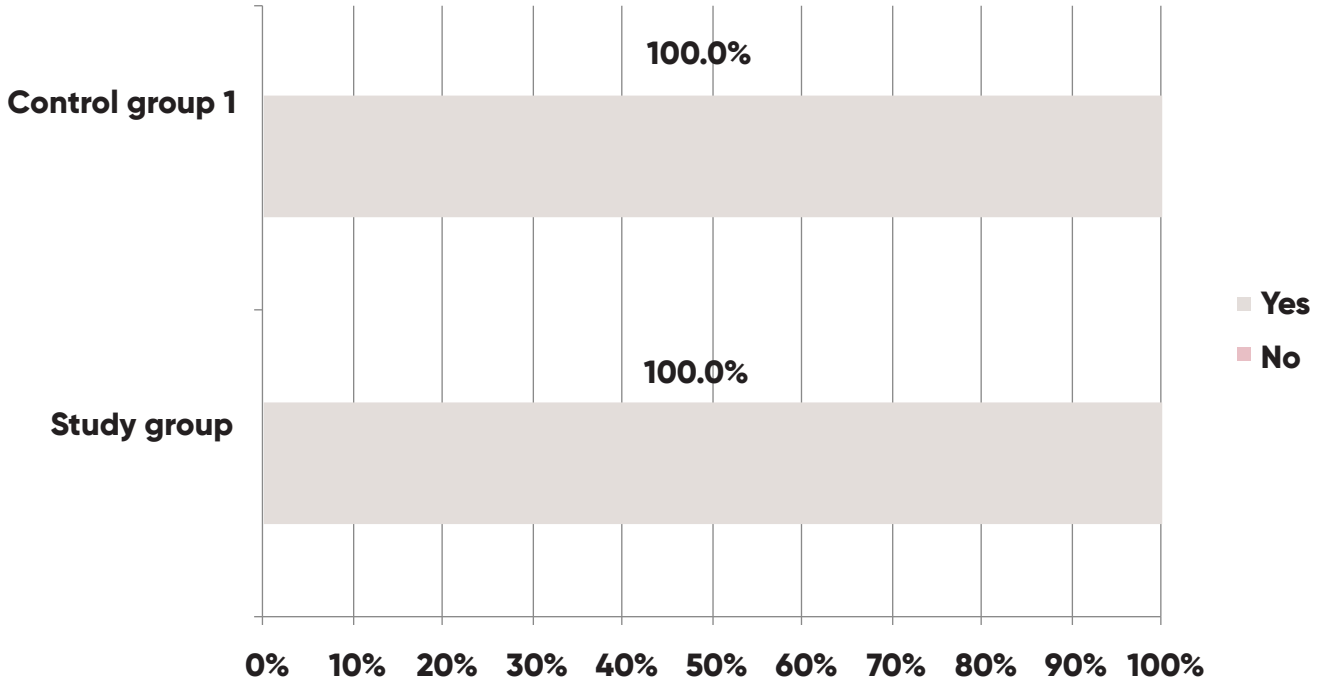


ART indication - endometriosis	Study group	Control group 1
Yes, n(%)	9 (7.4%)	2 (1.5%)
No, n(%)	113 (92.6%)	130 (98.5%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	5.26	1	0.022	0.029
Likelihood Ratio	5.61	1	0.018	
Fisher's Exact Test Continuity Correction	3.94	1	0.047	

The percentage values of the distribution of patients by ART indication for ovarian factor in the study group and control group 1 are given in Chart 24. The difference between data was nonsignificant (p=0.123).

Chart 24. The percentage values of the distribution of patients by ART indication for ovarian factor in the groups.

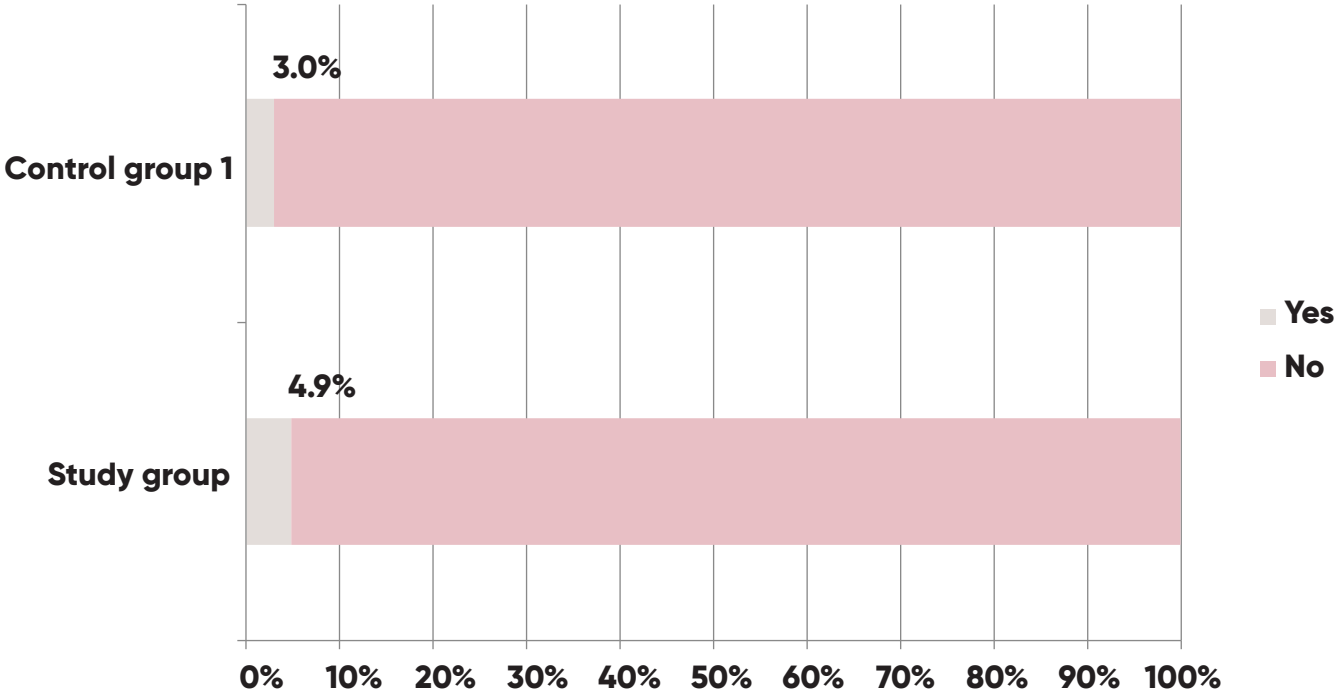


ART indication - ovarian factor	Study group	Control group 1
Yes, n(%)	122 (100.0%)	132 (100.0%)
No, n(%)	0 (0.0%)	0 (0.0%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.00	1	1.000	1.000
Likelihood Ratio	0.00	1	1.000	
Fisher's Exact Test Continuity Correction	0.00	1	1.000	

The percentage values of the distribution of patients by ART indication for tubal factor in the study group and control group 1 are given in the Chart 25. The difference between data was nonsignificant (p=0.528).

Chart 25. The percentage values of the distribution of patients by ART indication for tubal factor in the groups.

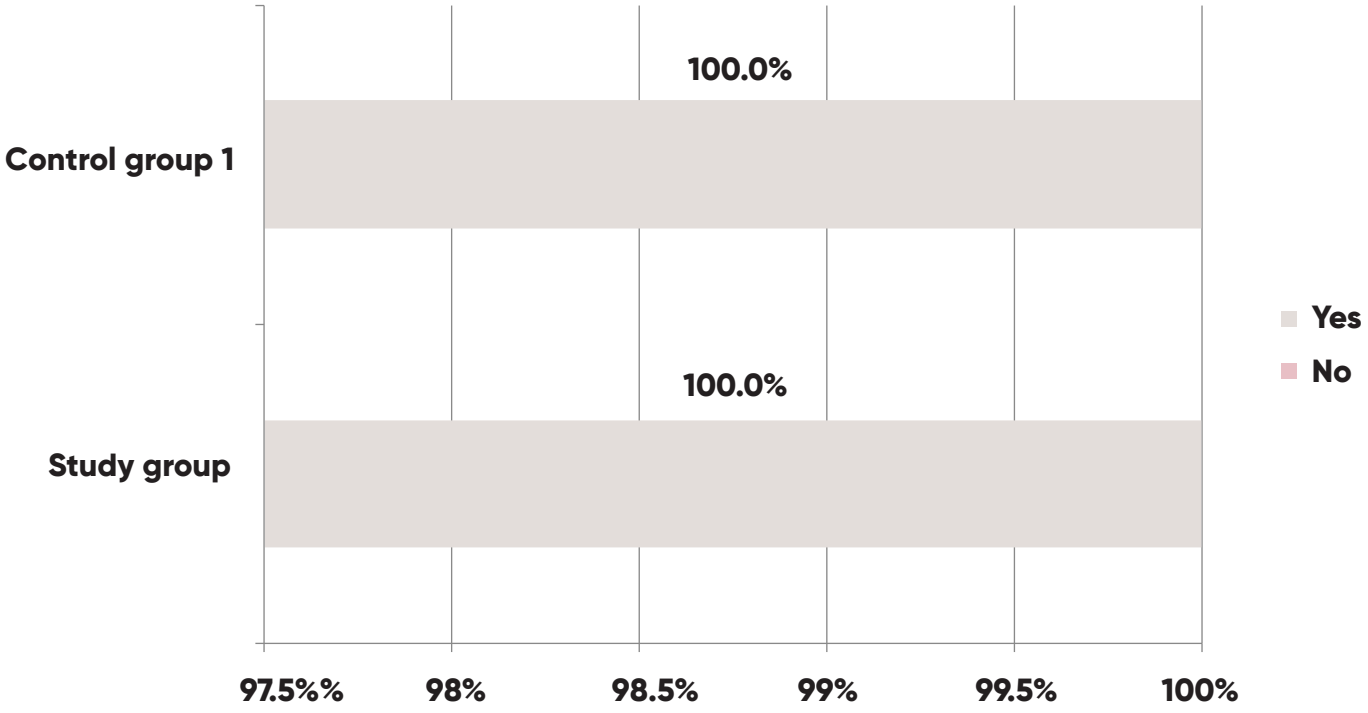


ART indication - tubal factor	Study group	Control group 1
Yes, n(%)	6 (4.9%)	4 (3.0%)
No, n(%)	116 (95.1%)	128 (97.0%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.60	1	0.440	0.528
Likelihood Ratio	0.60	1	0.439	
Fisher's Exact Test Continuity Correction	0.20	1	0.653	

The percentage values of the distribution of patients by ART indication for diminished ovarian reserve in the study group and control group 1 are given in the Chart 26. The difference between data was nonsignificant (p=0.499).

Chart 26. The percentage values of the distribution of patients by ART indicated by a diminished ovarian reserve in the groups.

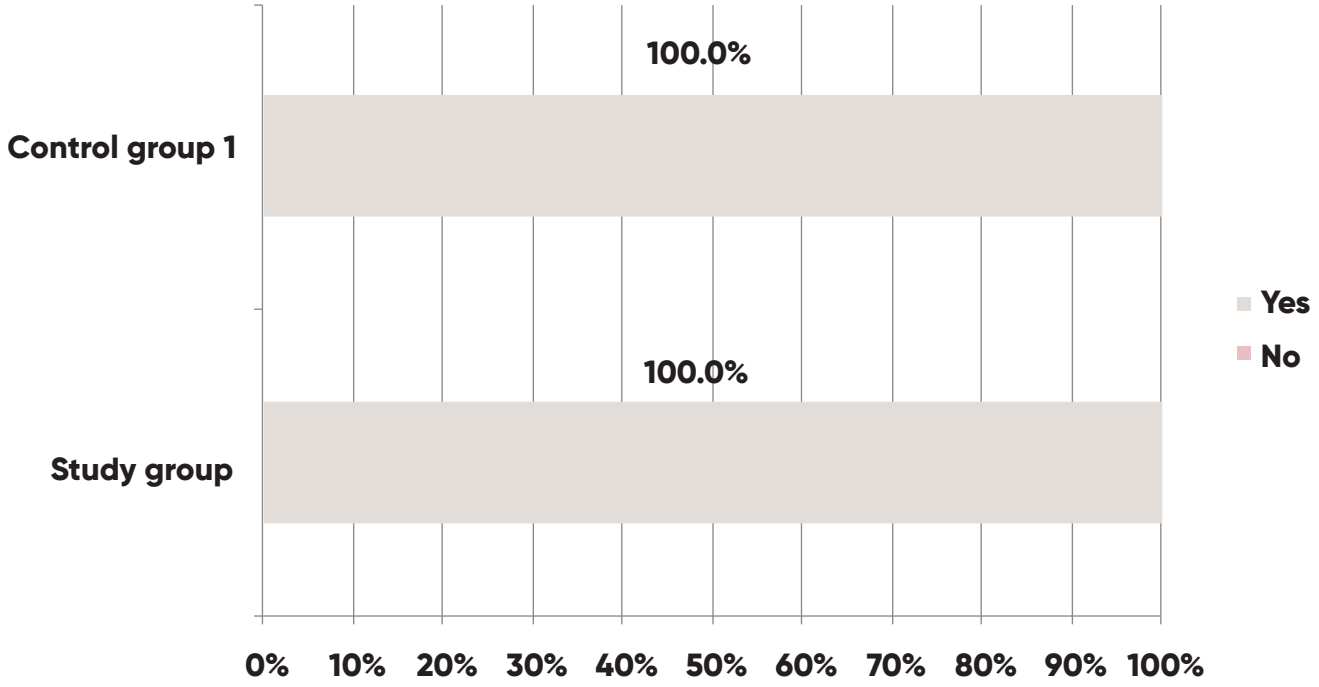


ART indication - diminished ovarian reserve	Study group	Control group 1
Yes, n(%)	122 (100.0%)	132 (100.0%)
No, n(%)	0 (0.0%)	0 (0.0%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.00	1	1.000	1.000
Likelihood Ratio	0.00	1	1.000	
Fisher's Exact Test Continuity Correction	0.00	1	1.000	

The percentage values of the distribution of patients by ART indication for RIF (>2 IVF cycles) in the study group and control group 1 are given in the Chart 27. The difference between data was significant (p=0.003).

Chart 27. The percentage values of the distribution of patients by ART indication for RIF (>2 IVF cycles) in the groups.

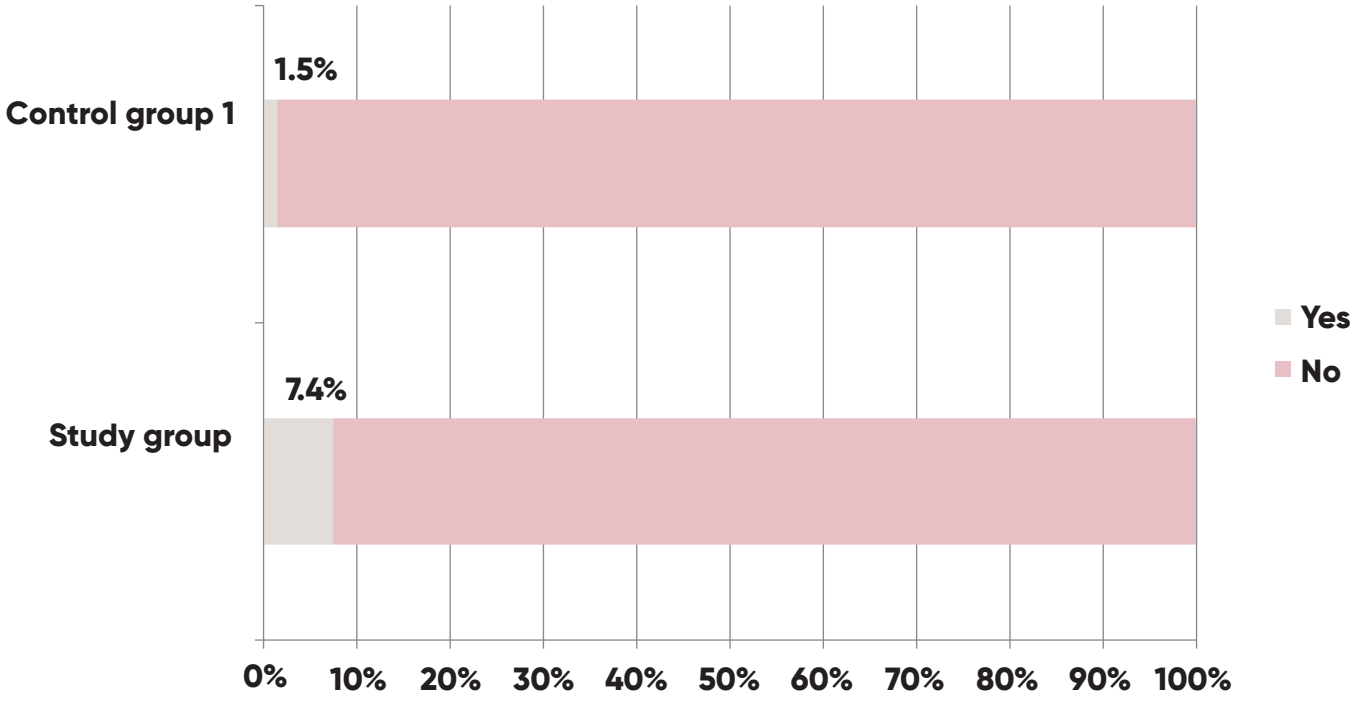


ART indication - RIF (>2 IVF cycles)	Study group	Control group 1
Yes, n(%)	122 (100.0%)	132 (100.0%)
No, n(%)	0 (0.0%)	0 (0.0%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.00	1	1.000	1.000
Likelihood Ratio	0.00	1	1.000	
Fisher's Exact Test Continuity Correction	0.00	1	1.000	

The percentage values of the distribution of patients by ART indication for RPL (>2) in the study group and control group 1 are given in the Chart 28. The difference between data was significant (p=0.029).

Chart 28. The percentage values of the distribution of patients by ART indication for RPL (>2) in the groups.

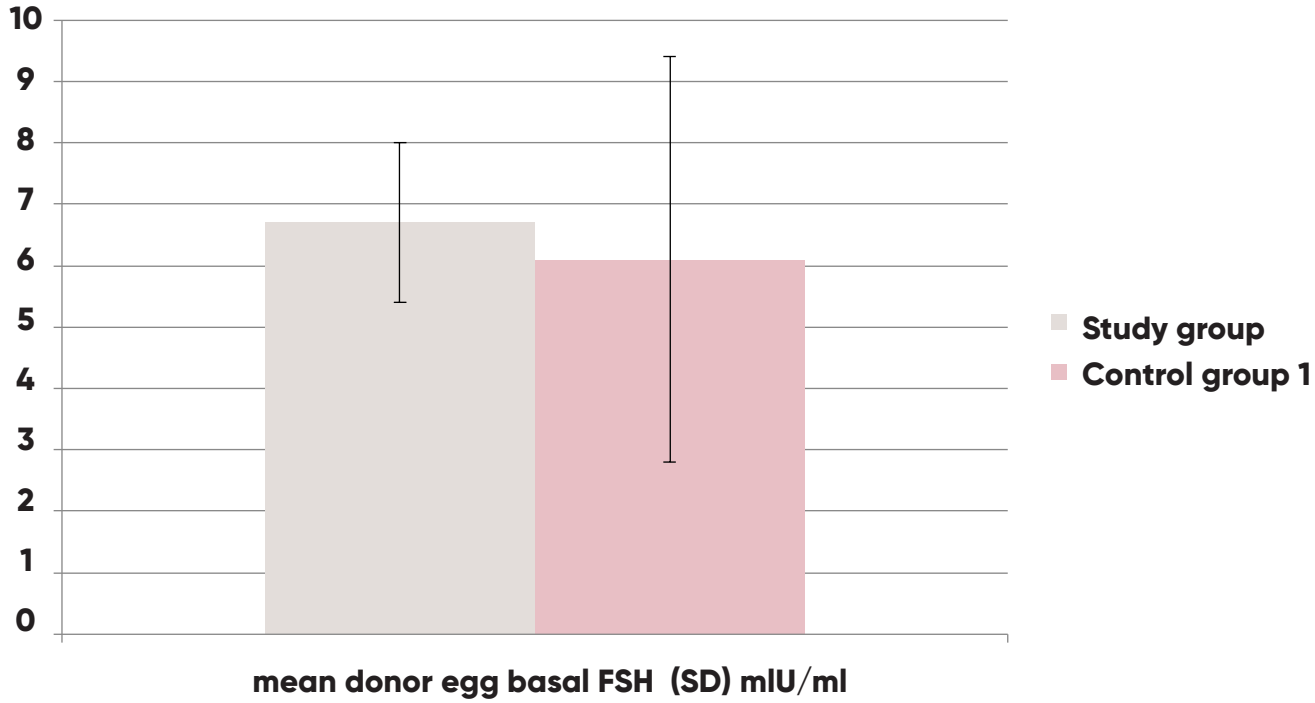


ART indication - RPL (>2)		Study group		Control group 1
Yes, n(%)		9 (7.4%)		2 (1.5%)
No, n(%)		113 (92.6%)		130 (98.5%)
Chi-square tests	**Value**	**df**	**P - Asymp. Sig. (2- tailed)**	**P - Exact Sig. (2- tailed)**
Pearson Chi- Square	5.26	1	0.022	0.029
Likelihood Ratio	5.61	1	0.018	
Fisher's Exact Test Continuity Correction	3.94	1	0.047	

4.2.6 Current ART

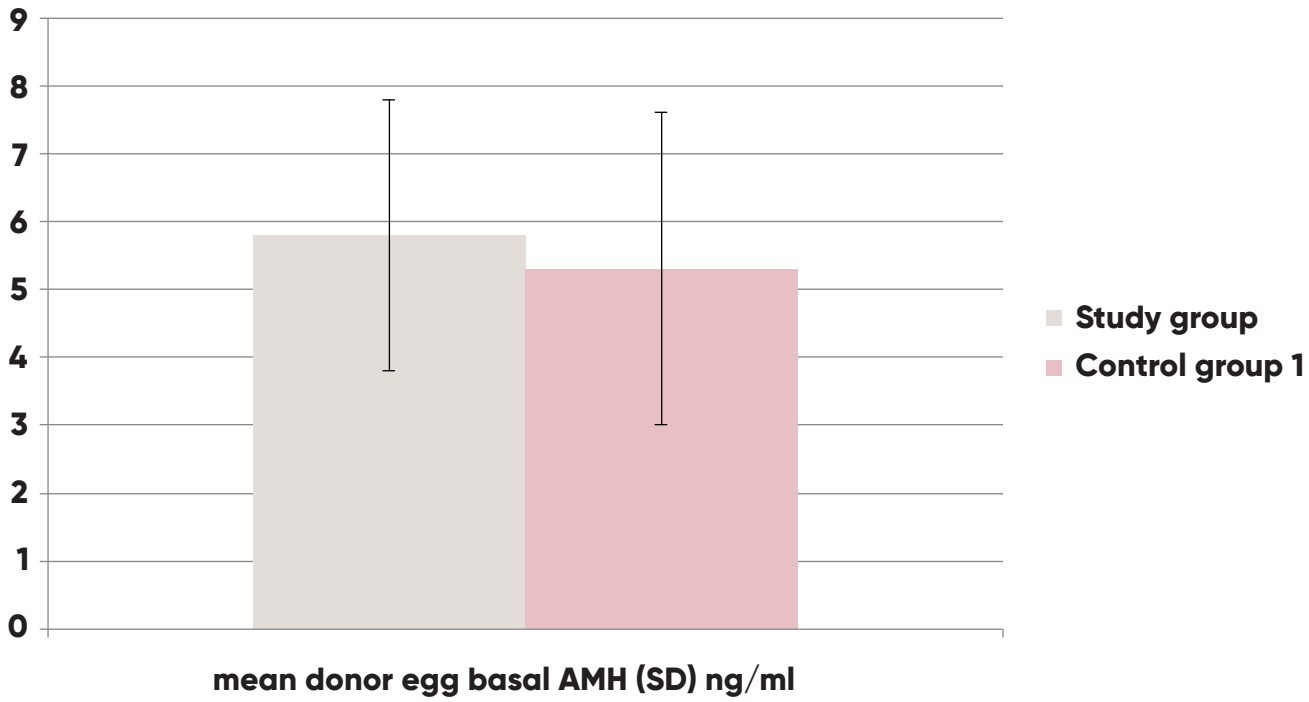
The basal FSH, AMH and AFC of the egg donors and sperm concentrations have been measured in both groups. Mean values of these parameters are given in the chart 29-32. The difference of these values between the groups was not significant (p>0.05).

Chart 29. The mean egg donor basal FSH concentrations in the groups



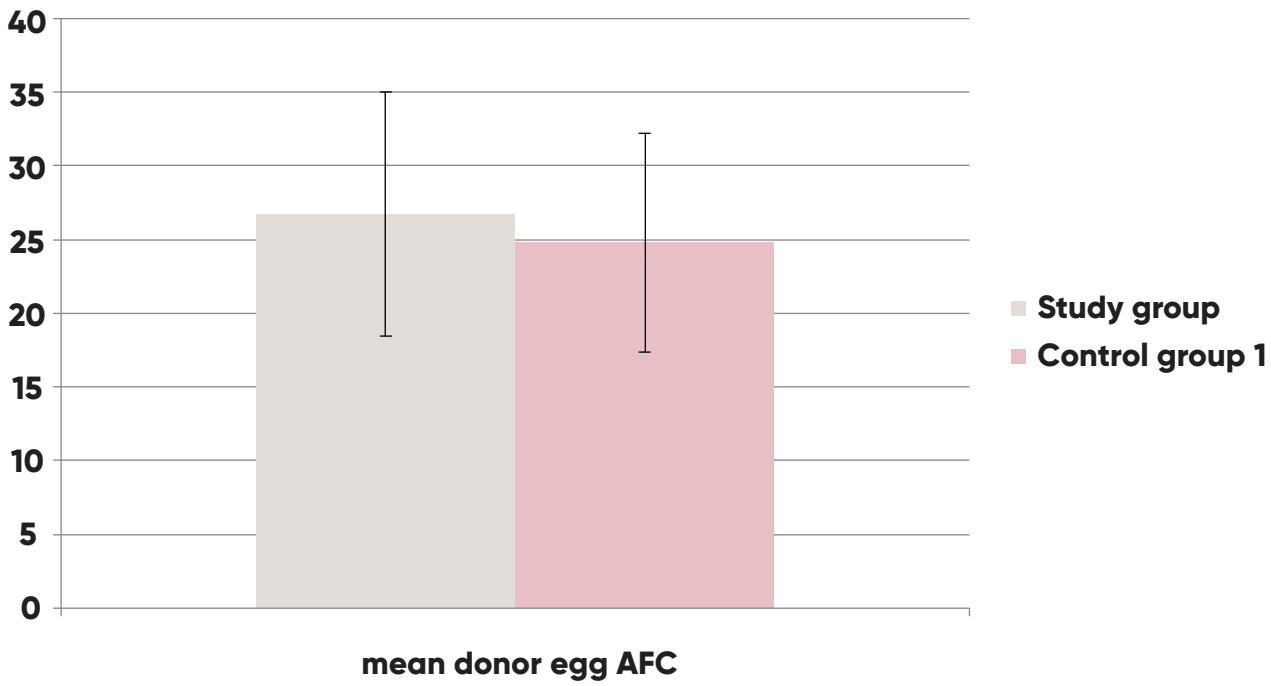
	Study group	Control group 1
Mean donor egg basal FSH (SD), mIU/ml	6.7 (1.3)	6.1 (3.3)
Levene's Test for Equality of Variances		
F-test=, p=	32.20, <0.001	
t-test for Equality of Means		
independent t-test=, p=	1.76, 0.079	

Chart 30. The mean egg donor basal AMH concentrations in the groups



	Study group	Control group 1
Mean donor egg basal AMH (SD), ng/ml	5.8 (2.0)	5.3 (2.3)
Levene's Test for Equality of Variances		
F-test=, p=	2.67, 0.104	
t-test for Equality of Means		
independent t-test=, p=	1.86, 0.064	

Chart 31. The mean egg donor AFC values in the groups



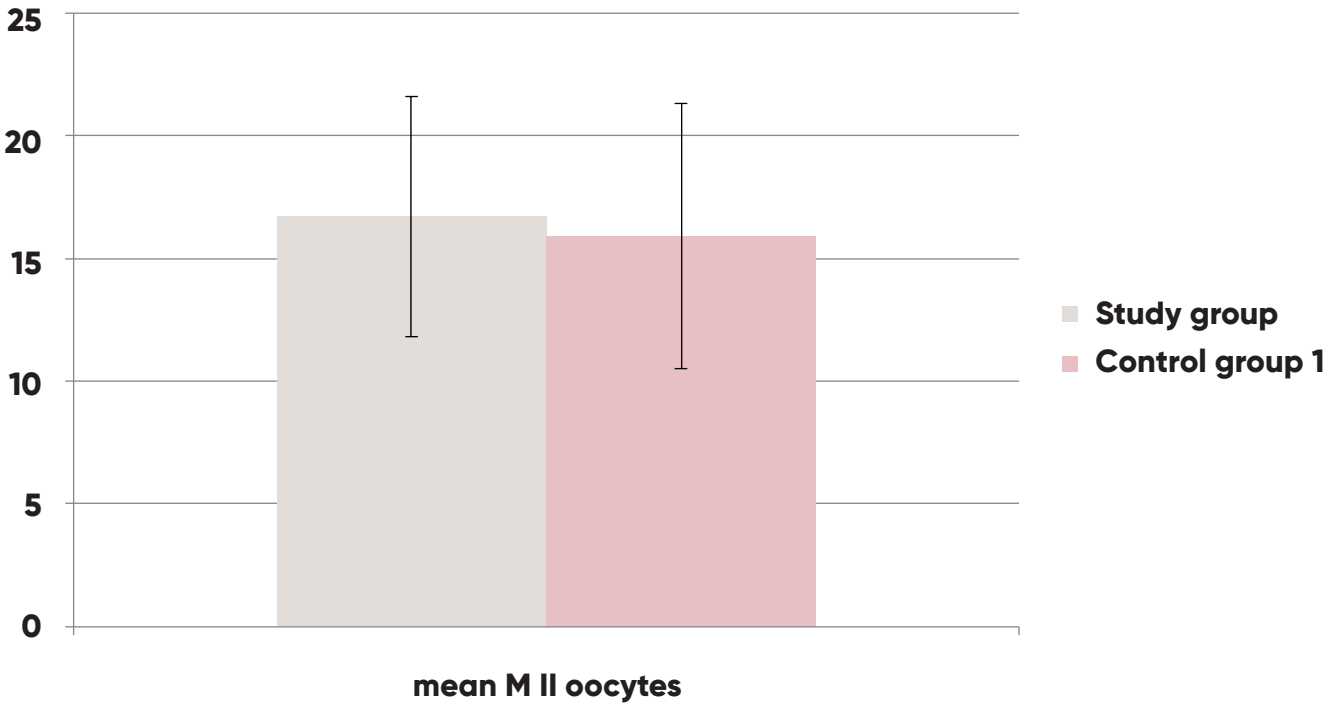
	Study group	Control group 1
Mean donor egg AFC (SD)	26.7 (8.3)	24.8 (7.4)
Levene's Test for Equality of Variances		
F-test=, p=	1.91, 0.168	
t-test for Equality of Means		
independent t-test=, p=	1.99, 0.048	

Chart 32. The mean sperm concentrations in the groups

	Study group	Control group 1
Mean sperm concentration (SD), mln	82.3 (51.7)	75.1 (45.1)
Levene's Test for Equality of Variances		
F-test=, p=	6.41, 0.012	
t-test for Equality of Means		
independent t-test=, p=	1.19, 0.235	

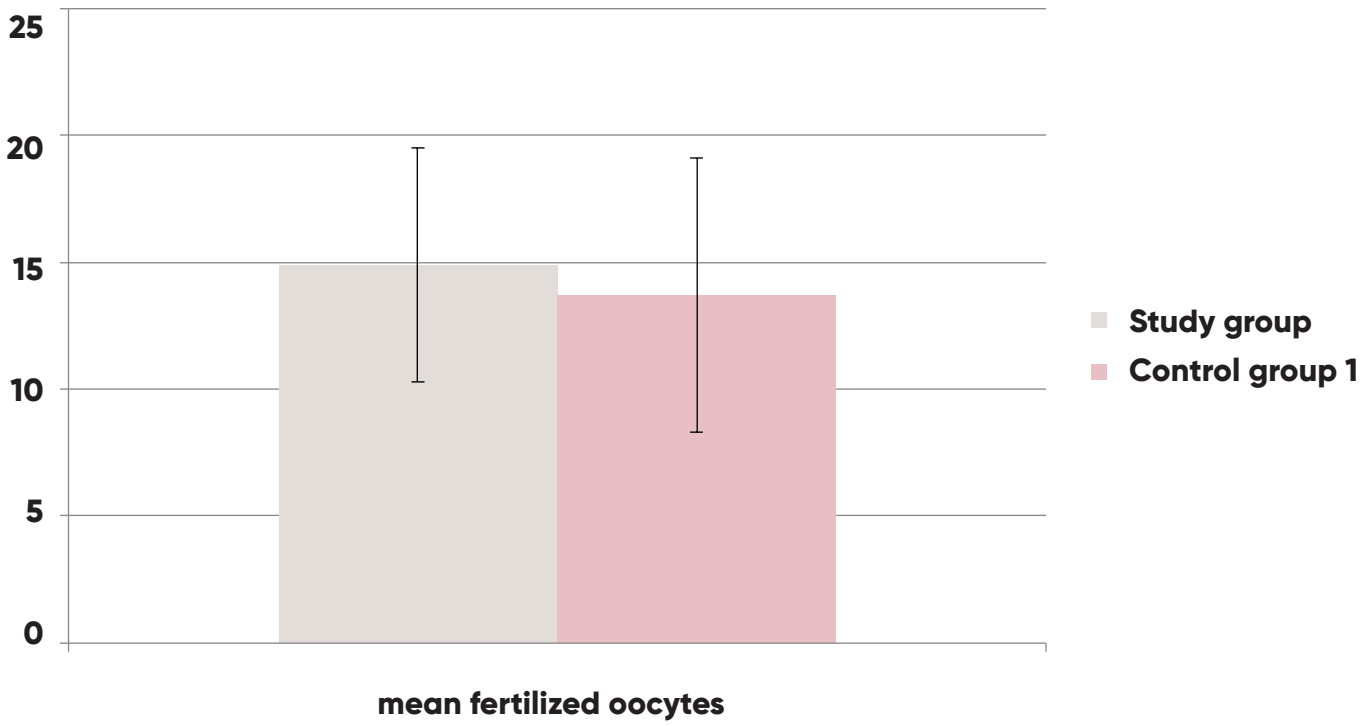
M II oocytes and fertilized oocytes of the egg donors have been measured and fertilization rate have been calculated in both groups. Mean values of these parameters are given n the Chart 33-35. The difference of these values between the groups was not significant (p>0.05).

Chart 33. The mean M II oocytes values in the groups



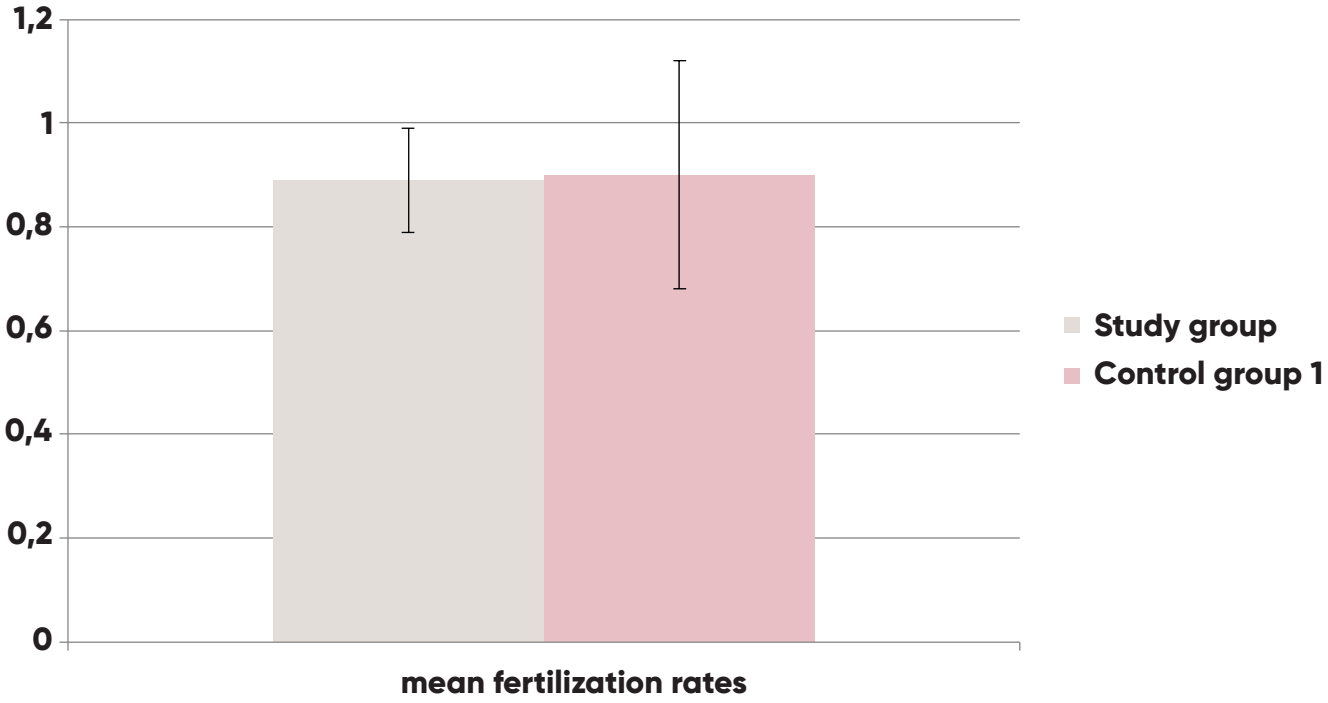
	Study group	Control group 1
Mean M II oocytes (SD)	16.7 (4.9)	15.9 (5.4)
Levene's Test for Equality of Variances		
F-test=, p=	1.37, 0.243	
t-test for Equality of Means		
independent t-test=, p=	1.29, 0.200	

Chart 34. The mean fertilized oocyte values in the groups



	Study group	Control group 1
Mean fertilized oocytes (SD)	14.9 (4.6)	13.7 (5.4)
Levene's Test for Equality of Variances		
F-test=, p=	4.62, 0.033	
t-test for Equality of Means		
independent t-test=, p=	1.94, 0.053	

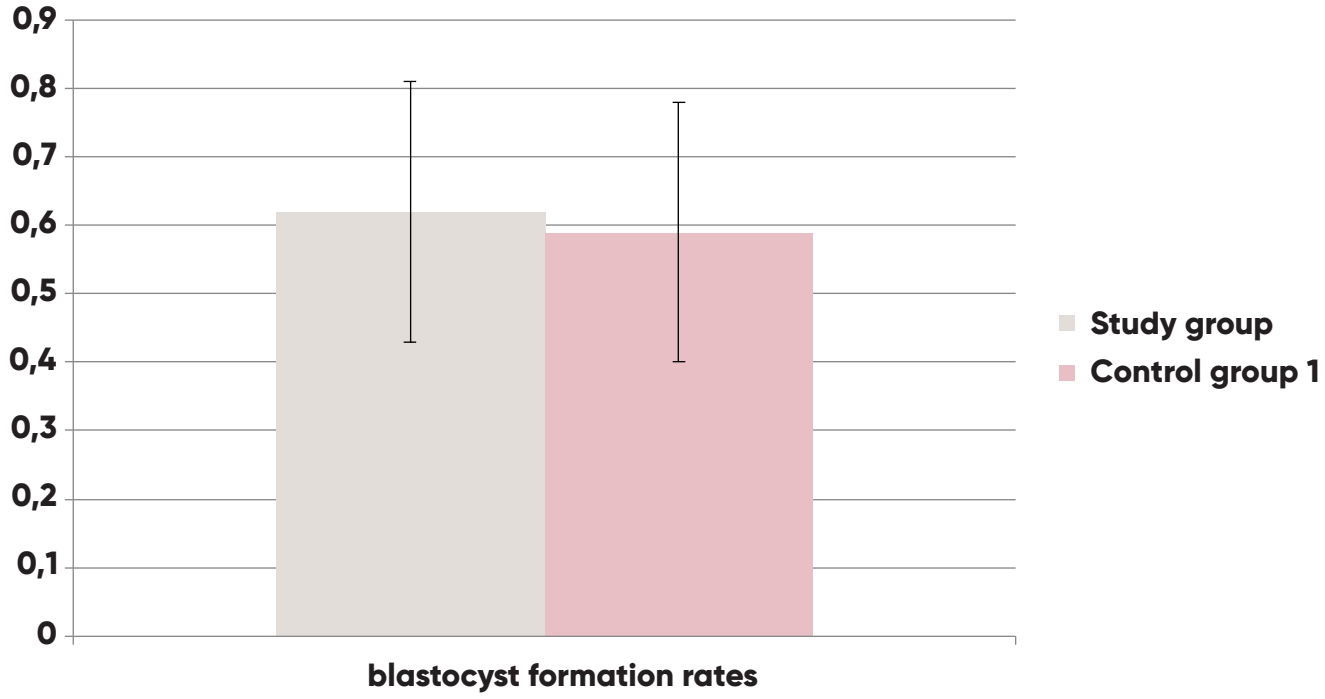
Chart 35. The mean fertilization rates in the groups



	Study group	Control group 1
Mean fertilization rates (SD)	0.89 (0.10)	0.90 (0.22)
Levene's Test for Equality of Variances		
F-test=, p=	1.37, 0.243	
t-test for Equality of Means		
independent t-test=, p=	1.29, 0.198	

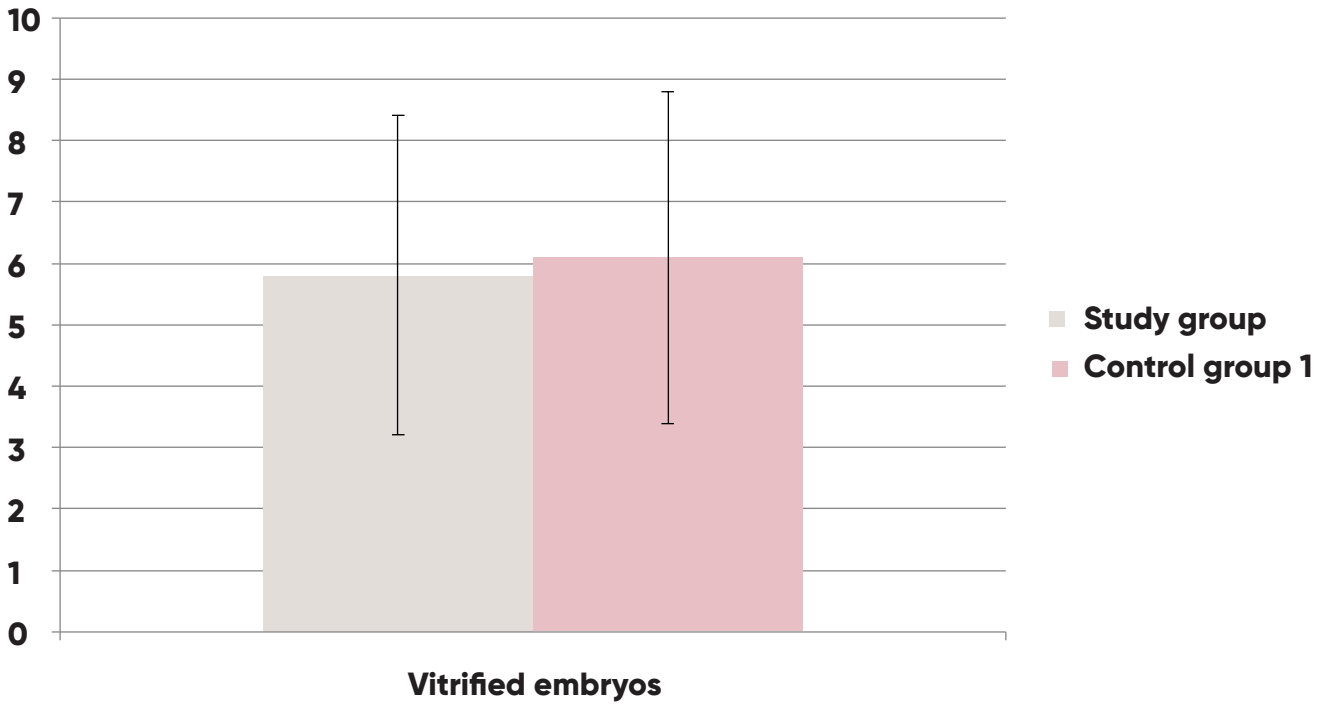
The number of via blastocyst formation rate and vitrified embryos have been measured, as well as PGT-A analyzed embryos have been calculated in both groups. The mean values of these parameters are given in the chart 36-38. The difference in these values between the groups was not significant (p>0.05).

Chart 36. The mean blastocyst formation rates in the groups



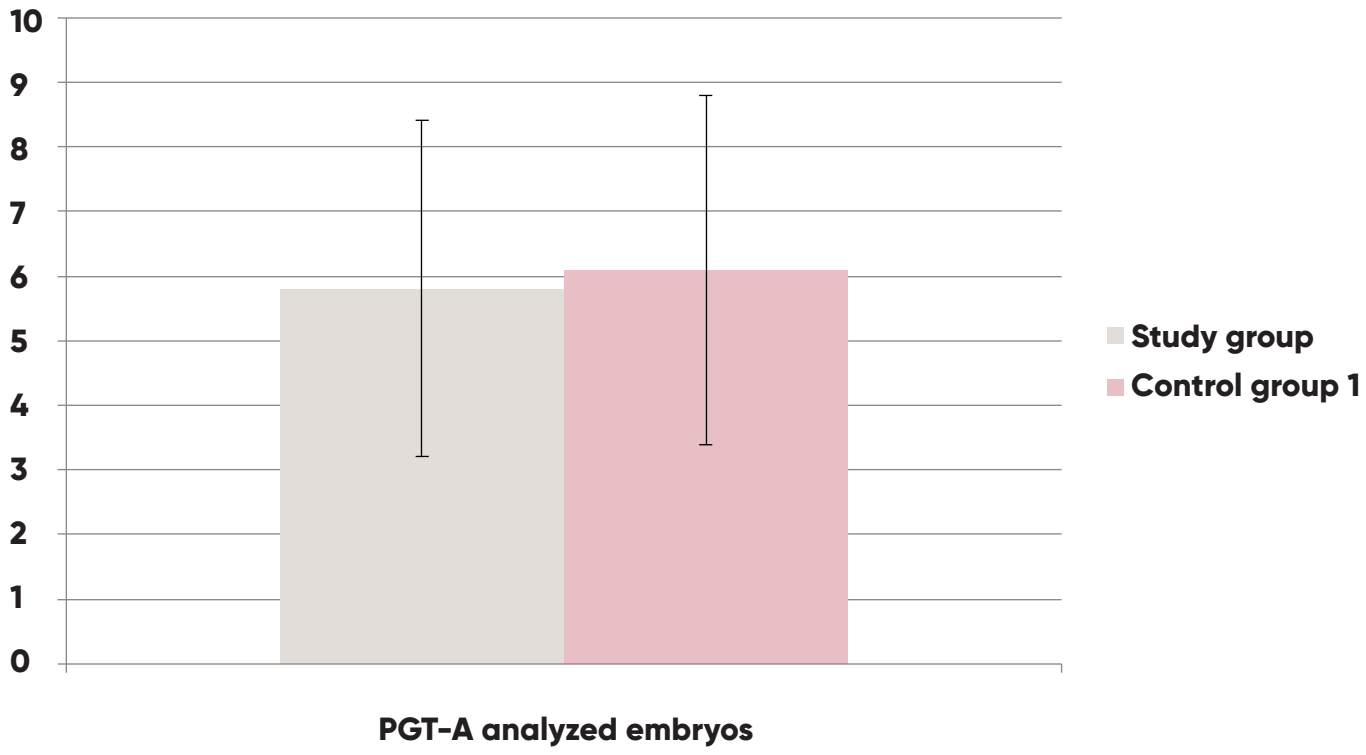
	Study group	Control group 1
Mean blastocyst formation rates (SD)	0.62 (0.19)	0.59 (0.19)
Levene's Test for Equality of Variances		
F-test=, p=	1.21, 0.273	
t-test for Equality of Means		
independent t-test=, p=	1.08, 0.280	

Chart 37. The mean vitrified embryos in the groups



	Study group	Control group 1
Mean vitrified embryos (SD)	9.1 (4.0)	7.7 (4.9)
Levene's Test for Equality of Variances		
F-test=, p=	1.73, 0.091	
t-test for Equality of Means		
independent t-test=, p=	1.78, 0.085	

Chart 38. The mean PGT-A analyzed embryos in the groups

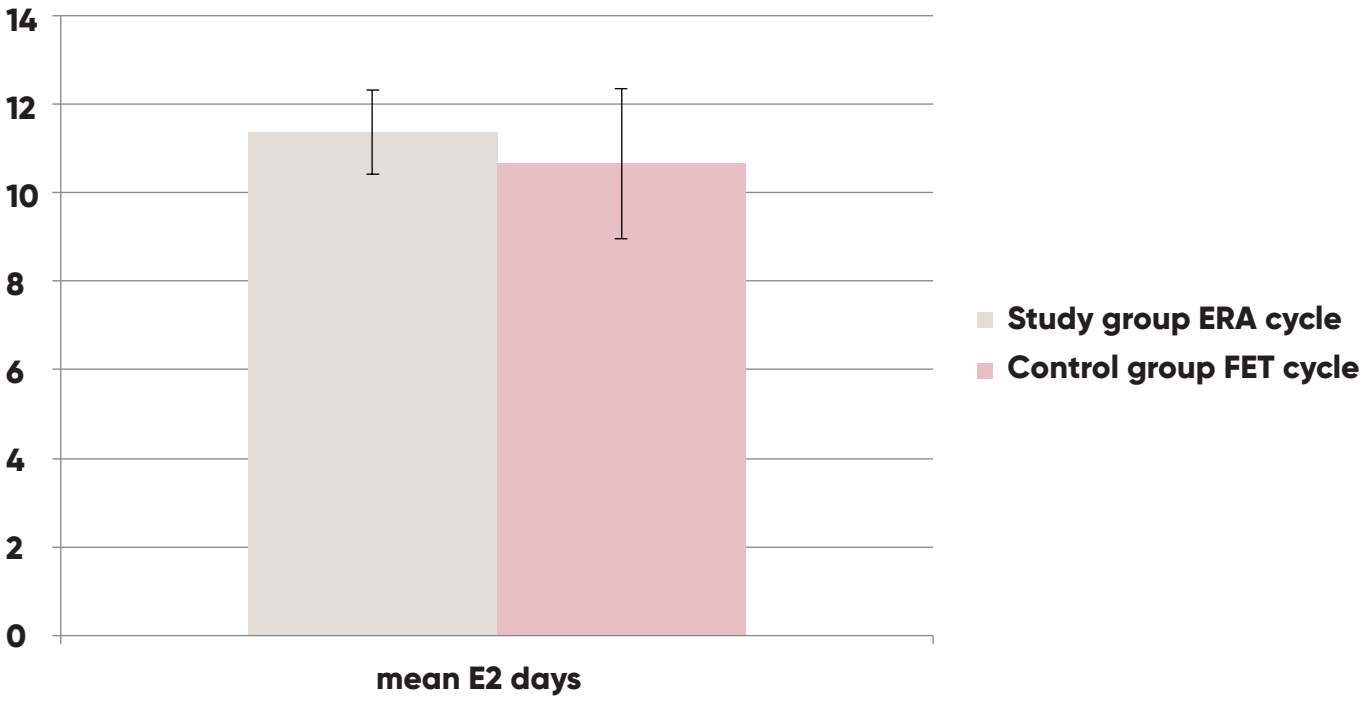


	Study group	Control group 1
Mean PGTA analyzed embryos (SD)	5.8 (2.6)	6.1 (2.7)
Levene's Test for Equality of Variances		
F-test=, p=	0.73, 0.393	
t-test for Equality of Means		
independent t-test=, p=	0.78, 0.439	

4.2.7 ERA cycle

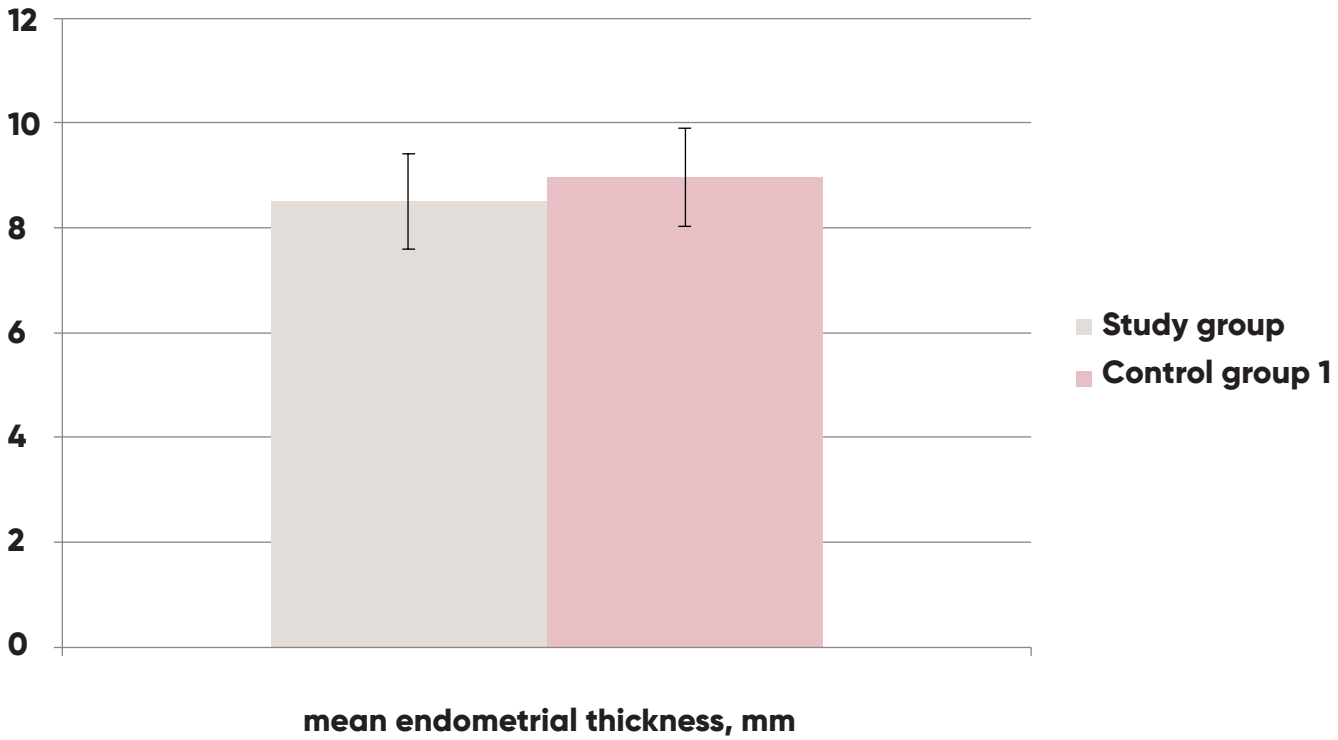
E2 days in ERA cycle have been measured in the study group, and E2 days in FET cycle in the control 1 group. Endometrial thicknesses and P4 levels 24h before starting the exogenous P4 have been also measured in both groups. Total hours from P4 initiation to ERA sample collection have been evaluated in the study group. The mean values of these parameters are given in the Chart 39-41. The difference in these values between the groups was not significant (p>0.001).

Chart 39. The mean E2 days in the study group



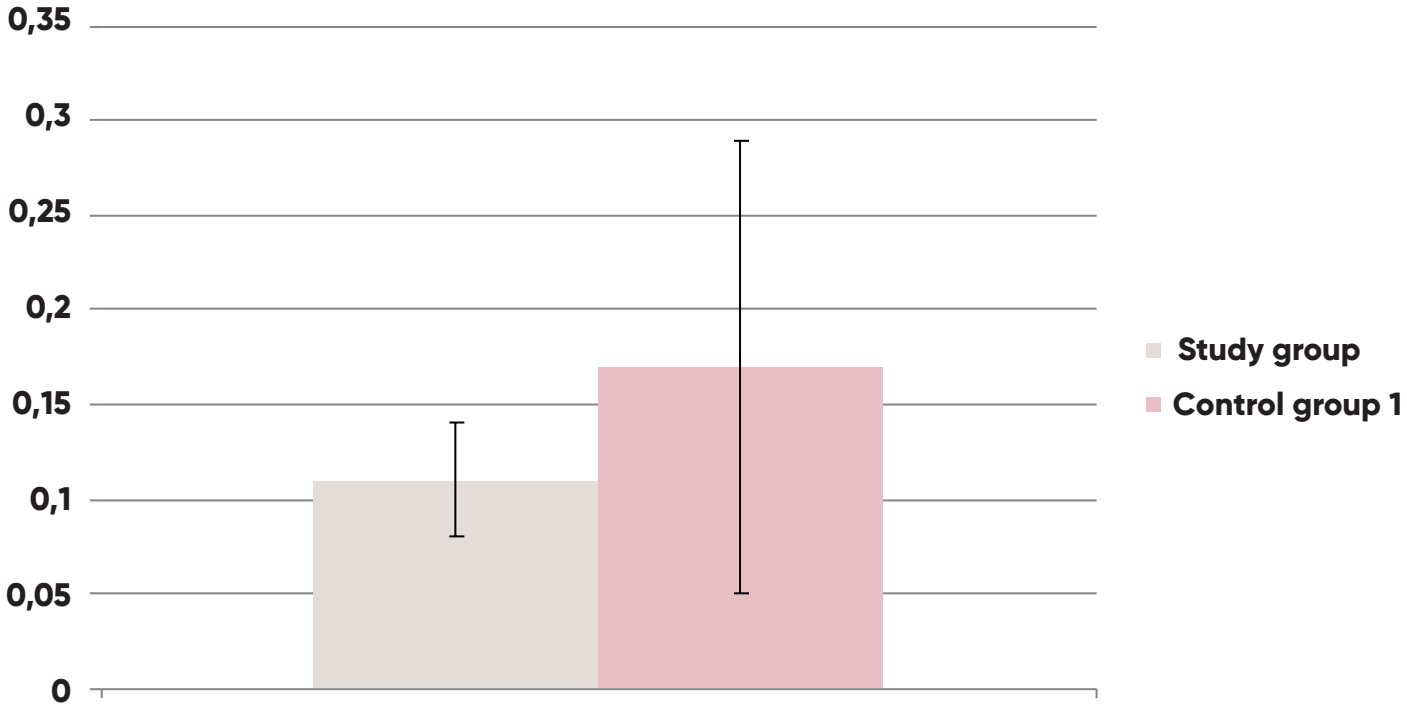
	Study group ERA cycle	Study group FET cycle
Mean E2 days (SD)	11.4 (0.9)	11.3 (0.9)
Levene's Test for Equality of Variances		
F-test=, p=	0.01, 0.979	
t-test for Equality of Means		
independent t-test=, p=	0.01, 0.979	

Chart 40. The mean endometrial thickness in the groups



	Study group ERA cycle	Control group 1 FET cycle
Mean endometrial thickness (SD), mm	8.51 (0.91)	8.96 (0.93)
Levene's Test for Equality of Variances		
F-test=, p=	0.27, 0.602	
t-test for Equality of Means		
independent t-test=, p=	3.90, <0.001	

Chart 41. The mean P4 levels 24h before starting the exogenous P4 in the groups

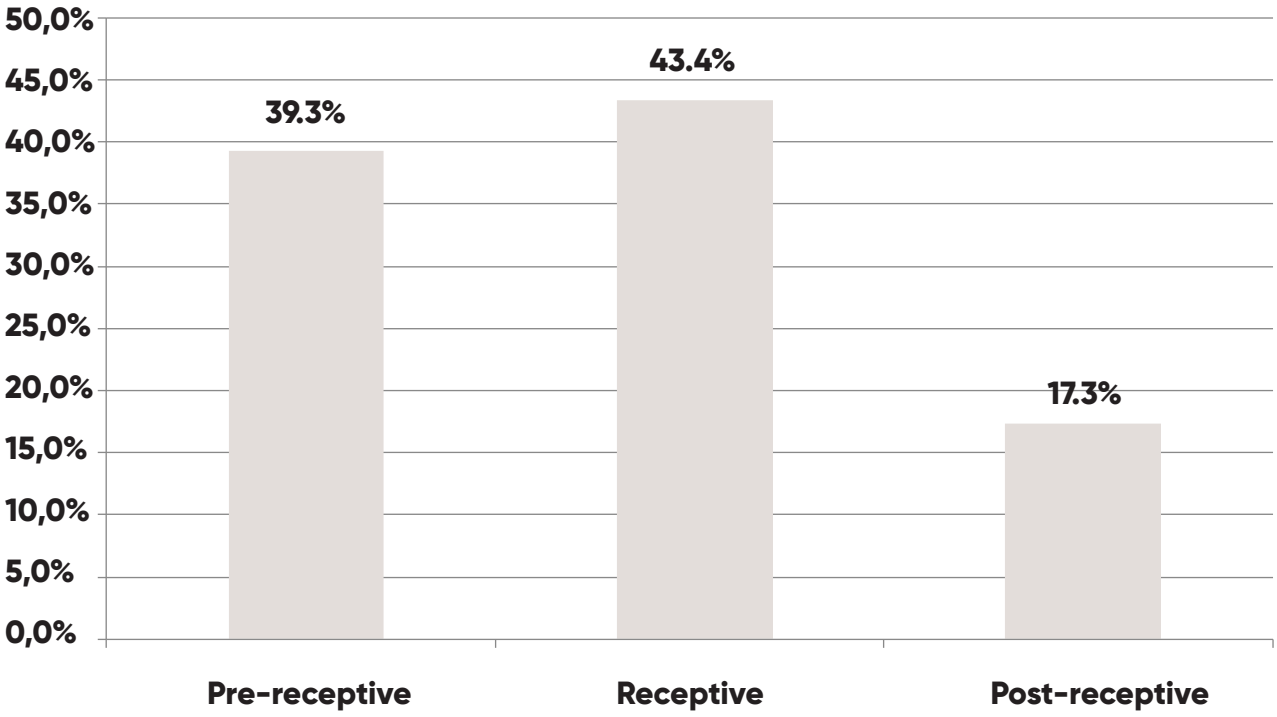


	Study group ERA cycle	Control group 1 FET cycle
Mean P4 level 24h before starting the exogenous P4 (SD), ng/ml	0.11 (0.03)	0.17 (0.12)
Levene's Test for Equality of Variances		
F-test=, p=	178.48, <0.001	
t-test for Equality of Means		
independent t-test=, p=	6.03, <0.001	

4.2.8. ERA result

The ERA results determined as pre-receptive, receptive, and post-receptive in the study group are given on Chart 42. Pre-receptive and receptive conditions were significantly prevailed (p=0.001).

Chart 42. ERA results in the study group.

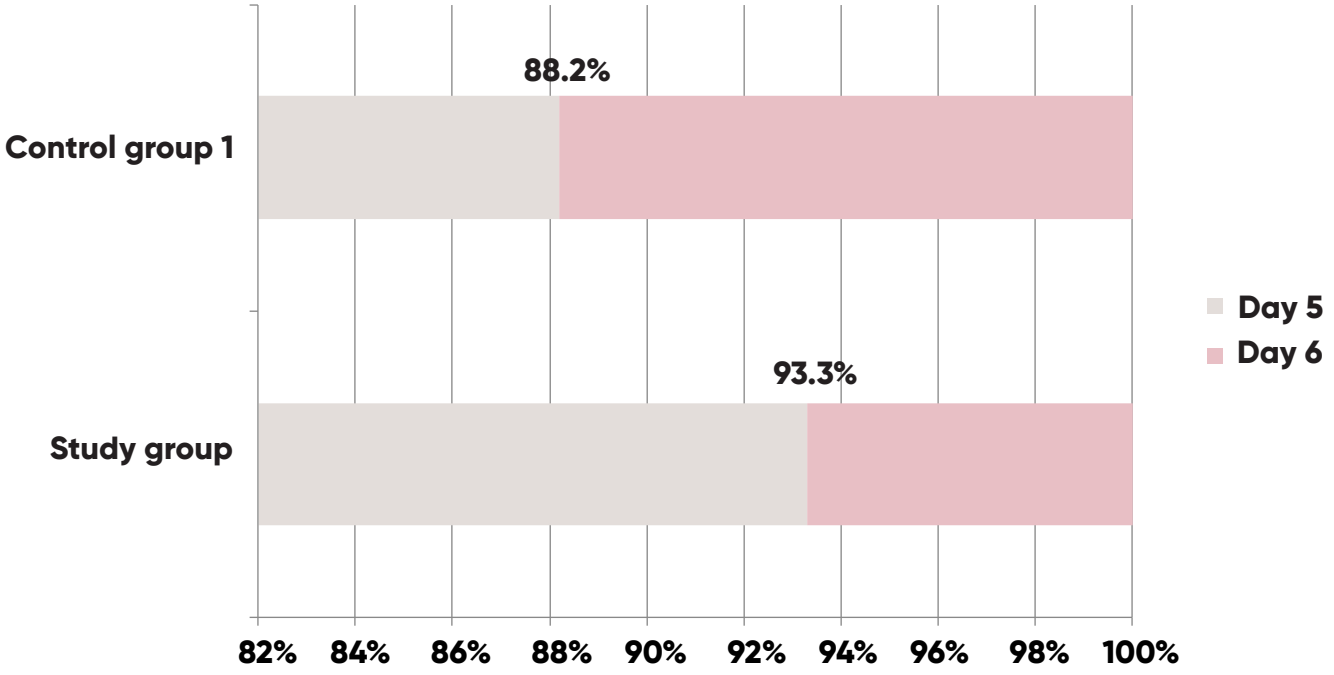


ERA result	Study group	Chi-Square	df	p-value, Asymp. Sig.
Pre-receptive	48 (39.3%)	14.57	2	0.001
Receptive	53 (43.4%)			
Post-receptive	21 (17.3%)			

4.2.9 FET cycle

The total number of developed embryos was 223 in the study group, and 220 – in the control group 1. The data regarding the day of embryo development at FET are given in the Chart 43. The distribution of the percentages of embryos developed on days 5 and 6 revealed the nonsignificant difference between the study and control groups.

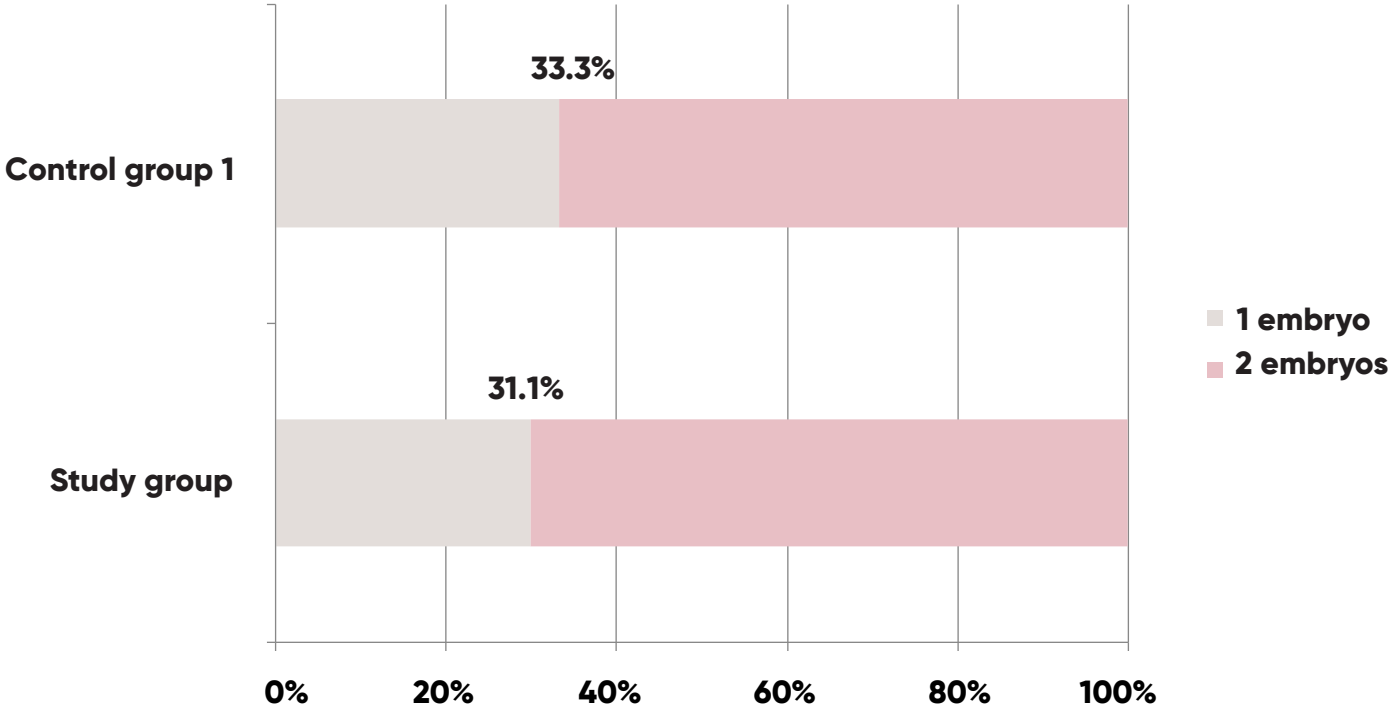
Chart 43. The data of embryo development day at FET in the groups.



Embryo development day at FET		Study group		Control group 1
Day 5, n(%)		208 (93.3%)		194 (88.2%)
Day 6, n(%)		15 (6.7%)		26 (11.8%)
Chi-square tests	**Value**	**df**	**P - Asymp. Sig. (2- tailed)**	**P - Exact Sig. (2- tailed)**
Pearson Chi- Square	3.41	1	0.061	0.065
Likelihood Ratio	3.42	1	0.061	
Fisher's Exact Test Continuity Correction	2.65	1	0.071	

The total number of transferred embryos was 114 in the study group, and 78 – in the control group 1. The data of embryos transferred by the quantity per patient at FET are given in the Chart 44. The distribution of percentages revealed the nonsignificant difference between the study and control groups.

Chart 44. The data of transferred embryos at FET in the groups.



Transferred embryos perpatient at FET		Study group		Control group 1
1 embryo, n(%)		38 (31.1%)		44 (33.3%)
2 embryos, n(%)		84 (68.9%)		88 (66.7%)
Chi-square tests	**Value**	**df**	**P - Asymp. Sig. (2- tailed)**	**P - Exact Sig. (2- tailed)**
Pearson Chi- Square	0.14	1	0.710	0.724
Likelihood Ratio	0.12	1	0.708	
Fisher's Exact Test Continuity Correction	0.08	1	0.735	

4.2.10 ART outcome

The pregnancy rates, defined by beta-hCG value, in the study and control groups are given in Chart 45. Odds ratio of the pregnancy in the study group vs. control group 1 was OR=2.59 (95%CI - 1.50-4.49). This value was significant (p=0.001).
The implantation rate in the study and control groups are given on Chart 46. Number of implanted embryos was 114 (54.1%) in the study group, and 78 – in the control group 1 (35.5%) Odds ratio of the successful implantation in the study group vs. control group 1 was OR=1.90 (95%CI - 1.30-2.79). This value was significant (p=0.001).

Chart 45. The pregnancy rates in the groups.

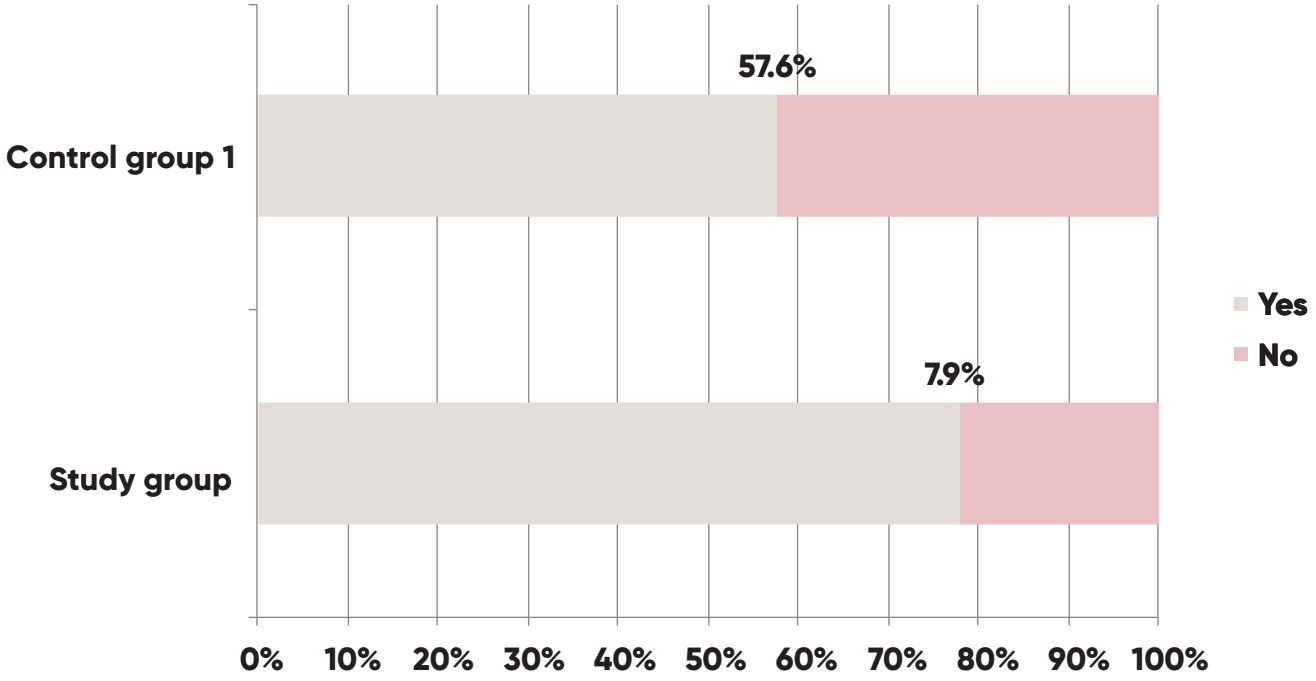
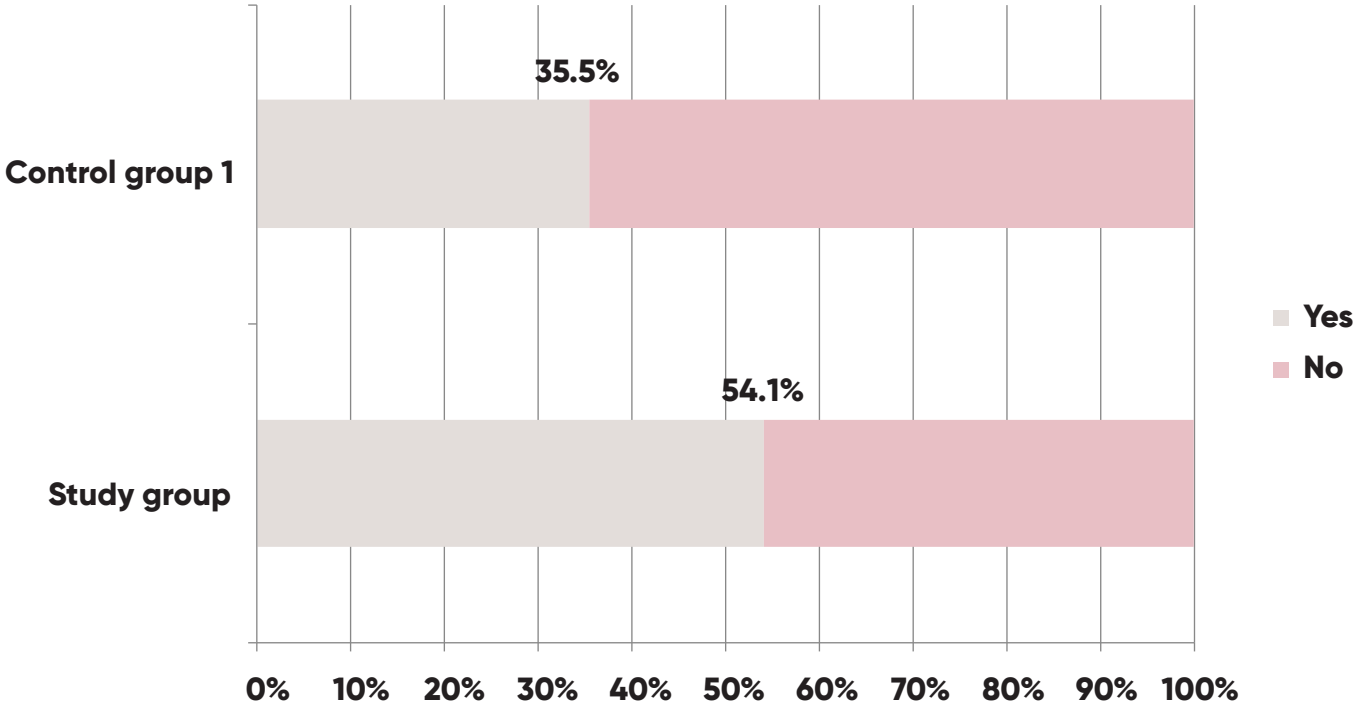
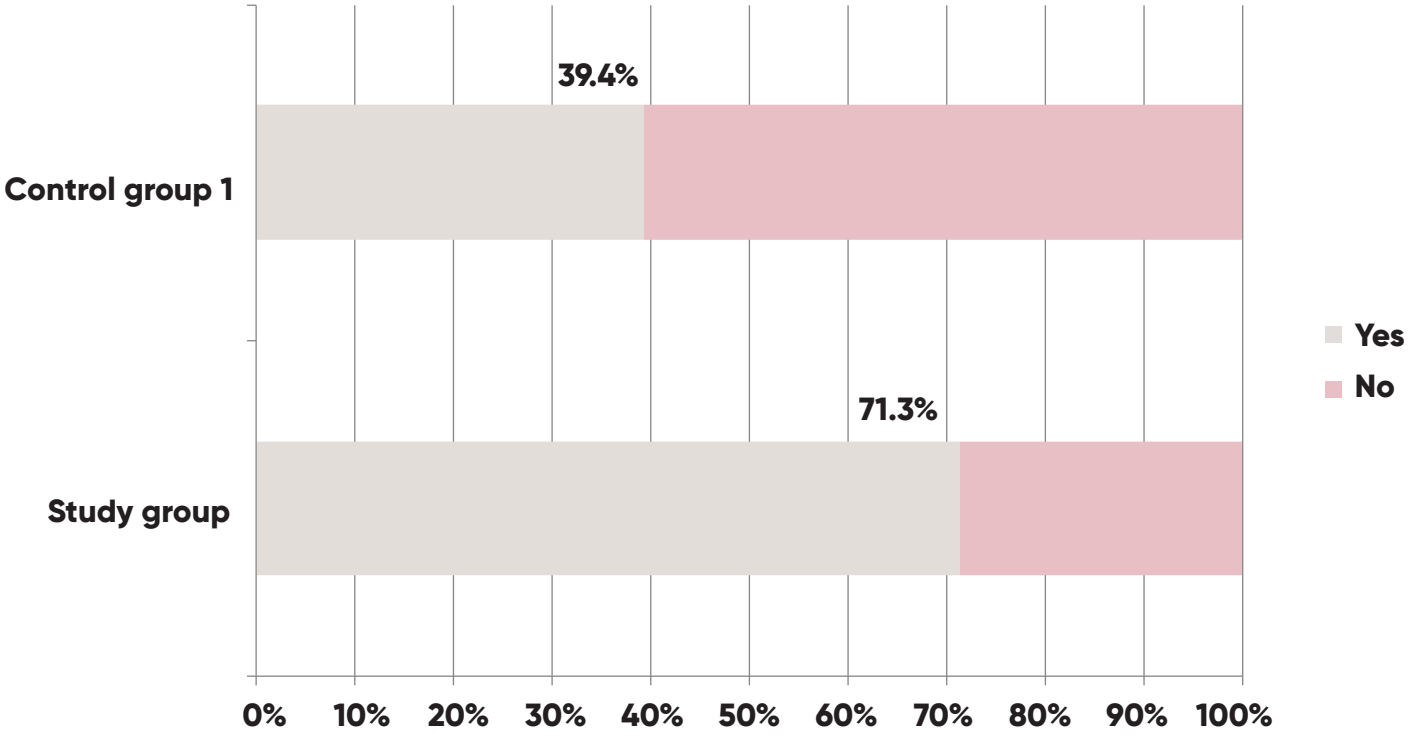


Chart 46. The implantation rates in the groups.



The live birth rate, defined by the successful delivery, in the study and control groups are given in Chart 47. Odds ratio of the Live birth rate in the study group vs. control group 1 was OR=3.82 (95%CI - 2.26-6.47). This value was significant (p<0.001).

Chart 47. The live birth rates in the groups.



The summarized data of reproductive outcomes in the groups are given in Table 8. The pregnancy and obstetrical complications in the groups are given in Table 9.

Table 8. Reproductive Outcomes at First ET during 1-year Follow-up: Intention-to-treat Analysis in the study and control Groups.

Outcome	Study group	Control group 1	study vs. control	
			Odds Ratio (95%CI)	P-value
Transfers, n	122	132		
Pregnancy rate, n (%)	95 (77.9%)	76 (57.6%)	**2.59 (1.50-4.49)**	**0.0007**
Implantation rate, n (%)	114/223 (54.1%)	78/220 (35.5%)	**1.90 (1.30-2.79)**	**0.0009**
Live birth rate n (%)	87 (71.3%)	52 (39.4%)	**3.82 (2.26-6.47)**	**<0.0001**
Singleton	63 (72.4%)	36 (69.3%)	1.17 (0.55-2.48)	0.69
Multiple (all twins)	24 (27.6%)	16 (30.7%)	0.86 (0.40-1.82)	0.69
Clinical miscarriages, n (%)	3/95 (3.2%)	8/76 (10.5%)	0.27 (0.07-1.08)	0.065
Biochemical pregnancies, n (%)	5/95 (5.3%)	10/76 (13.2%)	0.37 (0.12-1.12)	0.090

Table 9. Pregnancy and Obstetrical complications at First ET during 1-year Follow-up: Intention-to-treat Analysis in the study and control Groups.

Outcome	Study group	Control group 1	study vs. control	
			Odds Ratio (95%CI)	
Ectopic pregnancies, n (%)	0/95 (0.0%)	0/76 (0.0%)	N/A	N/A
Neonatal mortality, n (%)	0/95 (0.0%)	0/76 (0.0%)	N/A	N/A
Preeclampsia, n (%)	6/95 (6.3%)	0/76 (0.0%)	N/A	N/A
HBP, n (%)	0/95 (0.0%)	0/76 (0.0%)	N/A	N/A
Hyperemesis	0/95 (0.0%)	0/76 (0.0%)	N/A	N/A
IUGR	0/95 (0.0%)	0/76 (0.0%)	N/A	N/A
Metabolic Disorders	0/95 (0.0%)	0/76 (0.0%)	N/A	N/A
Infections	0/95 (0.0%)	0/76 (0.0%)	N/A	N/A
Gestational Diabetes	0/95 (0.0%)	2/76 (2.6%)	N/A	N/A
1st trimester Bleeding	36/95 (37.9%)	18/76 (23.7%)	1.85 (0.93-3.67)	0.078
3rd trimester Bleeding	0/95 (0.0%)	0/76 (0.0%)	N/A	N/A

4.3 The comparative analysis of PGT-A+ERA age group 35years vs. PGT-A+ERA age group <35years

4.3.1 Baseline demographic and clinical characteristics

Baseline demographics and clinical characteristics varied among the groups as it was expected according to the protocol. A comparison of reproductive parameters means such as basal FSH, AMH, AFC, and blastocyst formation rate showed a statistically significant difference between the study group and the control group 2. Between the parameters of sperm concentration and fertilization rate was not found to be a statistically significant difference **(Table 10).**

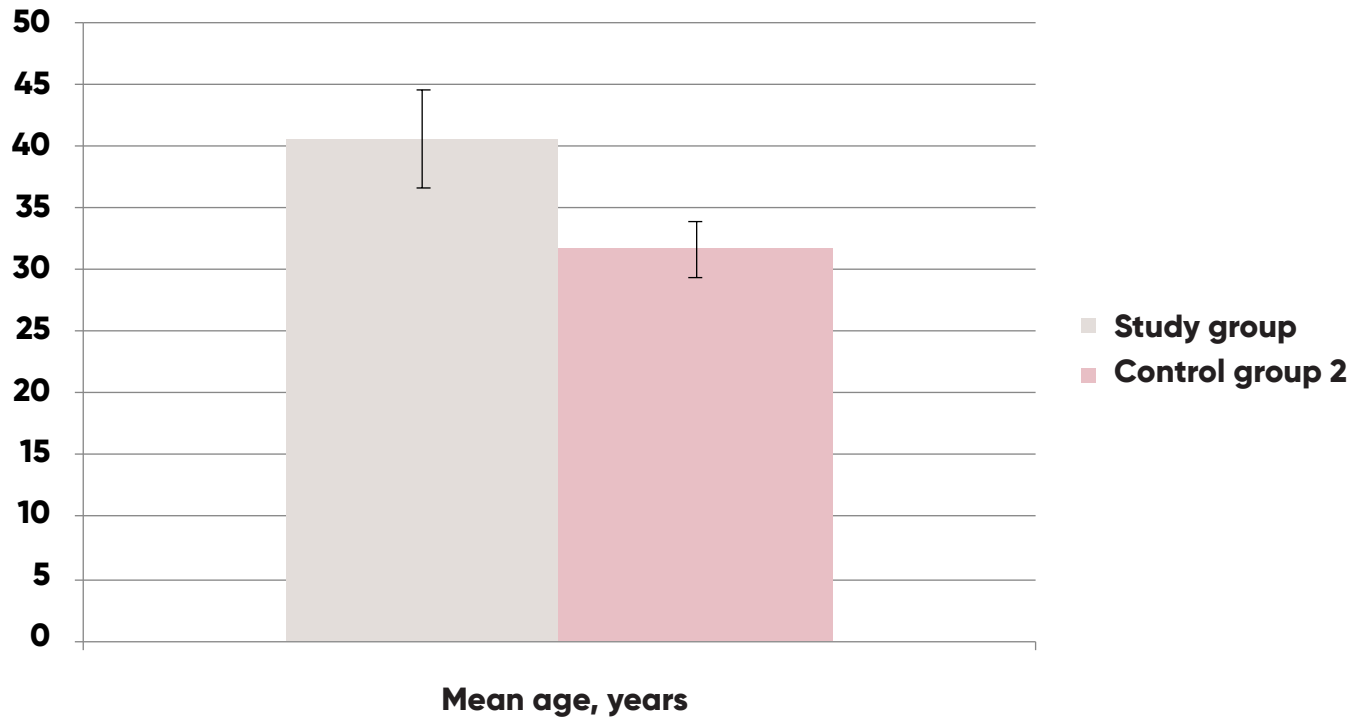
Table 10. Reproductive Parameters Comparison of means the study PGT-A+ERA Groups according to patient age

Parameter	PGT-A+ERA Age≥35years (n=122)	PGT-A+ERA Age<35years (n=44)	PGT-A+ERA vs. PGT-A	
	Mean ± SD	Mean± SD	Independent t-test	P-value
Egg Donor Age	23.4±3.7	28 ± 4.2	**13.96**	**0.001**
Egg Donor Basal FSH	6.7 ±1.3		**2.478**	**0.014**
Patient Basal FSH		7.3 ± 1.5		
Egg Donor AMH	5.8 ± 2.0		**10.026**	**<0.001**
Patient AMH		2.3± 1.8		
Sperm concentration	82.3 ± 51.7	71.8 ± 58.4	1.098	0.274
Egg Donor AFC	26.6 ± 8.4		**8.130**	**<0.001**
Patient AFC		14.8 ± 7.2		
Egg Donor M II	16.7 ± 4.9		**5.543**	**<0.001**
Patient M II		11.6 ± 5.8		
Fertilization rate - fertilized oocytes/M II	0.89 ± 0.10	0.89 ± 0.08	0.059	0.953
Blastocyst formation rate - Vitrified embryos/fertilized oocytes	0.62± 0.19	0.50 ± 0.14	**3.754**	**<0.001**

FSH - Follicle-stimulating hormone; AMH - Anti-Müllerian hormone; AFC - Antral follicle count; MII - Metaphase II; PGT-A - Preimplantation genetic testing for aneuploidy; ERA - Endometrial receptivity analysis; Standard deviation (SD)

The values of mean ages of patients in the study group (Age> 35 years) and control group 2 (Age< 35 years) are given in Chart 48.

Chart 48. The values of the mean ages of patients in the groups

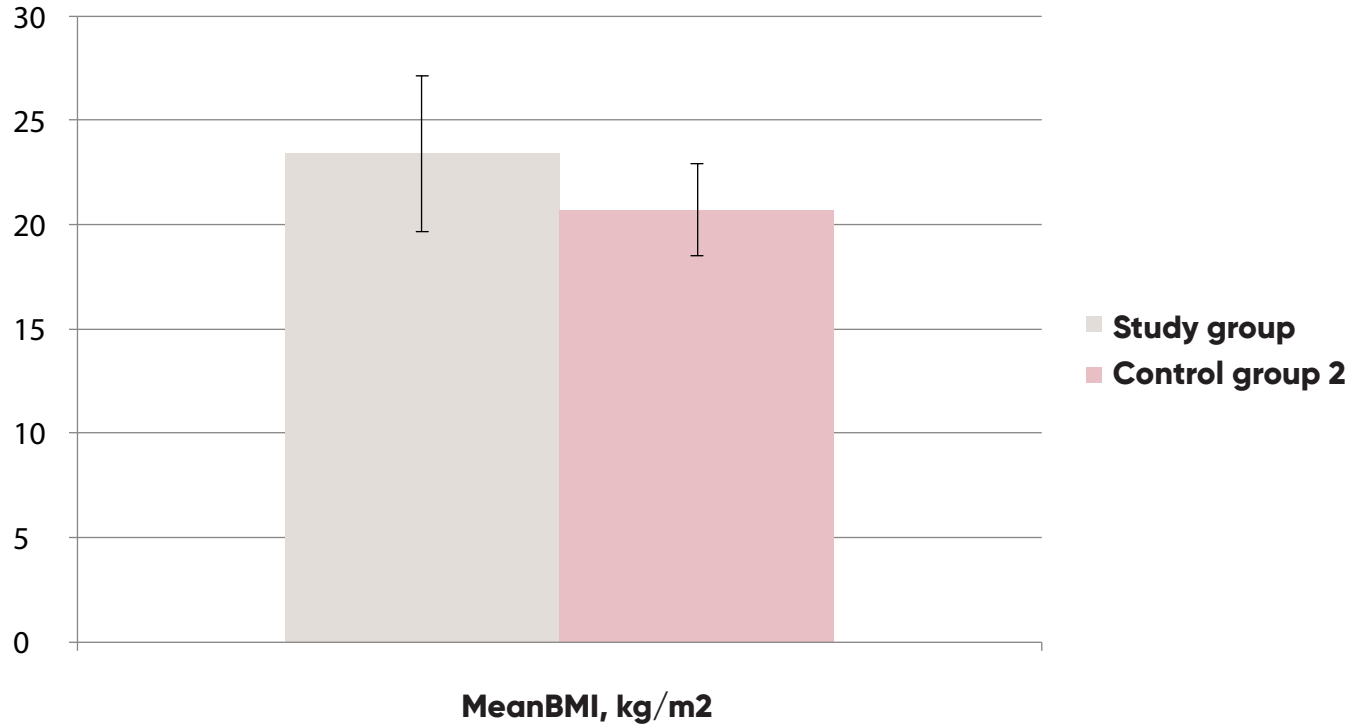


	Study group	Control group 2
Mean Age (SD), years	40.5 (4.0)	31.6 (2.2)
Levene's Test for Equality of Variances		
F-test=, p=	12.44, <0.001	
t-test for Equality of Means		
independent t-test=, p=	11.85, <0.001	

Statistically significant age differences were identified between the study group and control group 2, as outlined in the procedure.

The values of mean BMIs of patients in the study group and control group 2 are given in Chart 49.

Chart 49. The values of mean BMIs of patients in the groups



	Study group	Control group 2
Mean BMI (SD), kg/m²	23.4 (3.7)	20.7 (2.2)
Levene's Test for Equality of Variances		
F-test=, p=	8.81, <0.001	
t-test for Equality of Means		
independent t-test=, p=	7.92, <0.001	

There was no statistically significant difference in BMI parameters.

4.3.2 Personal history

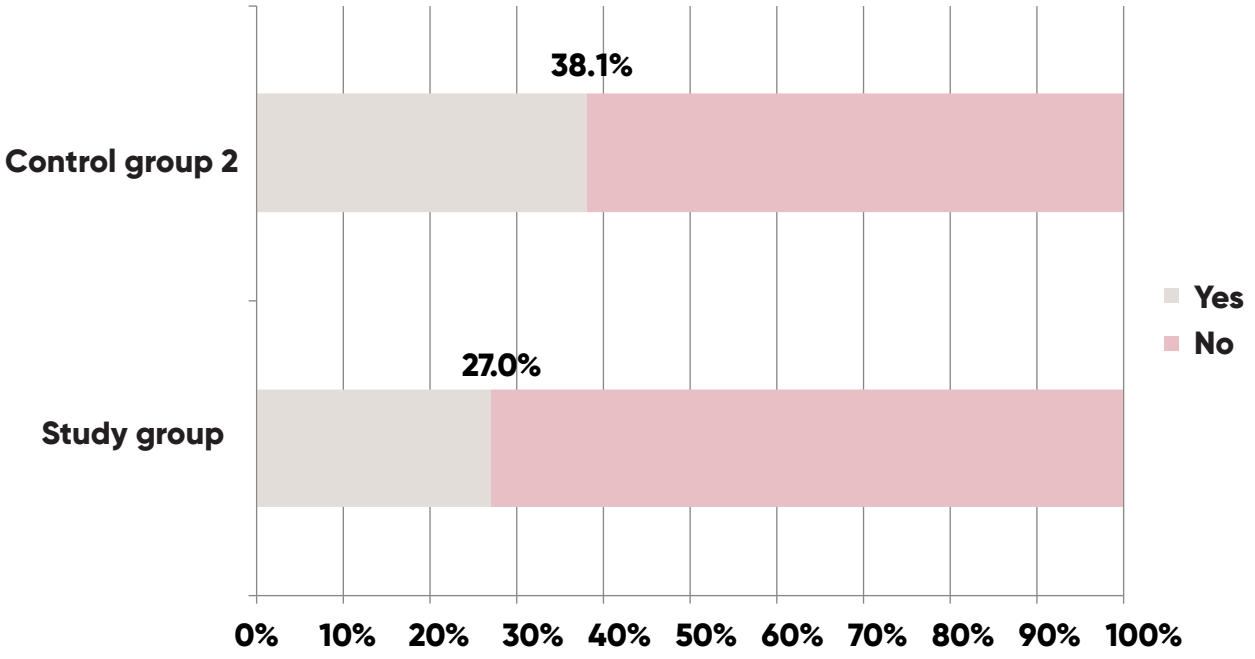
The percentage values of the distribution of patients by personal history data in the study group and control group 2 are given in Table 11 (statistically non-significant data) and in Chart 50. Statistically significant differences were revealed in the data regarding the presence of exercise (p=0.038).

Table 11. The percentage values of the distribution of patients by personal history data in the groups.

#	Personal history data	Study group, n(%)	Control group 2, n(%)	Chi-square test, (P-value)
1	HTA	0 (0.0%)	0 (0.0%)	N/A
2	Diabetes	0 (0.0%)	0 (0.0%)	N/A
3	Hypercholesterinemia	0 (0.0%)	0 (0.0%)	N/A
4	Cardiovascular	0 (0.0%)	0 (0.0%)	N/A
5	Neurologic	0 (0.0%)	0 (0.0%)	N/A
6	Allergic	6 (4.9%)	0 (0.0%)	N/A
7	Immunologic	0 (0.0%)	0 (0.0%)	N/A
8	Oncologic	0 (0.0%)	0 (0.0%)	N/A
9	Alcohol	0 (0.0%)	0 (0.0%)	N/A
10	Drugs	0 (0.0%)	0 (0.0%)	N/A
11	Psychiatric	0 (0.0%)	0 (0.0%)	N/A
12	Low Ovarian reserve	100 (100.0%)	22 (52.4%)	N/A

HTA - Health Technology Assessment

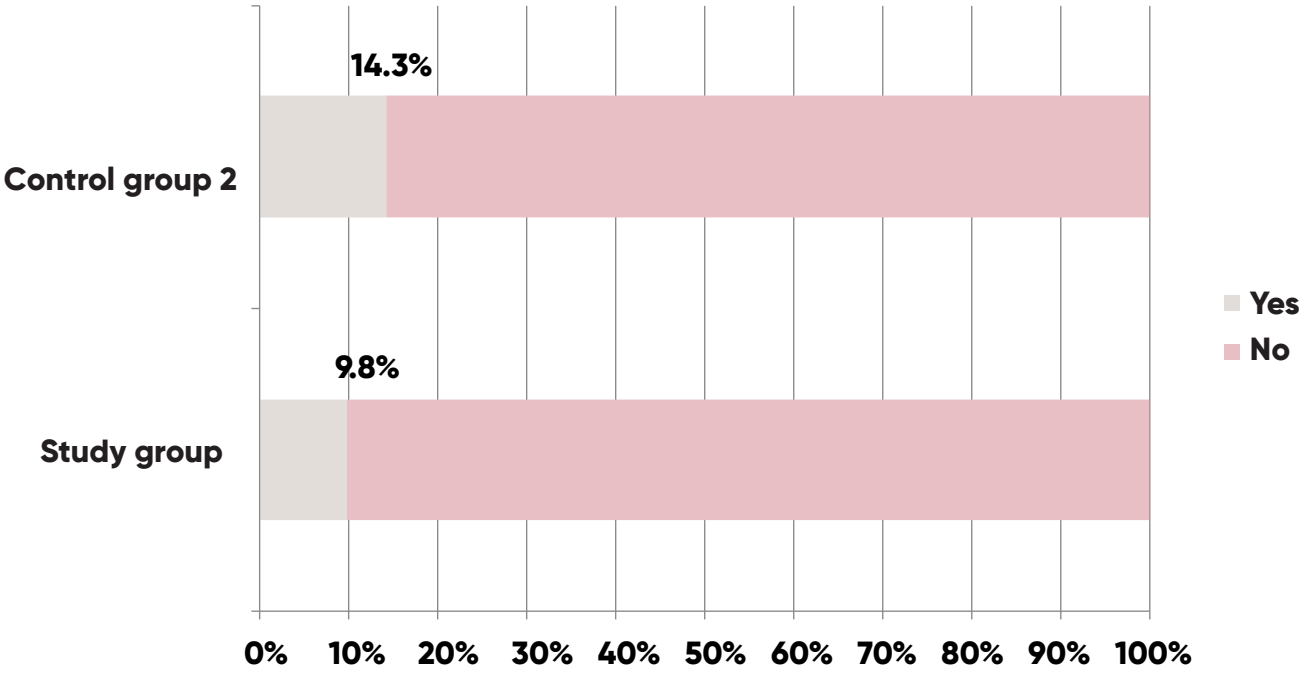
Chart 50. The percentage values of the distribution of patients by presence of surgery in the groups.



Surgery	Study group	Control group 2
Yes, n(%)	33 (27.0%)	16 (38.1%)
No, n(%)	89 (73.0%)	28 (61.9%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	10.96	1	0.238	0.247
Likelihood Ratio	11.06	1	0.236	
Fisher's Exact Test Continuity Correction	10.79	1	0.251	

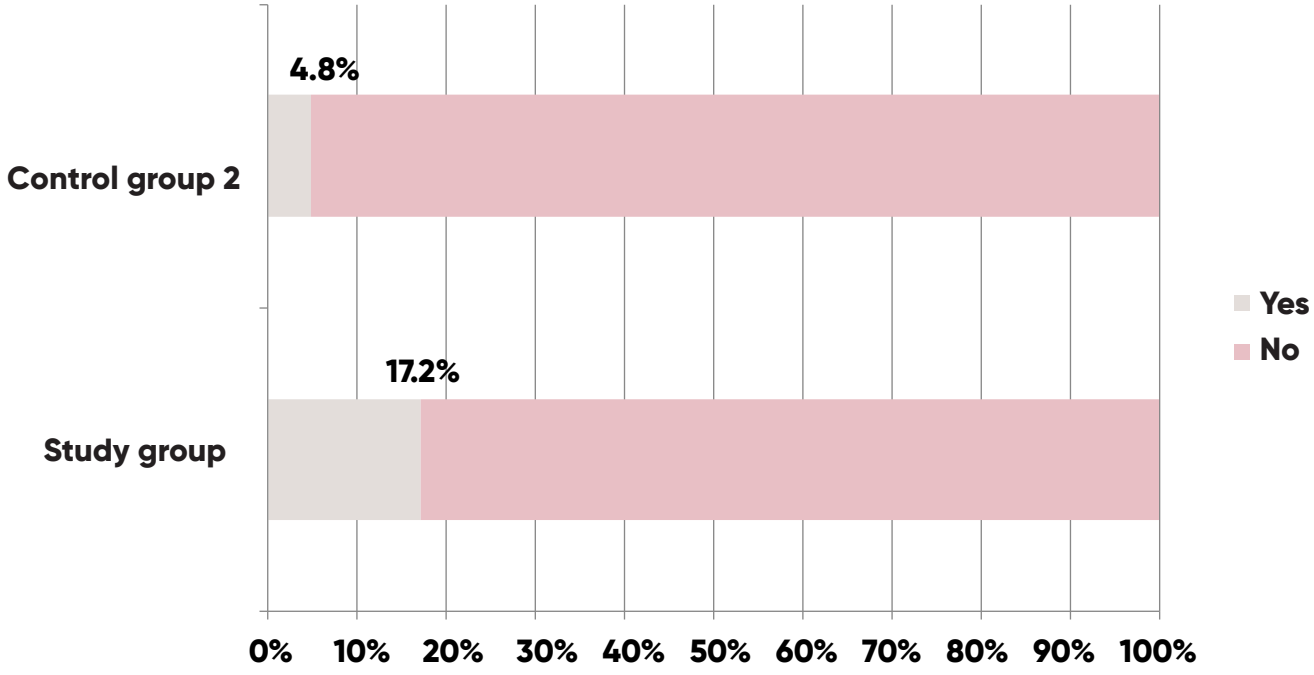
Chart 51. The percentage values of the distribution of patients by the presence of Tobacco users in the groups.



Tobacco user	Study group	Control group 2
Yes, n(%)	12 (9.8%)	6 (14.3%)
No, n(%)	110 (90.2%)	38 (85.7%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.47	1	0.435	0.488
Likelihood Ratio	0.48	1	0.434	
Fisher's Exact Test Continuity Correction	0.33	1	0.511	

Chart 52. The percentage values of the distribution of patients by the presence of Exercise in the groups.



Exercise	Study group	Control group 2
Yes, n(%)	21 (17.2%)	2 (4.8%)
No, n(%)	101 (82.8%)	42 (95.2%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	4.10	1	0.035	0.038
Likelihood Ratio	4.12	1	0.034	
Fisher's Exact Test Continuity Correction	3.95	1	0.041	

4.3.3 Family history

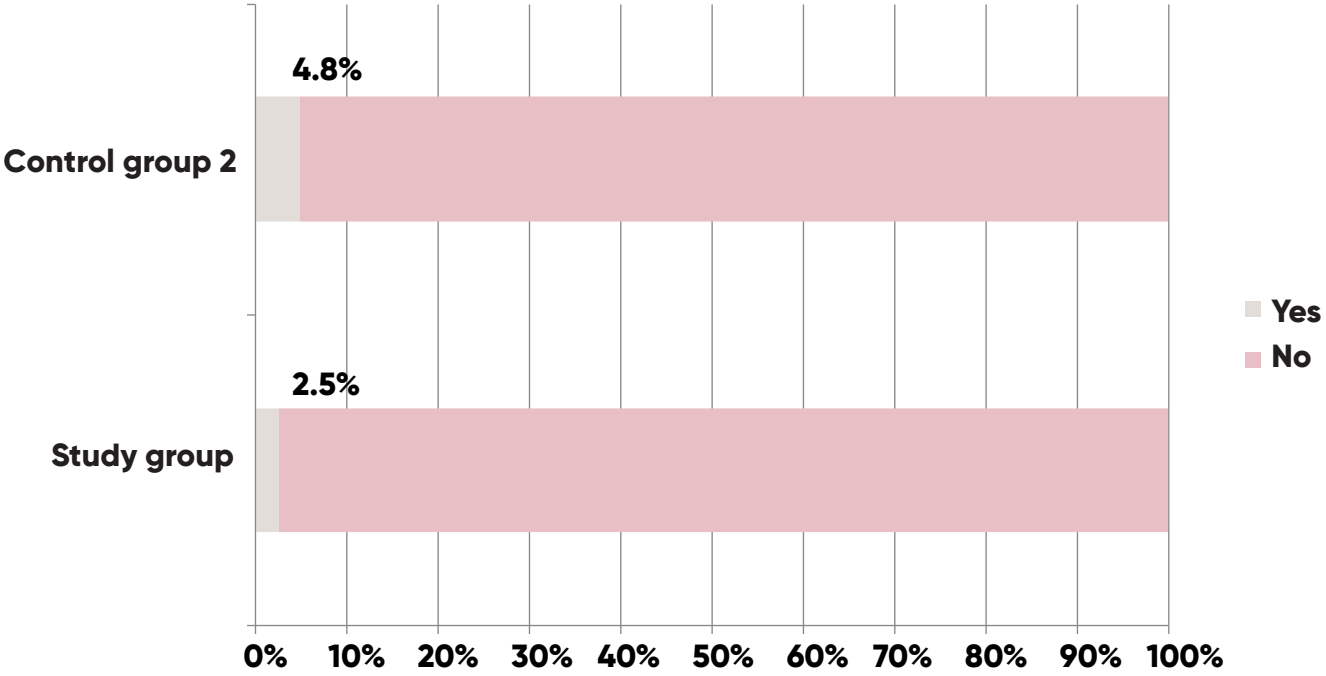
The percentage values of the distribution of patients by family history data in the study group and control group 2 are given in the Table 12 (statistically non-significant data) and in the Chart 53-56.

Table 12. The percentage values of the distribution of patients by family history data in the groups.

#	Family history data	Study group, n(%)	Control group 2, n(%)	Chi-square test, (P-value)
1	HTA	0 (0.0%)	0 (0.0%)	N/A
2	Genetics	0 (0.0%)	0 (0.0%)	N/A
3	Immunologic	0 (0.0%)	0 (0.0%)	N/A
4	Oncologic	0 (0.0%)	0 (0.0%)	N/A
5	Twins in Family	0 (0.0%)	0 (0.0%)	N/A
6	Azoospermia	0 (0.0%)	0 (0.0%)	N/A

HTA, Health Technology Assessment

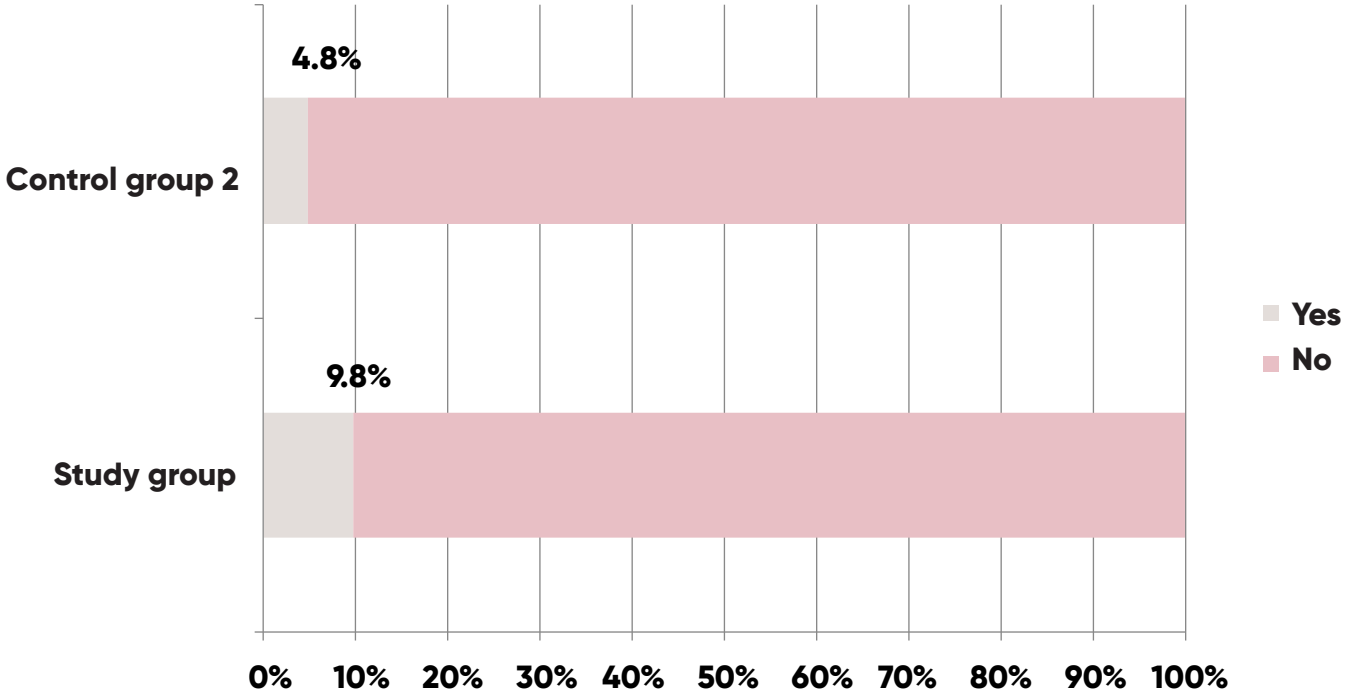
Chart 53. The percentage values of the distribution of patients by the presence of Fertility problems in family history in the groups.



Fertility problems in family history	Study group	Control group 2
Yes, n(%)	3 (2.5%)	2 (4.8%)
No, n(%)	119 (97.5%)	42 (95.2%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.45	1	0.485	0.489
Likelihood Ratio	0.44	1	0.484	
Fisher's Exact Test Continuity Correction	0.51	1	0.491	

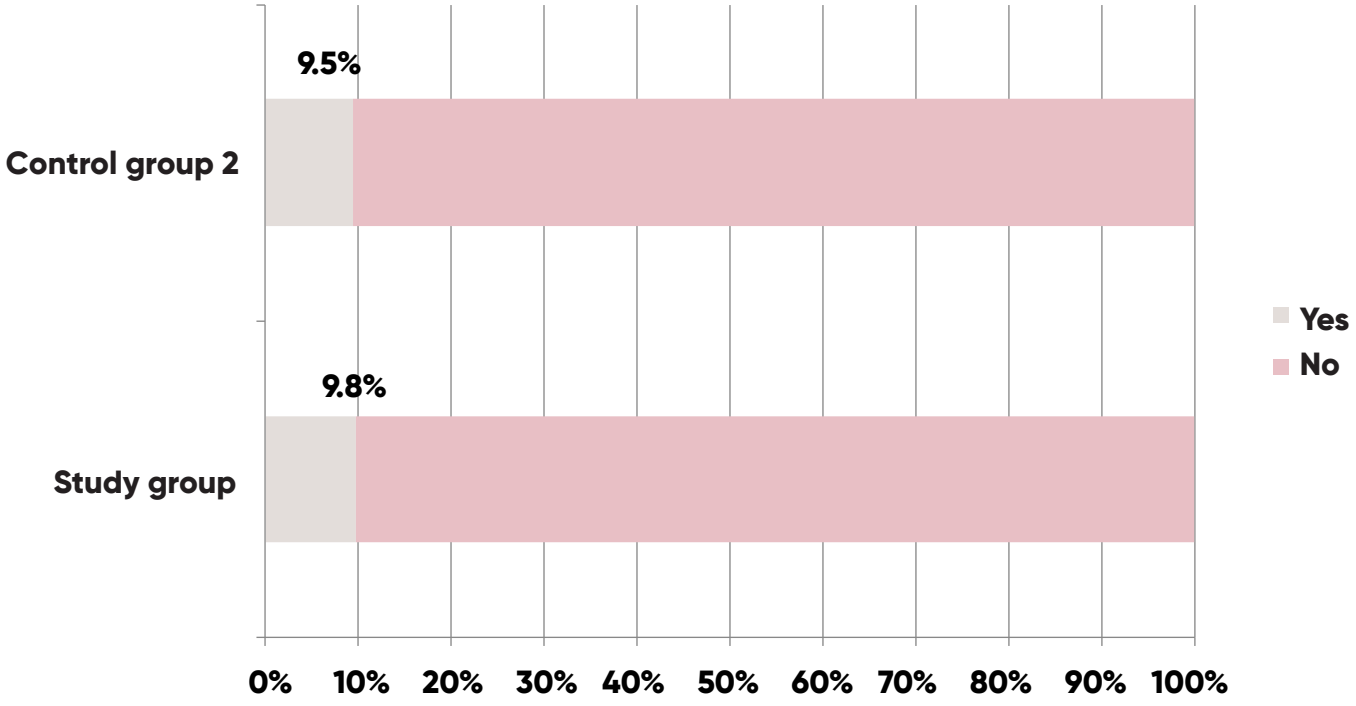
Chart 54. The percentage values of the distribution of patients by the presence of diabetes in family history in the groups.



Diabetes in family history	Study group	Control group 2
Yes, n(%)	12 (9.8%)	2 (4.8%)
No, n(%)	110 (90.2%)	42 (95.2%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	1.17	1	0.223	0.280
Likelihood Ratio	1.16	1	0.221	
Fisher's Exact Test Continuity Correction	1.05	1	0.297	

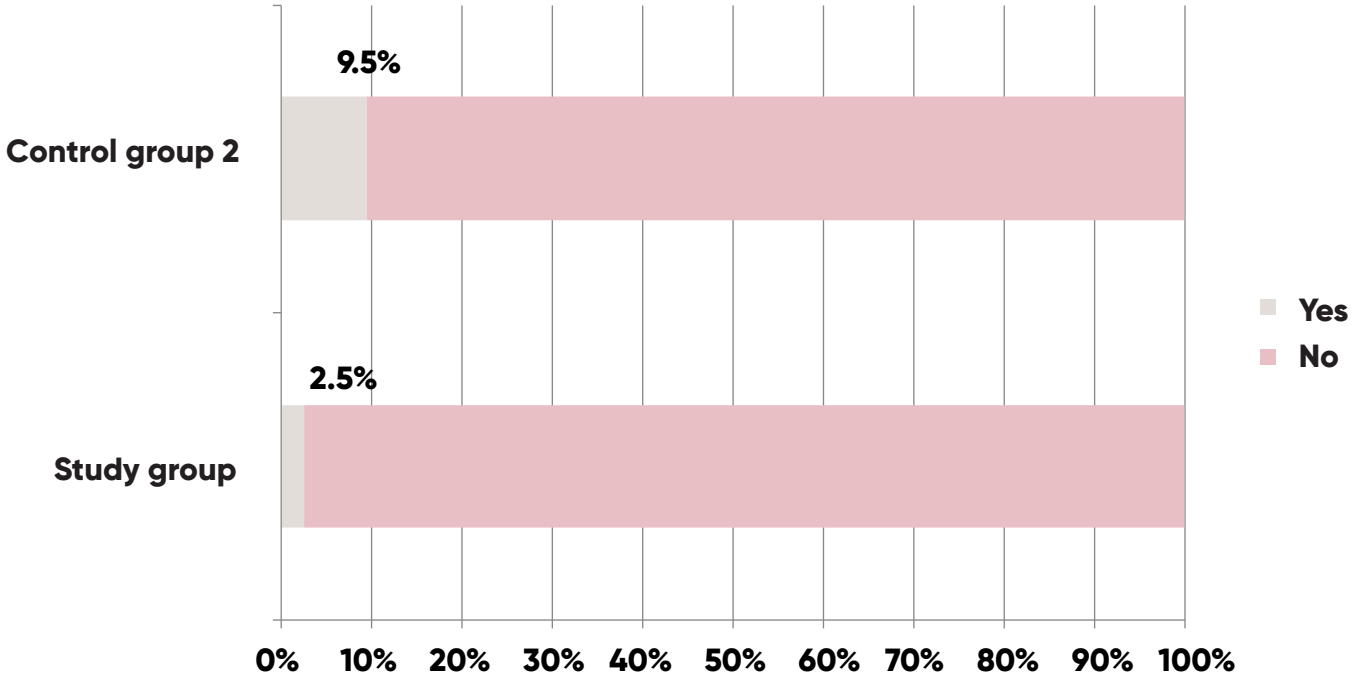
Chart 55. The percentage values of the distribution of patients by the presence of cardiovascular diseases in family history in the groups.



Cardiovascular diseases in family history	Study group	Control group 2
Yes, n(%)	12 (9.8%)	4 (9.5%)
No, n(%)	110 (90.2%)	40 (90.5%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.03	1	0.885	0.886
Likelihood Ratio	0.04	1	0.884	
Fisher's Exact Test Continuity Correction	0.01	1	0.902	

Chart 56. The percentage values of the distribution of patients by the presence of male infertility in family history in the groups.



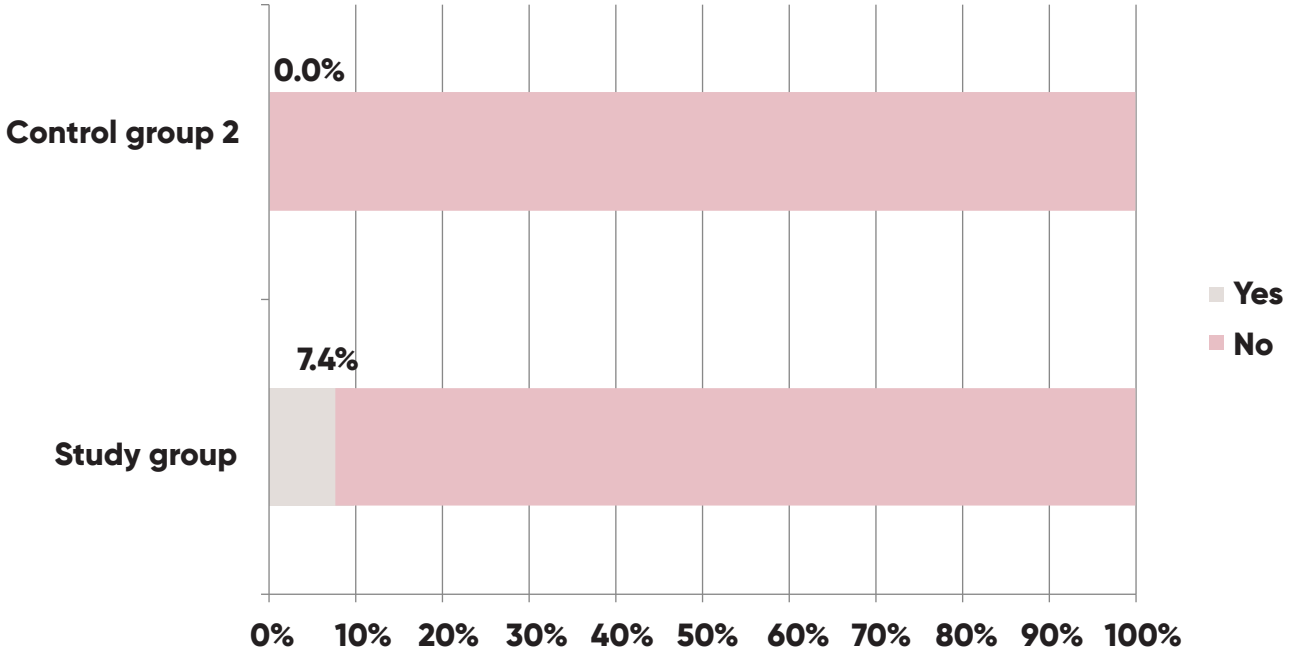
Male infertility in family history	Study group	Control group 2
Yes, n(%)	3 (2.5%)	4 (9.5%)
No, n(%)	119 (97.5%)	40 (90.5%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	3.51	1	0.059	0.061
Likelihood Ratio	3.52	1	0.058	
Fisher's Exact Test Continuity Correction	2.99	1	0.063	

4.3.4 Gynecology and Obstetrical Anamnesis

Gynecology anamnesis

Chart 57. The percentage values of the distribution of patients by regularity of menstrual cycle in the groups.



Regularity of menstrual cycle	Study group	Control group 2
Yes, n(%)	9 (7.4%)	0 (0.0%)
No, n(%)	113 (92.6%)	44 (100.0%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.01	1	0.912	0.915
Likelihood Ratio	0.01	1	0.911	
Fisher's Exact Test Continuity Correction	0.01	1	0.922	

The prevalence of regular and irregular menstrual cycles did not significantly differ between the study and control group 2.

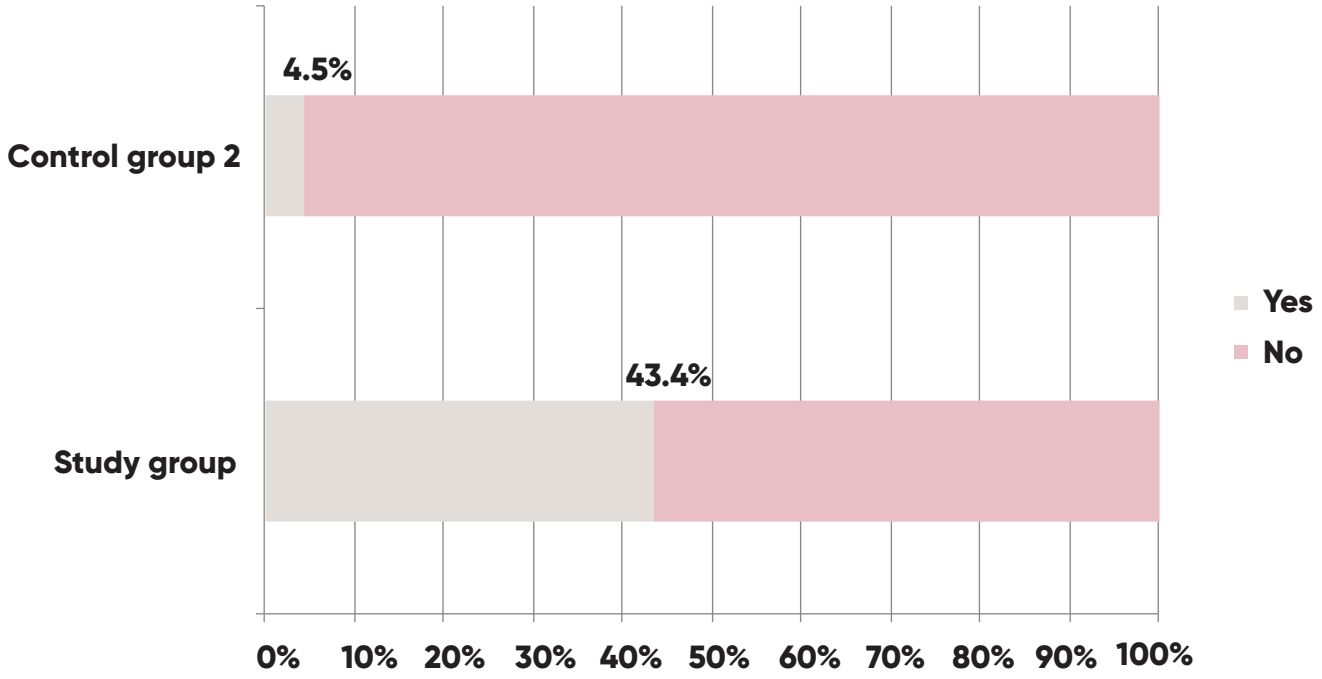
Uterine pathologies

The percentage values of the distribution of patients by uterine pathologies in the study group and control group 2 are given in Table 13 (statistically non-significant data) and in Chart 58. Statistically significant differences were revealed in the data on the presence of adenomyosis ($p<0.001$).

Table 13. The percentage values of the distribution of patients by gynecology history data in the groups.

#	Personal history data	Study group, n(%)	Control group 2, n(%)	Chi-square test, (P-value)
1	Fibroids	0 (0.0%)	0 (0.0%)	N/A
2	Mulerian	0 (0.0%)	0 (0.0%)	N/A
3	Polyps	0 (0.0%)	0 (0.0%)	N/A
4	Endometritis	0 (0.0%)	0 (0.0%)	N/A
5	Myoma	0 (0.0%)	0 (0.0%)	N/A
6	Other	3 (2.5%)	0 (0.0%)	N/A

Chart 58. The percentage values of the distribution of patients by adenomyosis in the groups.



Uterine pathologies - adenomyosis	Study group	Control group 2
Yes, n(%)	53 (43.4%)	2 (4.5%)
No, n(%)	69 (56.6%)	42 (95.5%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	21.95	1	<0.001	<0.001
Likelihood Ratio	21.99	1	<0.001	
Fisher's Exact Test Continuity Correction	21.15	1	<0.001	

Previous pregnancies and obstetric history

The percentage values of the distribution of patients by the data of previous pregnancies in the study group and control group 2 are given in Table 14 and Table 15. The comparative analysis revealed that the groups were homogenous according to these data.

Table 14. The percentage values of the distribution of patients by data of previous pregnancies in the groups.

#	Previous pregnancies	Study group, n(%)	Control group 2, n(%)	Chi-square test, (P-value)
1	**Previous Pregnancies**	**75 (61.5%)**	**24 (57.1%)**	0.64 (0.423)
	1	72 (59.0%)	24 (57.1%)	
	2	0 (0.0%)	0 (0.0%)	
	3	3 (2.5%)	0 (0.0%)	
2	**Previous Term Pregnancies**	**18 (14.8%)**	**12 (28.6%)**	3.40 (0.065)
	1	15 (12.3%)	12 (28.6%)	
	2	0 (0.0%)	0 (0.0%)	
	3	3 (2.5%)	0 (0.0%)	
3	**Previous preterm Pregnancies**	0 (0.0%)	0 (0.0%)	N/A
	1	0 (0.0%)	0 (0.0%)	
4	**Previous miscarriages**	**48 (39.3%)**	**10 (23.8%)**	**3.90 (0.048)**
	1	39 (32.0%)	10 (23.8%)	
	2	3 (2.5%)	0 (0.0%)	
	3	6 (4.9%)	0 (0.0%)	
5	**Previous stillbirths**	0 (0.0%)	0 (0.0%)	N/A
	1	0 (0.0%)	0 (0.0%)	
6	**Previous live births**	**18 (14.8%)**	**12 (28.6%)**	3.40 (0.065)
	1	15 (12.3%)	12 (28.6%)	
	2	0 (0.0%)	0 (0.0%)	
	3	3 (2.5%)	0 (0.0%)	
7	**Previous curettages**	**33 (27.0%)**	**6 (14.3%)**	3.22 (0.073)
	1	30 (24.6%)	6 (14.3%)	
	2	0 (0.0%)	0 (0.0%)	
	3	3 (2.5%)	0 (0.0%)	

Table 15. The percentage values of the distribution of patients by data of previous pregnancies and obstetric history in the groups.

#	Previous pregnancies and obstetric history	Study group, n(%)	Control group 2, n(%)	Chi-square test, (P-value)
1	Previous chromosomal abnormalities	0 (0.0%)	0 (0.0%)	N/A
2	Fetal Malformations	0 (0.0%)	0 (0.0%)	N/A
3	Preeclampsia	6 (4.9%)	0 (0.0%)	N/A
4	Preterm birth	0 (0.0%)	0 (0.0%)	N/A
5	Twins	0 (0.0%)	0 (0.0%)	N/A
6	Molar pregnancy	0 (0.0%)	0 (0.0%)	N/A
7	Anembryonic pregnancy	6 (4.9%)	0 (0.0%)	N/A
8	Prematurity	0 (0.0%)	0 (0.0%)	N/A

4.3.5 ART history

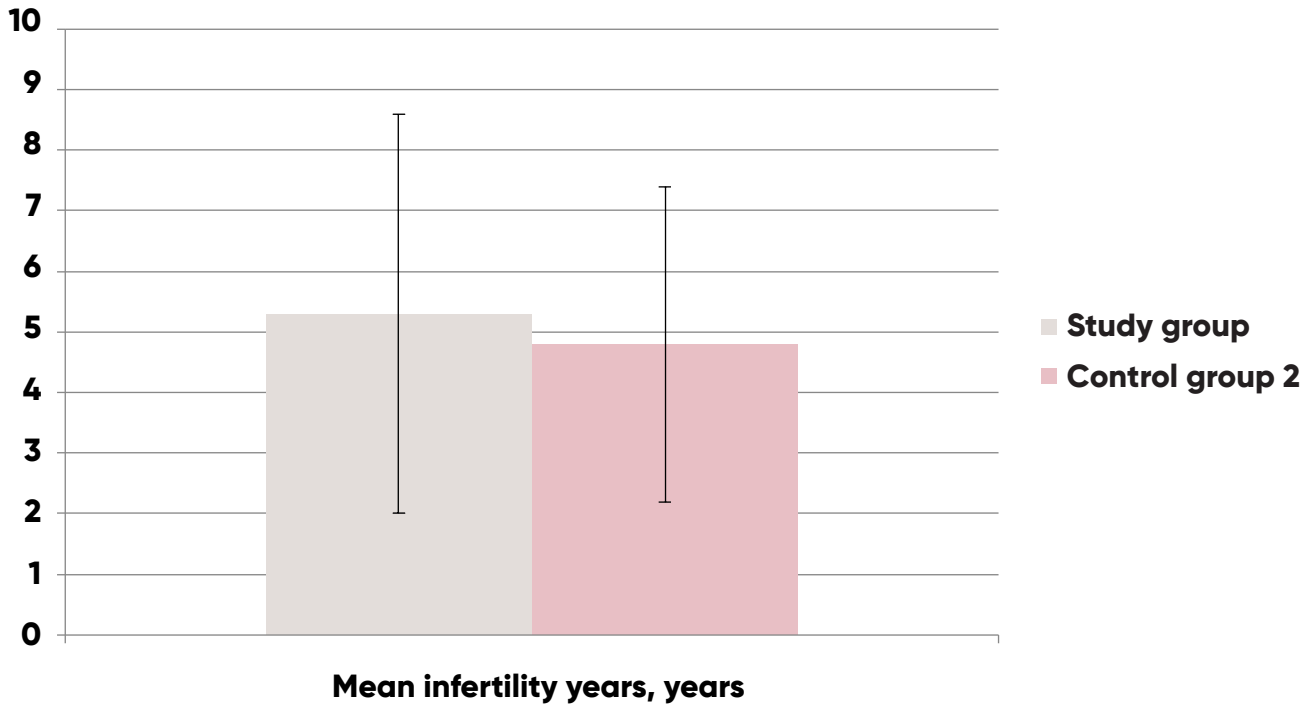
The percentage values of the distribution of patients by the data of previous ART history in the study group and control group 2 are given in the Table 16. The comparative analysis revealed that the groups were homogenous according to these data.

Table 16. The percentage values of the distribution of patients by data of previous ART history in the groups.

#	Previous ART history	Study group, n(%)	Control group 2, n(%)	Chi-square test, (P-value)
1	**Previous implantation failure**	**116 (95.1%)**	**42 (95.5%)**	0.01 (0.921)
	1	0 (0.0%)	0 (0.0%)	
	2	47 (38.5%)	6 (13.6%)	
	3	48 (39.3%)	18 (40.9%)	
	4	15 (12.3%)	16 (36.4%)	
	5	6 (4.9%)	8 (18.2%)	
	6	0 (0.0%)	0 (0.0%)	
	7	0 (0.0%)	0 (0.0%)	
	8	0 (0.0%)	0 (0.0%)	
2	**Previous implantation failure with PGT-A**	**119 (97.5%)**	**42 (95.5%)**	0.48 (0.489)
	1	0 (0.0%)	6 (13.6%)	
	2	98 (80.3%)	28 (63.6%)	
	3	21 (17.2%)	6 (13.6%)	

The mean values of infertility years in the study and control group 2 are given in the chart 59.

Chart 59. The values of infertility years of patients in the groups

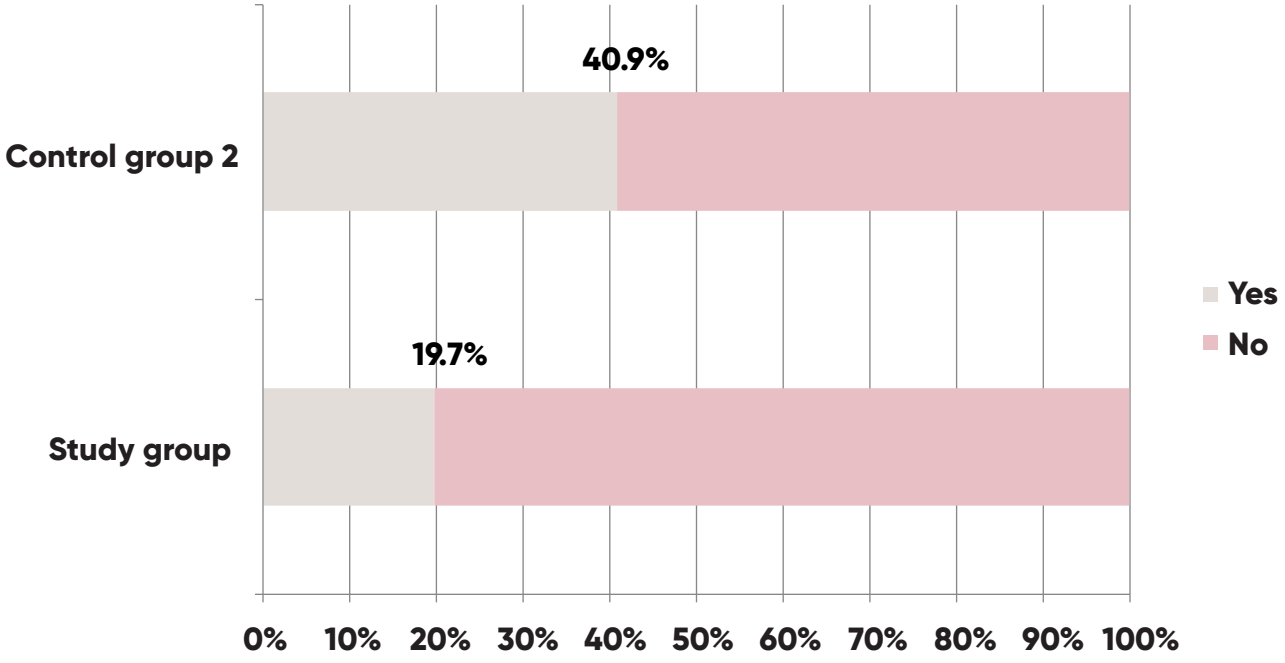


	Study group	Control group 2
Mean infertility years (SD), years	5.3 (3.3)	4.8 (2.6)
Levene's Test for Equality of Variances		
F-test=, p=	12.65, <0.001	
t-test for Equality of Means		
independent t-test=, p=	0.91, 0.365	

ART indication

The percentage values of the distribution of patients by ART indication for male factor in the study group and control group 2 are given in the Chart 60. The difference between data was significant (p=0.006).

Chart 60. The percentage values of the distribution of patients by ART indication for male factor in the groups.

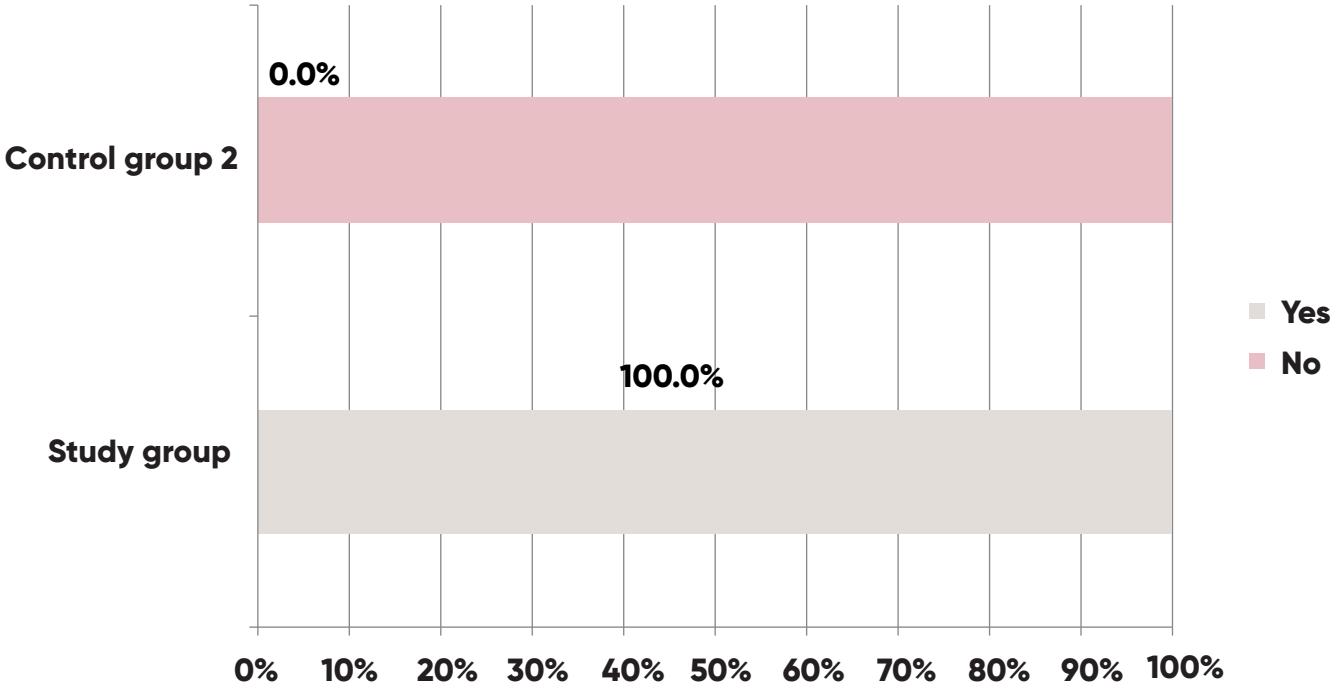


ART indication - male factor	Study group	Control group 2
Yes, n(%)	24 (19.7%)	18 (40.9%)
No, n(%)	98 (80.3%)	26 (59.1%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	7.67	1	0.006	0.006
Likelihood Ratio	7.68	1	0.006	
Fisher's Exact Test Continuity Correction	7.51	1	0.007	

The percentage values of the distribution of patients by ART indication for advanced maternal age in the study group and control group 2 are given in the Chart 61.

Chart 61. The percentage values of the distribution of patients by ART indication for advanced maternal age in the groups.

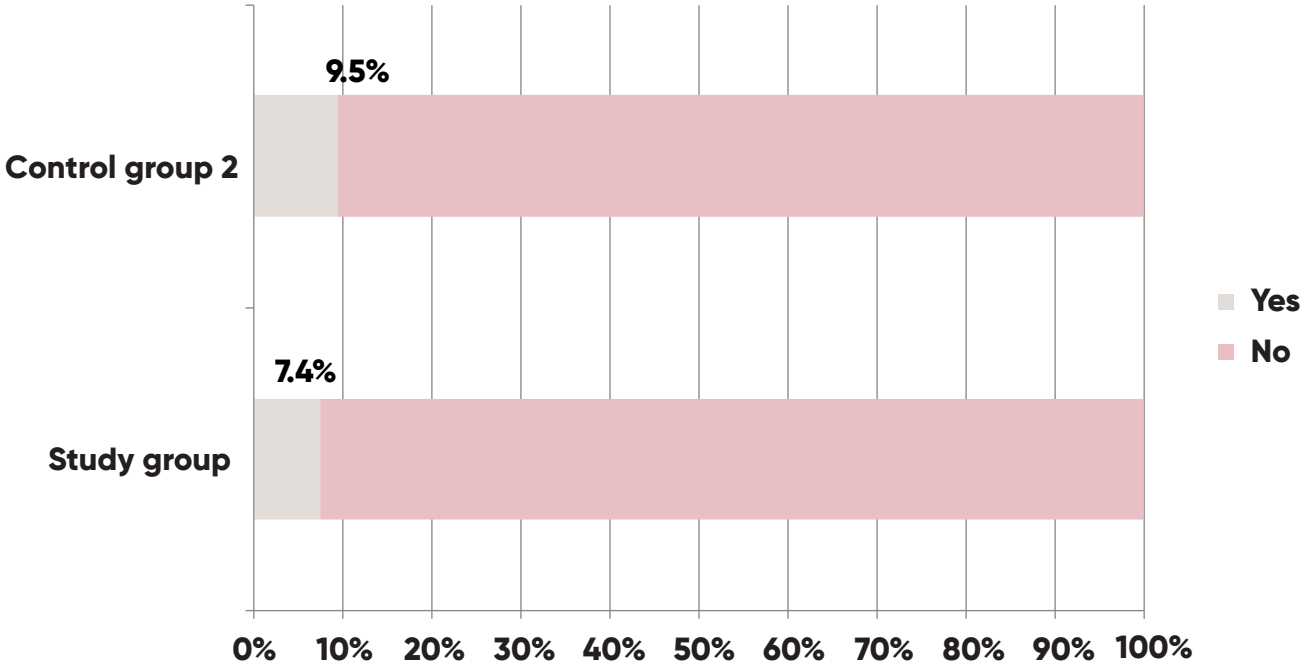


ART indication - advanced maternal age	Study group	Control group 2
Yes, n(%)	122 (100.0%)	0 (0.0%)
No, n(%)	0 (0.0%)	44 (100.0%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.00	1	N/A	N/A
Likelihood Ratio	0.00	1	N/A	
Fisher's Exact Test Continuity Correction	0.00	1	N/A	

The percentage distribution of patients by ART indication for endometriosis in the study group and control group 2 is presented in Chart 62. The disparity between the results was not statistically significant (p=0.718).

Chart 62. The percentage values of the distribution of patients by ART indication for endometriosis in the groups.

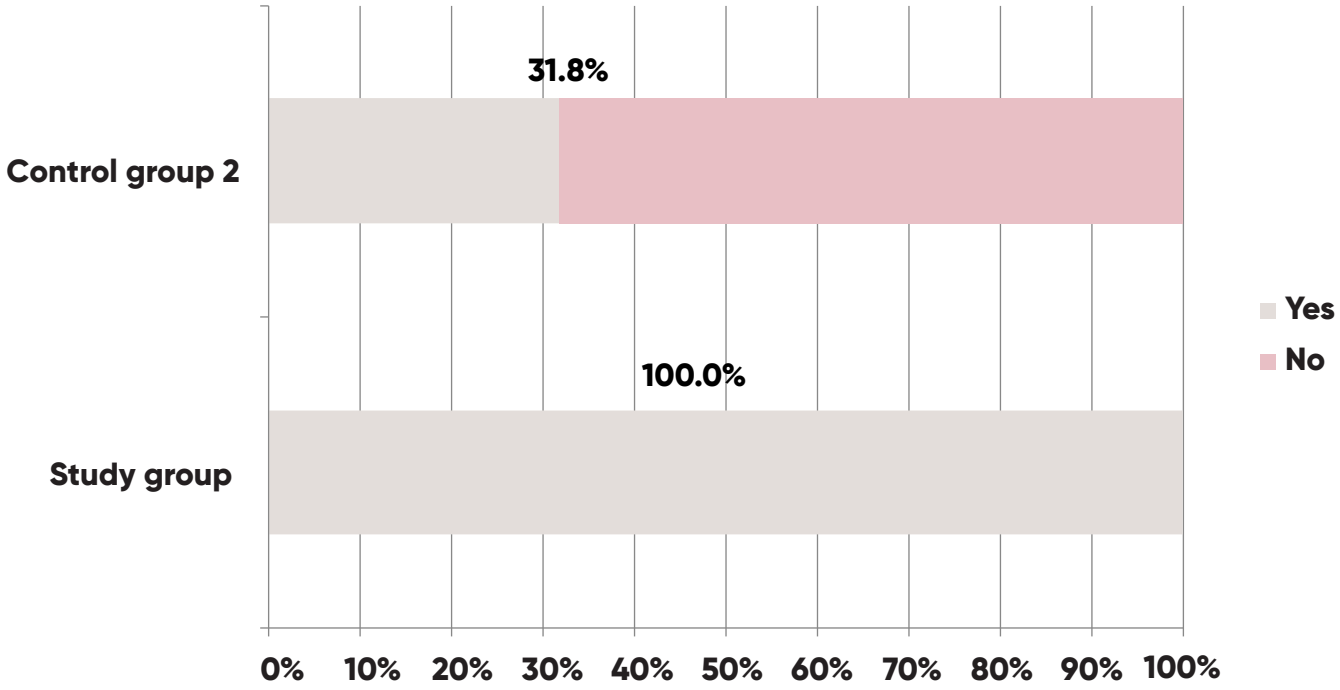


ART indication - endometriosis	Study group	Control group 2
Yes, n(%)	9 (7.4%)	4 (9.5%)
No, n(%)	113 (92.6%)	40 (90.5%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.13	1	0.717	0.718
Likelihood Ratio	0.13	1	0.716	
Fisher's Exact Test Continuity Correction	0.15	1	0.719	

The percentage values of the distribution of patients by ART indication for ovarian factor in the study group and control group 2 are given in the Chart 63. The difference between data was significant (p<0.001).

Chart 63. The percentage values of the distribution of patients by ART indication for ovarian factor in the groups.

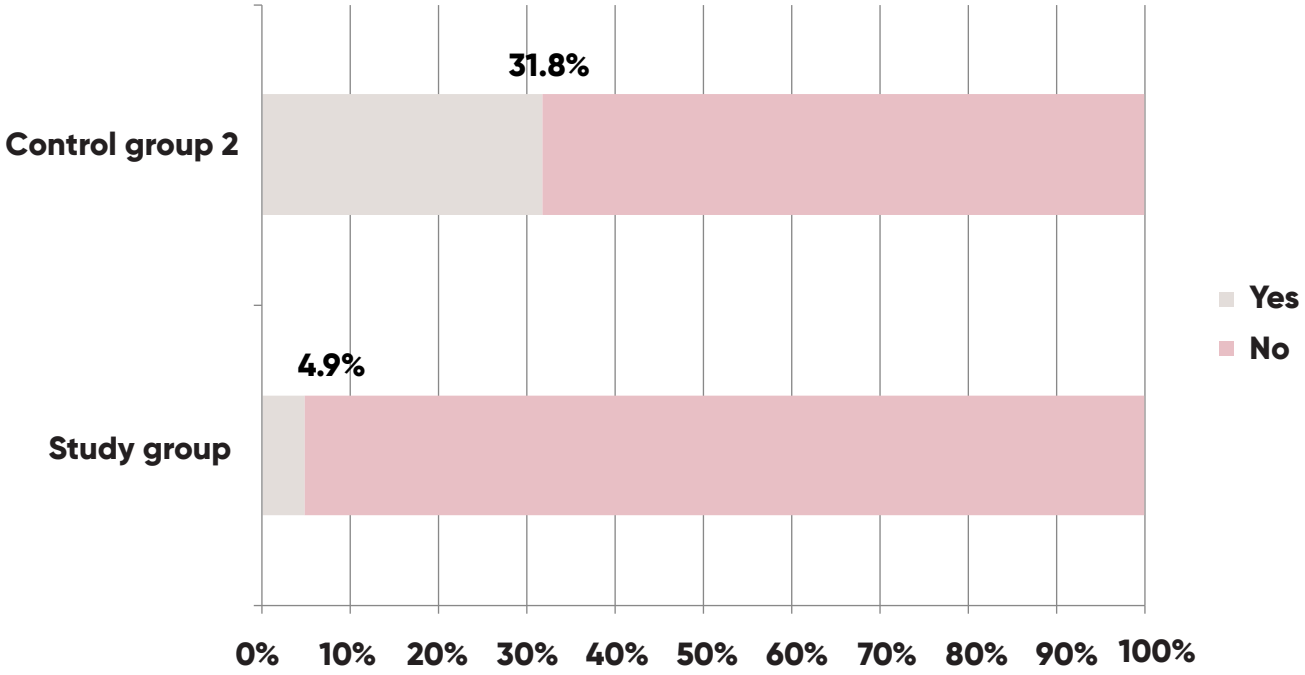


ART indication - ovarian factor	Study group	Control group 2
Yes, n(%)	122 (100.0%)	14 (31.8%)
No, n(%)	0 (0.0%)	30 (68.2%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	100.9	1	<0.001	<0.001
Likelihood Ratio	101.2	1	<0.001	
Fisher's Exact Test Continuity Correction	98.51	1	<0.001	

The percentage values of the distribution of patients by ART indication for tubal factor in the study group and control group 2 are given in the Chart 64. The difference between data was significant (p<0.001).

Chart 64. The percentage values of the distribution of patients by ART indication for tubal factor in the groups.

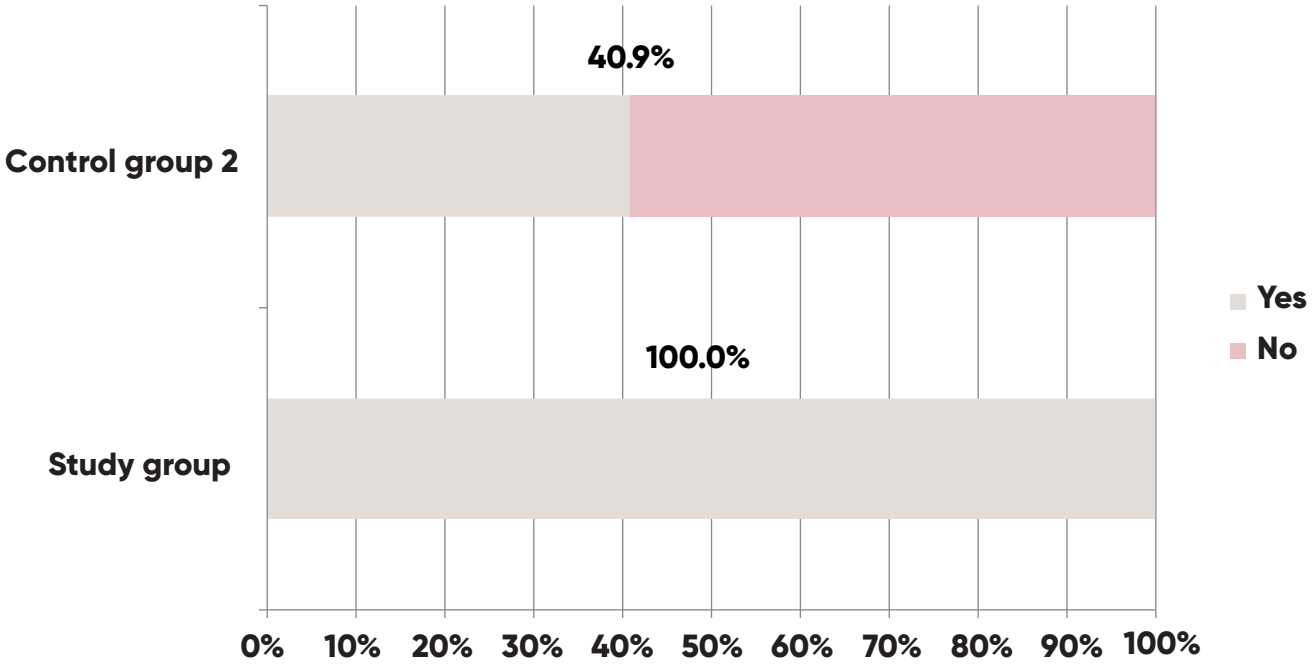


ART indication - tubal factor	Study group	Control group 2
Yes, n(%)	6 (4.9%)	14 (31.8%)
No, n(%)	116 (95.1%)	30 (68.2%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	21.95	1	<0.001	<0.001
Likelihood Ratio	21.98	1	<0.001	
Fisher's Exact Test Continuity Correction	20.90	1	<0.001	

The percentage values of the distribution of patients by ART indication for diminished ovarian reserve in the study group and control group 2 are given in the Chart 65. The difference between data was significant (p<0.001).

Chart 65. The percentage values of the distribution of patients by ART indication for diminished ovarian reserve in the groups.

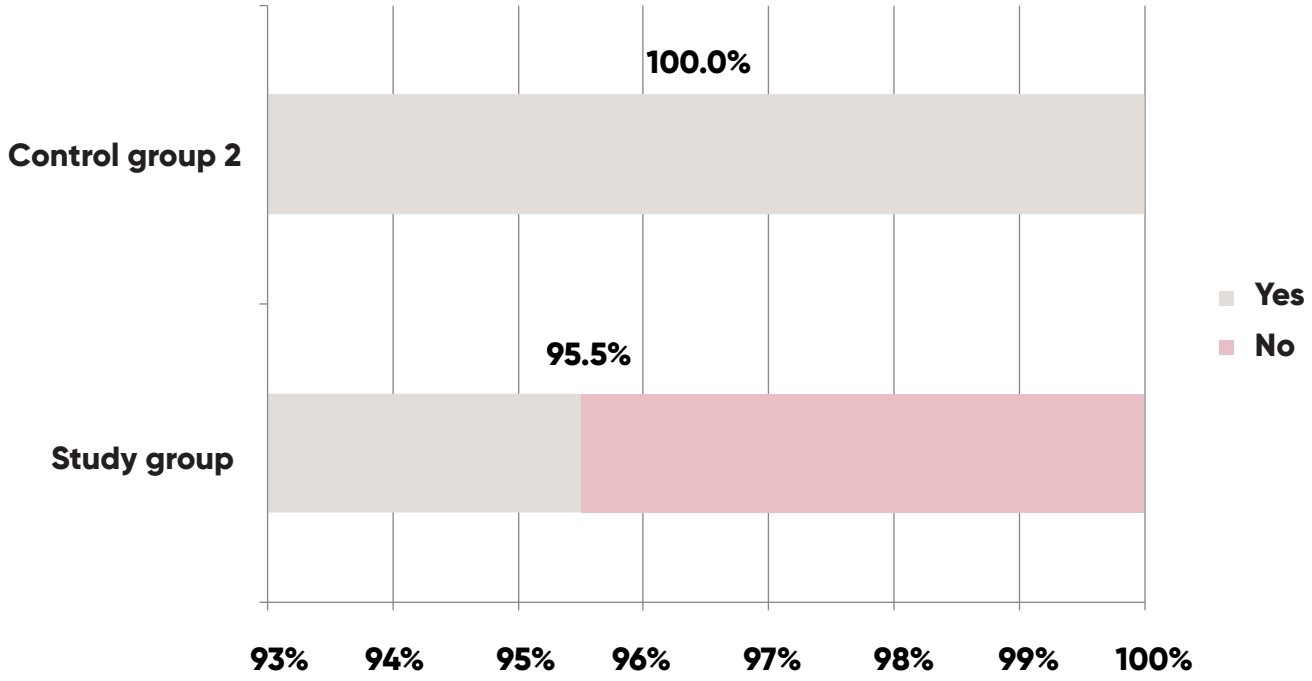


ART indication - diminished ovarian reserve	Study group	Control group 2
Yes, n(%)	122 (100.0%)	18 (40.9%)
No, n(%)	0 (0.0%)	26 (59.1%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	84.96	1	<0.001	<0.001
Likelihood Ratio	85.05	1	<0.001	
Fisher's Exact Test Continuity Correction	79.95	1	<0.001	

The percentage distribution of patients by ART indication for RIF (>2 IVF cycles) in the study group and control group 2 is presented in Chart 66. The disparity between the results was not statistically significant (p=0.925).

Chart 66. The percentage values of the distribution of patients by ART indication for RIF (>2 IVF cycles) in the groups.

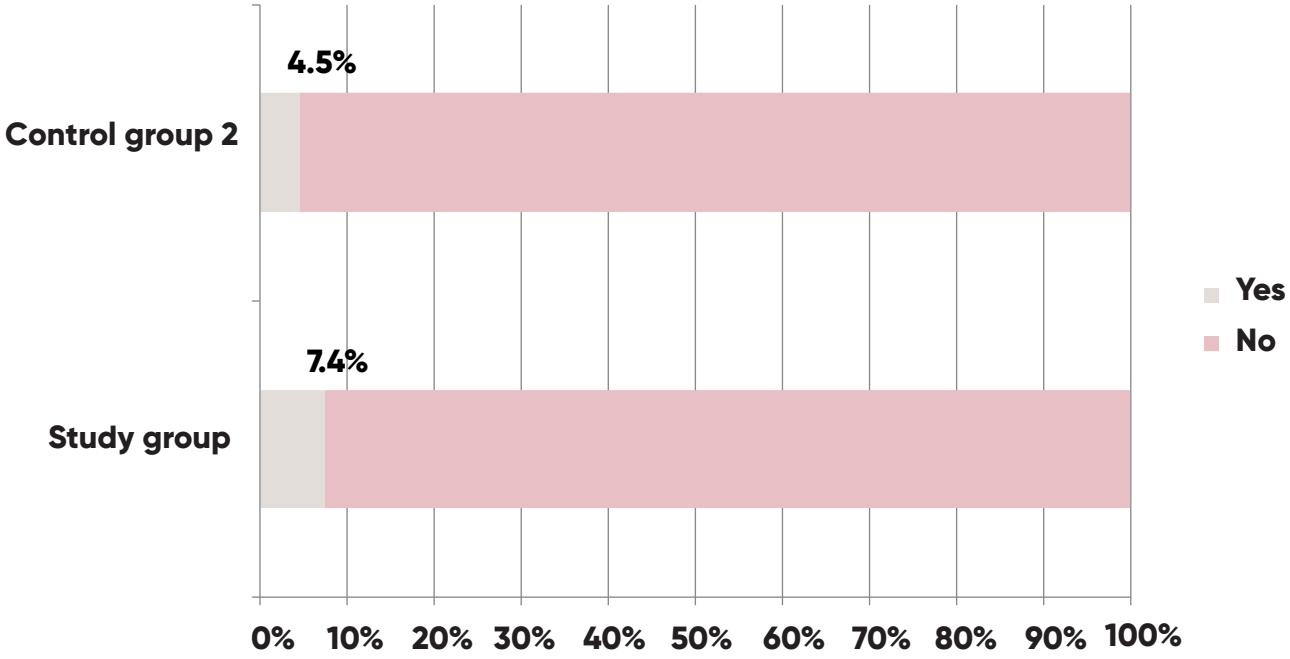


ART indication - RIF (>2 IVF cycles)	Study group	Control group 2
Yes, n(%)	122 (100.0%)	42 (95.5%)
No, n(%)	0 (0.0%)	2 (4.5%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.02	1	0.921	0.925
Likelihood Ratio	0.02	1	0.920	
Fisher's Exact Test Continuity Correction	0.01	1	0.940	

The percentage values of the distribution of patients by ART indication for recurrent pregnency loss (>2) in the study group and control group 2 are given in the Chart 67. The difference between data was significant (p=0.029).

Chart 67. The percentage values of the distribution of patients by ART indication for RPL (>2) in the groups.



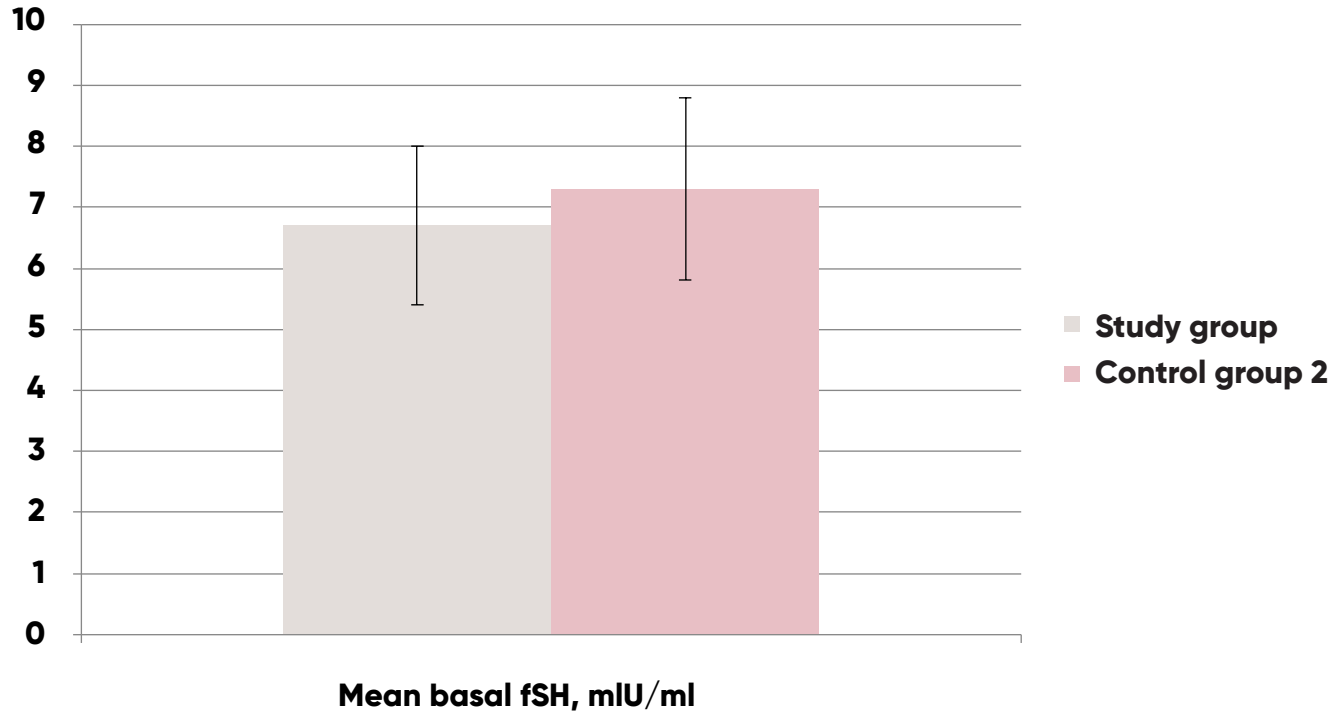
ART indication - recurrent pregnency loss (>2)	Study group	Control group 2
Yes, n(%)	9 (7.4%)	2 (4.5%)
No, n(%)	113 (92.6%)	42 (95.5%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	0.42	1	0.516	0.519
Likelihood Ratio	0.44	1	0.515	
Fisher's Exact Test Continuity Correction	0.39	1	0.522	

4.3.6 Current ART

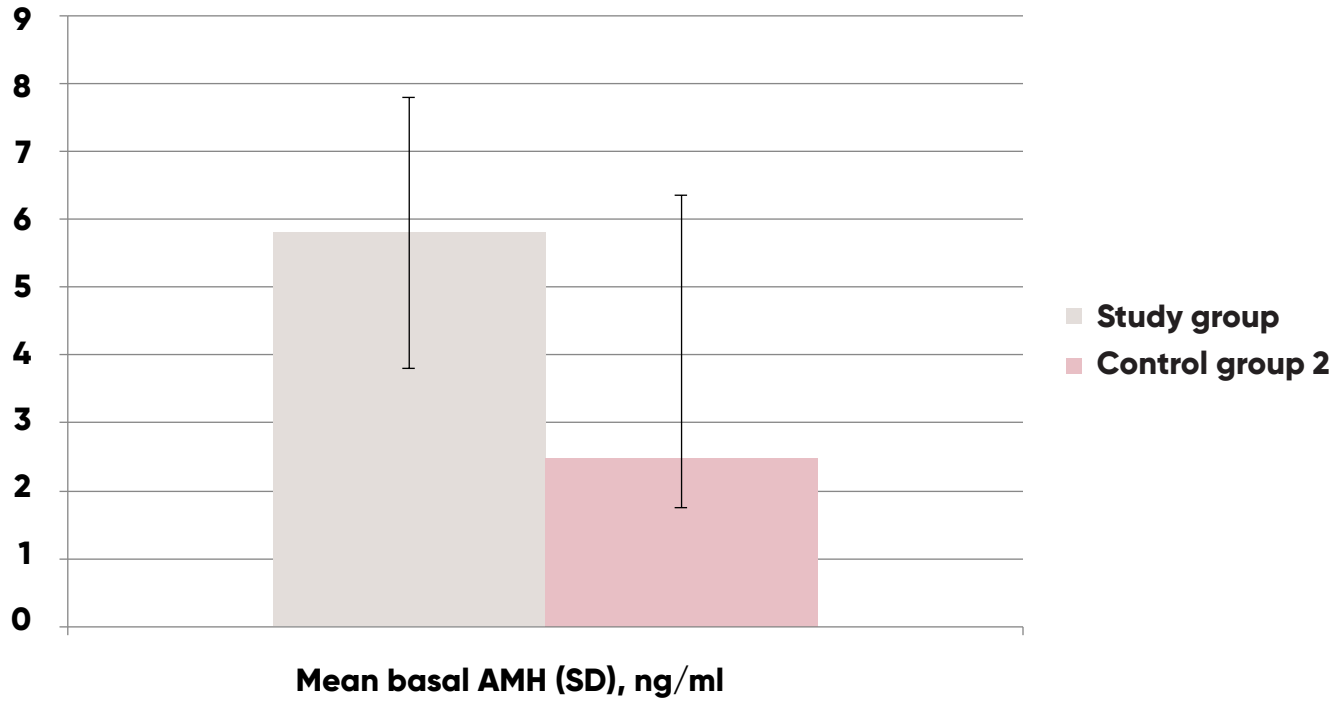
The basal FSH, AMH and AFC levels of egg donors and sperm concentrations were measured in the study group. Similarly, the basal FSH and AMH levels of patients utilizing own oocytes and sperm concentrations were measured in control group 2. The mean values of these parameters are presented in Charts 68-71. With the exception of sperm concentration, the differences in the remaining values between the groups were significant ($p < 0.05$).

Chart 68. The mean egg donor basal FSH concentration in the study group and FSH concentration of patients utilizing their own oocytes in the control group 2.



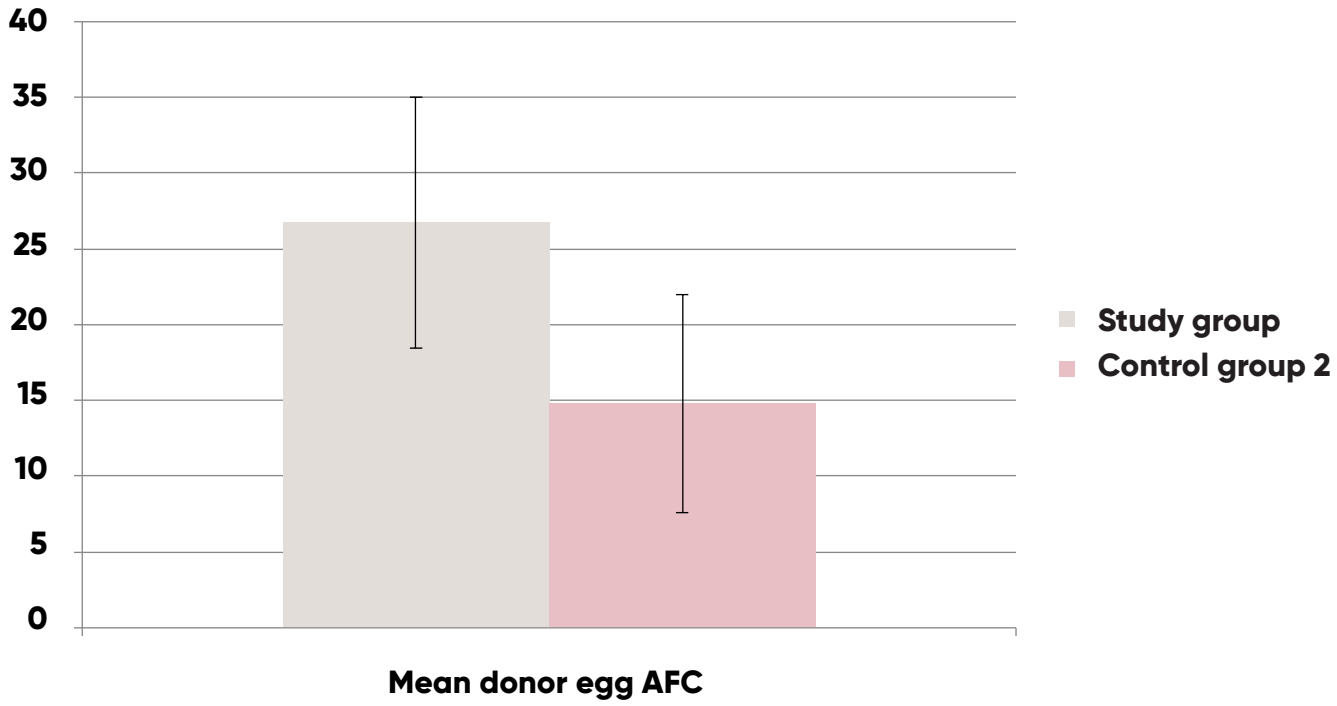
	Study group	Control group 2
Mean donor egg basal FSH (SD), mIU/ml	6.7 (1.3)	
Mean own oocyte basal FSH (SD), mIU/ml		7.3 (1.5)
Levene's Test for Equality of Variances		
F-test=, p=	32.20, <0.001	
t-test for Equality of Means		
independent t-test=, p=	2.48, 0.014	

Chart 69. The mean egg donor basal AMH concentration in the study group and own oocyte basal FSH concentration in the control group 2.



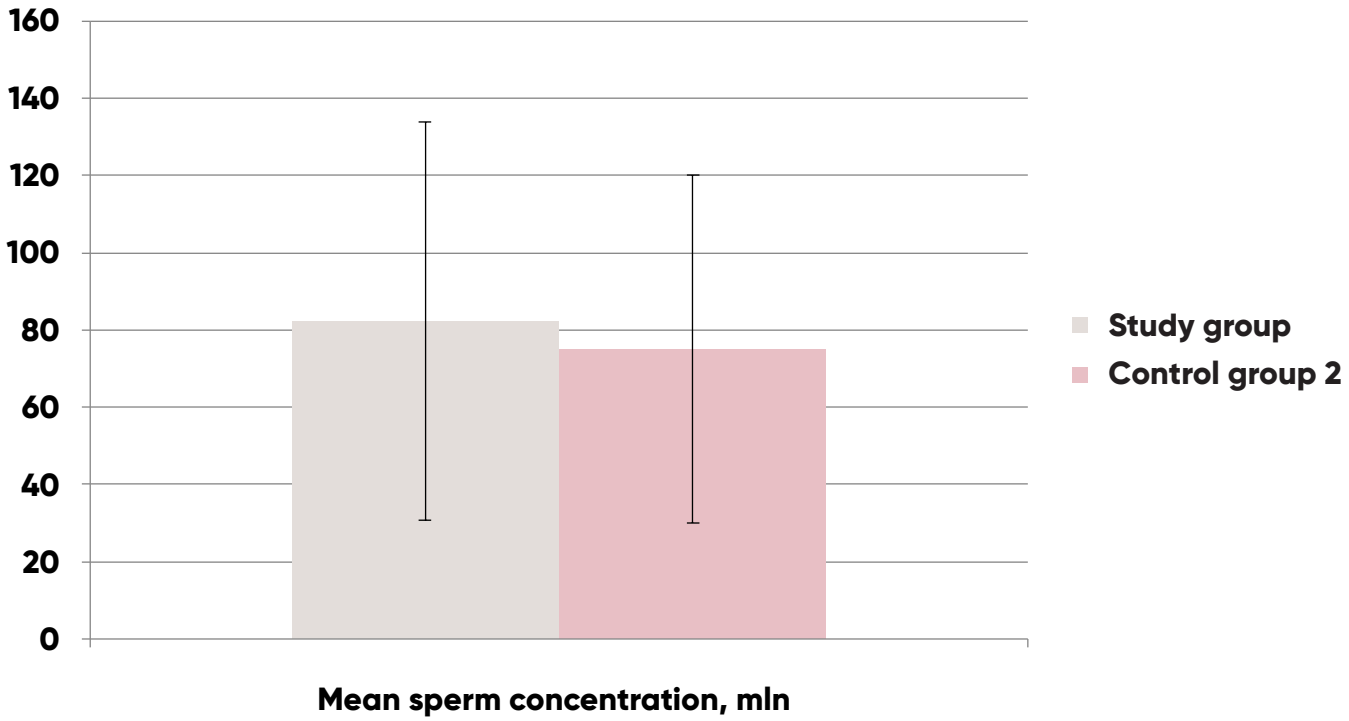
	Study group	Control group 2
Mean donor egg basal AMH (SD), ng/ml	5.8 (2.0)	
Mean own oocyte basal AMH (SD), ng/ml		2.3 (1.8)
Levene's Test for Equality of Variances		
F-test=, p=	8.75, <0.001	
t-test for Equality of Means		
independent t-test=, p=	10.03, <0.001	

Chart 70. The mean egg donor AFC in the study group and patients' own AFC in the control group 2.



	Study group	Control group 2
Mean donor egg AFC (SD)	26.7 (8.3)	
Patient own oocyte AFC		14.8 (7.2)
Levene's Test for Equality of Variances		
F-test=, p=	7.92, <0.001	
t-test for Equality of Means		
independent t-test=, p=	8.13, <0.001	

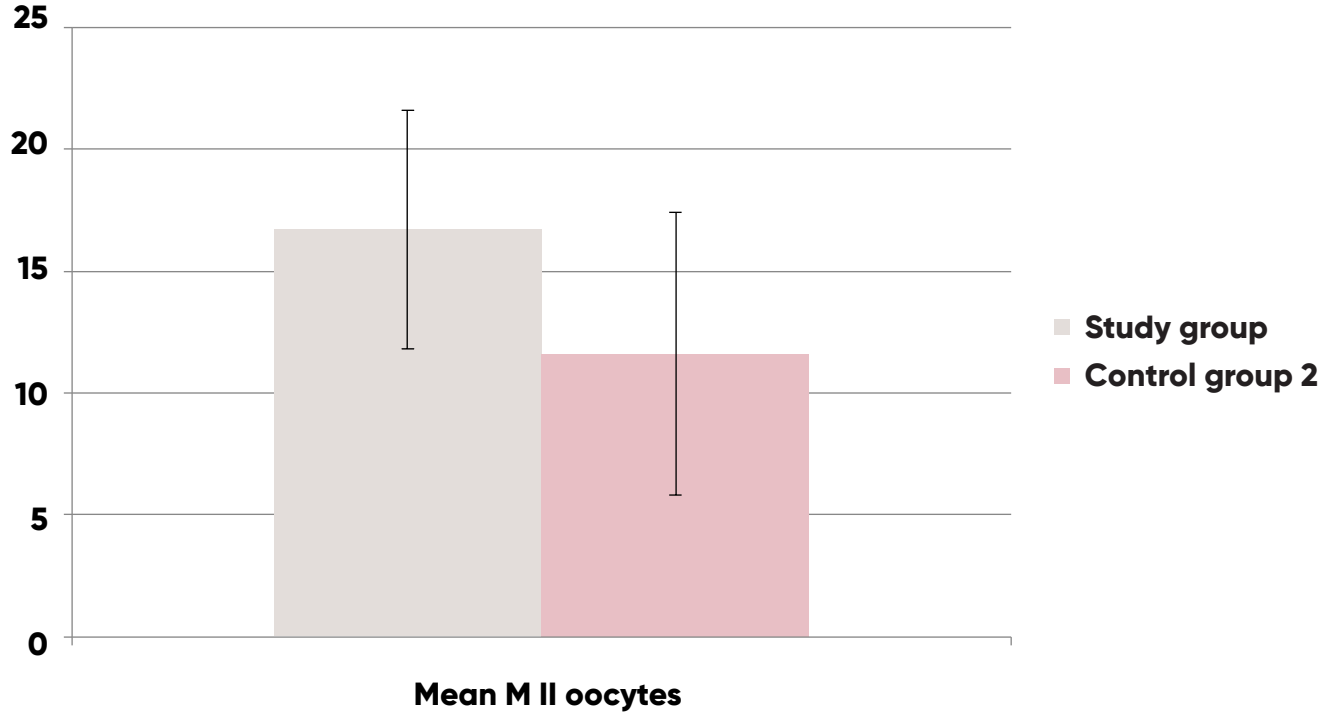
Chart 71. The mean sperm concentrations in the groups



	Study group	Control group 2
Mean sperm concentration (SD), mln	82.3 (51.7)	71.8 (58.4)
Levene's Test for Equality of Variances		
F-test=, p=	6.58, 0.015	
t-test for Equality of Means		
independent t-test=, p=	1.10, 0.274	

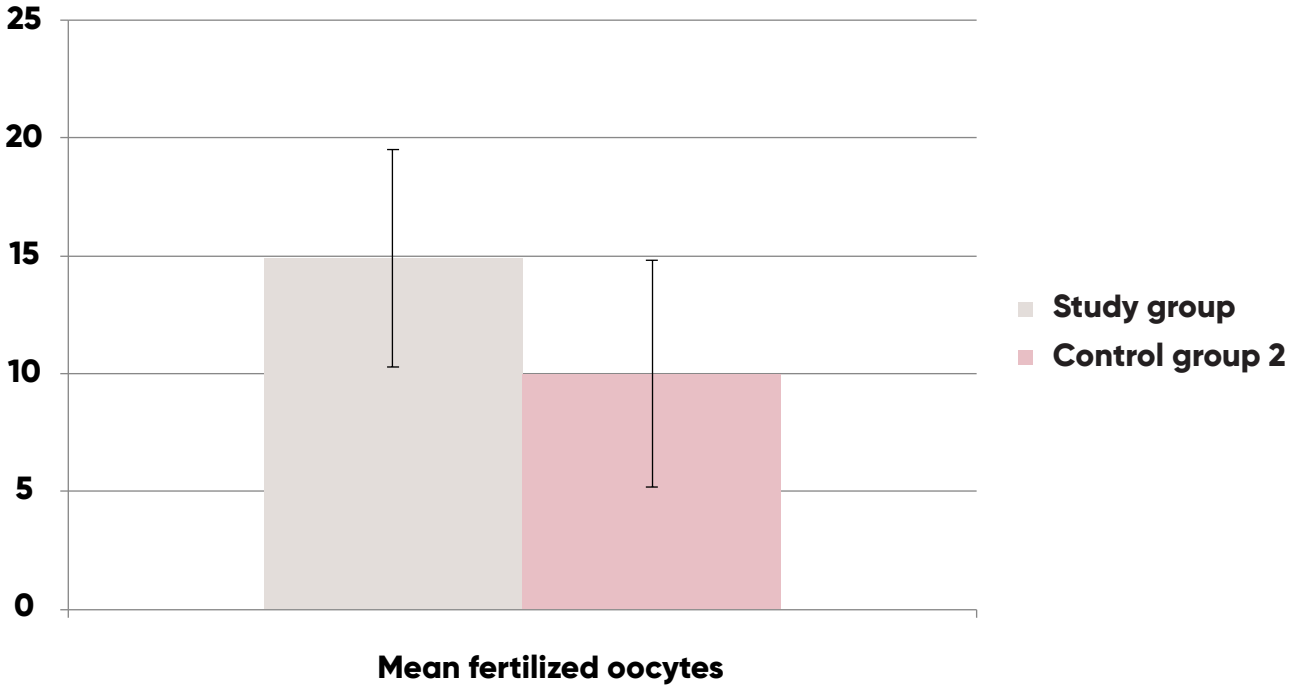
The M II oocytes, and fertilized oocytes for egg donors (study group) and patients utilizing own oocytes (control group 2) were measured, and the fertilization rate was calculated in both groups. The mean values of these parameters are presented in Charts 72-75. The difference of these values between the groups was not significant (p>0.05).

Chart 72. The mean M II oocytes values in the groups



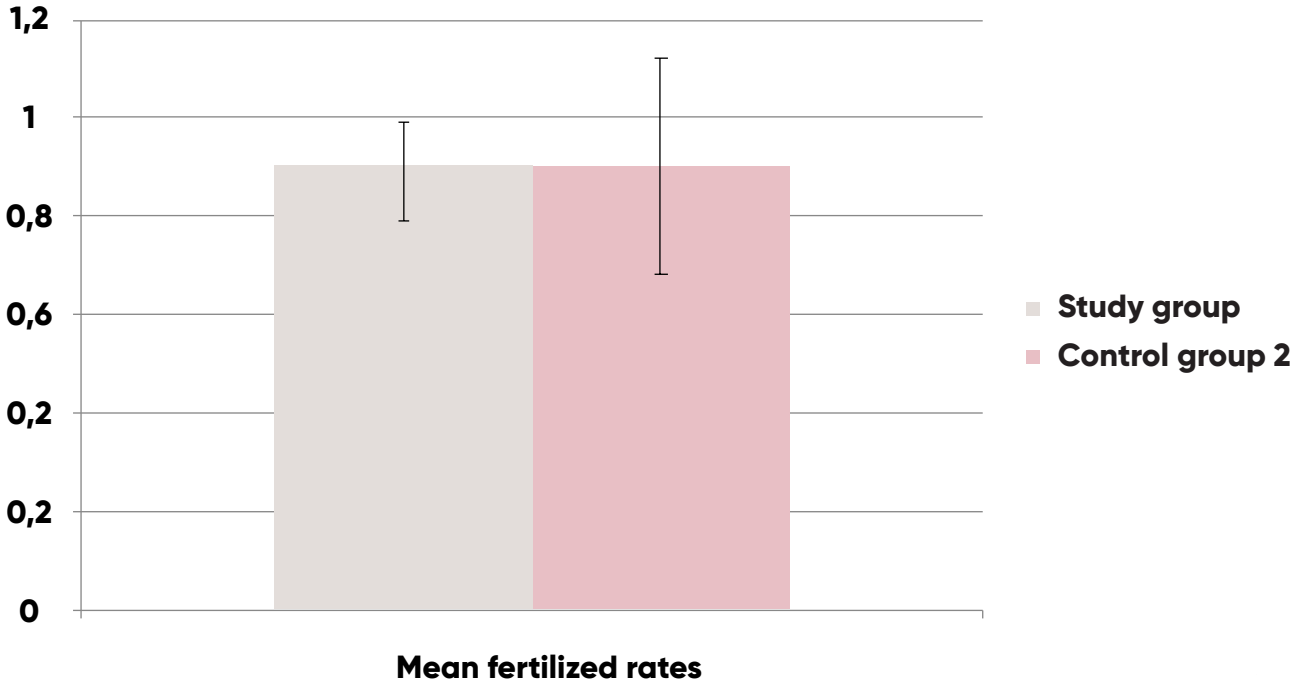
	Study group	Control group 2
Mean M II oocytes (SD)	16.7 (4.9)	11.6 (5.8)
Levene's Test for Equality of Variances		
F-test=, p=	6.34, <0.001	
t-test for Equality of Means		
independent t-test=, p=	5.54, <0.001	

Chart 73. The mean fertilized oocytes values in the groups



	Study group	Control group 2
Mean fertilized oocytes (SD)	14.9 (4.6)	10.0 (4.8)
Levene's Test for Equality of Variances		
F-test=, p=	5.62, <0.001	
t-test for Equality of Means		
independent t-test=, p=	5.94, <0.001	

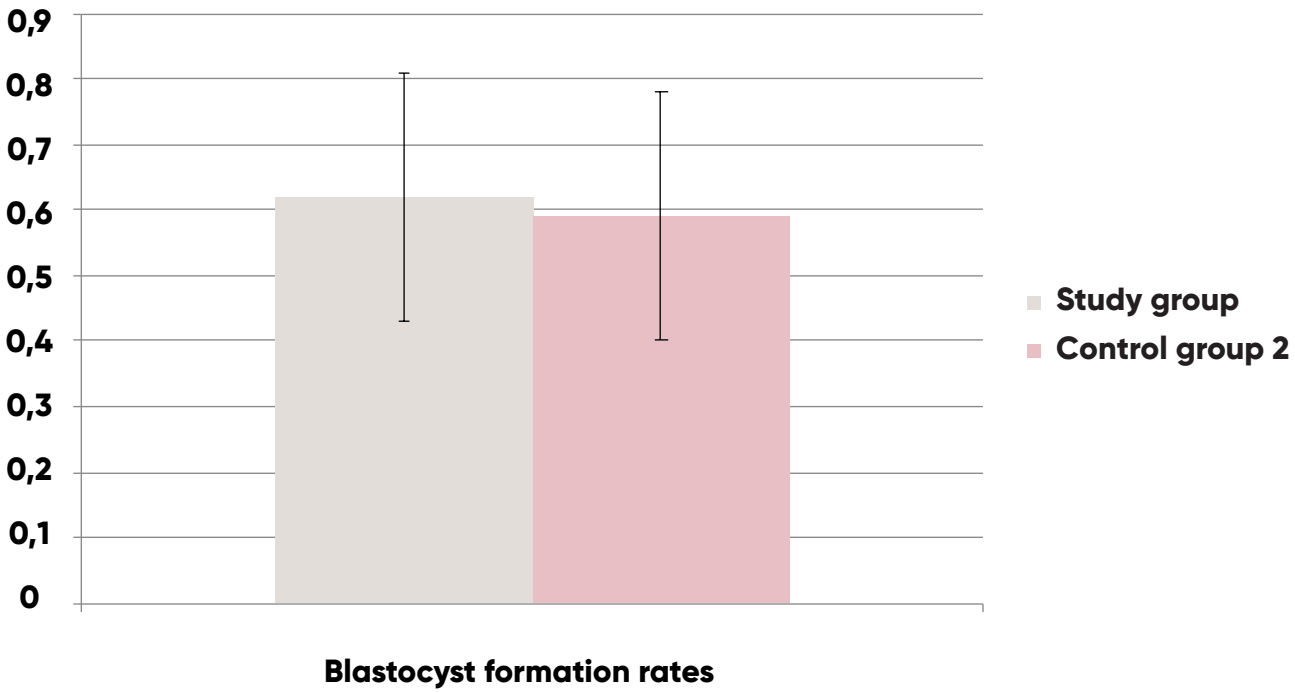
Chart 74. The mean fertilization rates in the groups



	Study group	Control group 2
Mean fertilization rates (SD)	0.89 (0.10)	0.89 (0.08)
Levene's Test for Equality of Variances		
F-test=, p=	1.07, 0.354	
t-test for Equality of Means		
independent t-test=, p=	0.06, 0.953	

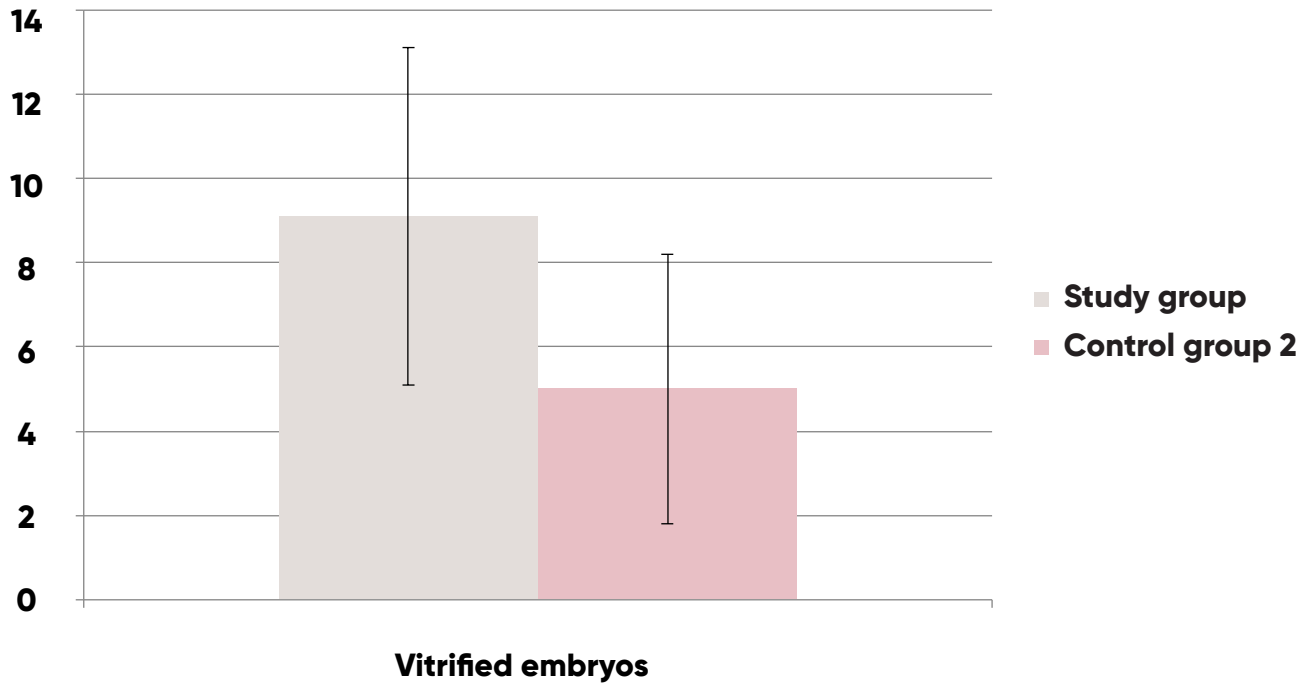
The number of via blastocyst formation rate and vitrified embryos have been measured, , as well as PGT-A analyzed embryos have been calculated in both groups. Mean values of these parameters are given in the chart 75-77. The difference of these values between the groups was not significant (p>0.05).

Chart 75. The mean blastocyst formation rates in the groups



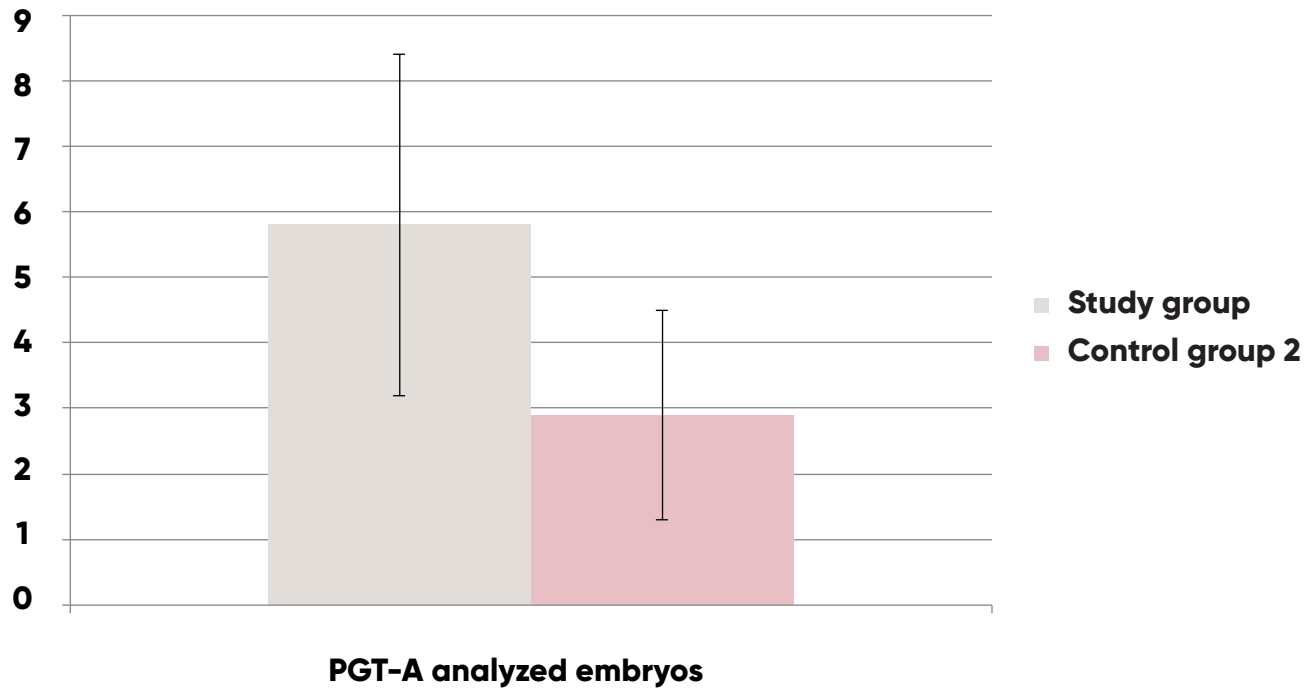
	Study group	Control group 2
Mean blastocyst formation rates (SD)	0.62 (0.19)	0.50 (0.14)
Levene's Test for Equality of Variances		
F-test=, p=	3.77, <0.001	
t-test for Equality of Means		
independent t-test=, p=	3.75, <0.001	

Chart 76. The mean vitrified embryos in the groups



	Study group	Control group 2
Mean vitrified embryos (SD)	9.1 (4.0)	5.0 (3.2)
Levene's Test for Equality of Variances		
F-test=, p=	6.19, <0.001	
t-test for Equality of Means		
independent t-test=, p=	6.12, <0.001	

Chart 77. The mean PGT-A analyzed embryos in the groups



	Study group	Control group 2
Mean PGT-A analyzed embryos (SD)	5.8 (2.6)	2.9 (1.6)
Levene's Test for Equality of Variances		
F-test=, p=	6.76, <0.001	
t-test for Equality of Means		
independent t-test=, p=	6.93, <0.001	

A comparative analysis of reproductive parameters, including basal FSH, AMH, antral follicle count (AFC), and blastocyst formation rate, revealed a statistically significant difference between the study group and the control group. No statistically significant difference was seen between sperm concentration and fertilization rate (Table 17).

Table 17. Reproductive Parameters Comparison of means the study PGT-A+ERA Groups according to patient age

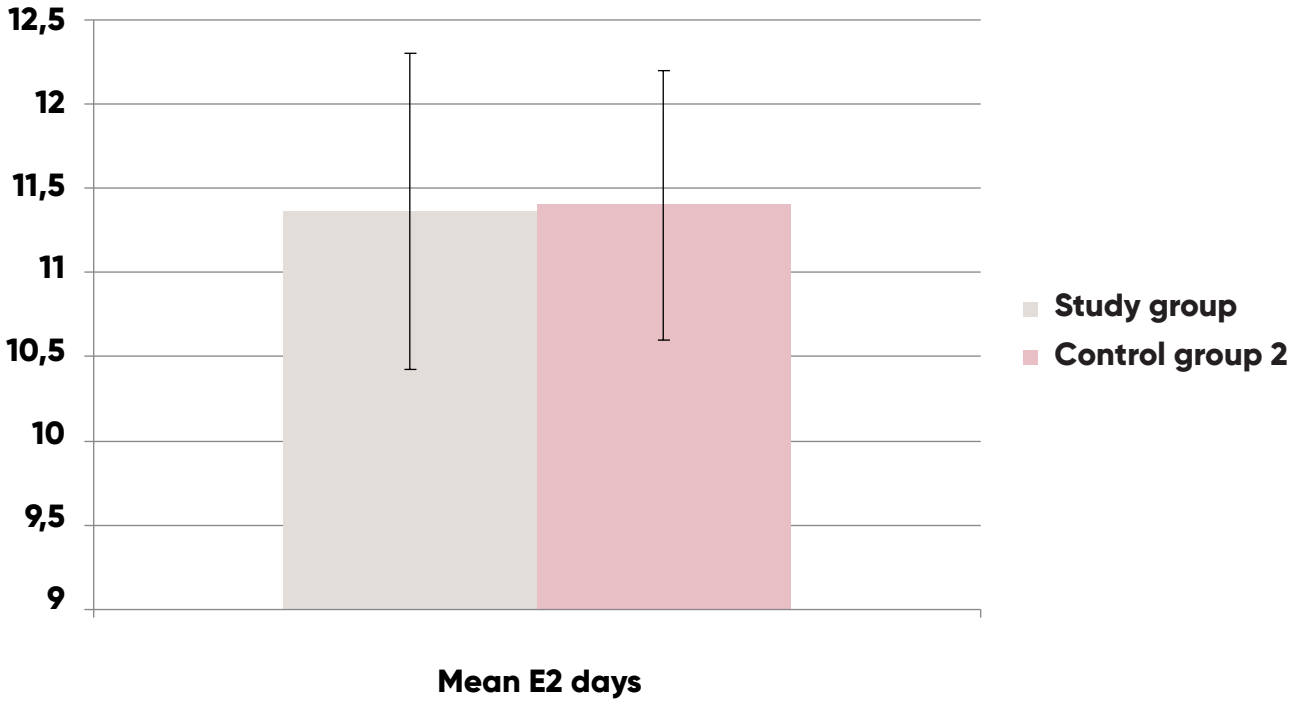
Parameter	PGT-A+ERA Age≥35years (n=122)	PGT-A+ERA Age<35years (n=44)	PGT-A+ERA vs. PGT-A	
	Mean ± SD	Mean± SD	Independent t-test	P-value
Egg Donor Age	23.4±3.7	28 ± 4.2	13.96	0.001
Egg Donor Basal FSH	6.7 ±1.3		2.478	0.014
Patient Basal FSH		7.3 ± 1.5		
Egg Donor AMH	5.8 ± 2.0		10.026	<0.001
Patient AMH		2.3± 1.8		
Sperm concentration	82.3 ± 51.7	71.8 ± 58.4	1.098	0.274
Egg Donor AFC	26.6 ± 8.4		8.130	<0.001
Patient AFC		14.8 ± 7.2		
Egg Donor M II	16.7 ± 4.9		5.543	<0.001
Patient M II		11.6 ± 5.8		
Fertilization rate - fertilized oocytes/M II	0.89 ± 0.10	0.89 ± 0.08	0.059	0.953
Blastocyst formation rate - Vitrified embryos/fertilized oocytes	0.62± 0.19	0.50 ± 0.14	3.754	<0.001

FSH - Follicle-stimulating hormone; AMH - Anti-Müllerian hormone; AFC - Antral follicle count; MII - Metaphase II; PGT-A - Preimplantation genetic testing for aneuploidy; ERA - Endometrial receptivity analysis; Standard deviation (SD)

4.3.7 ERA cycle

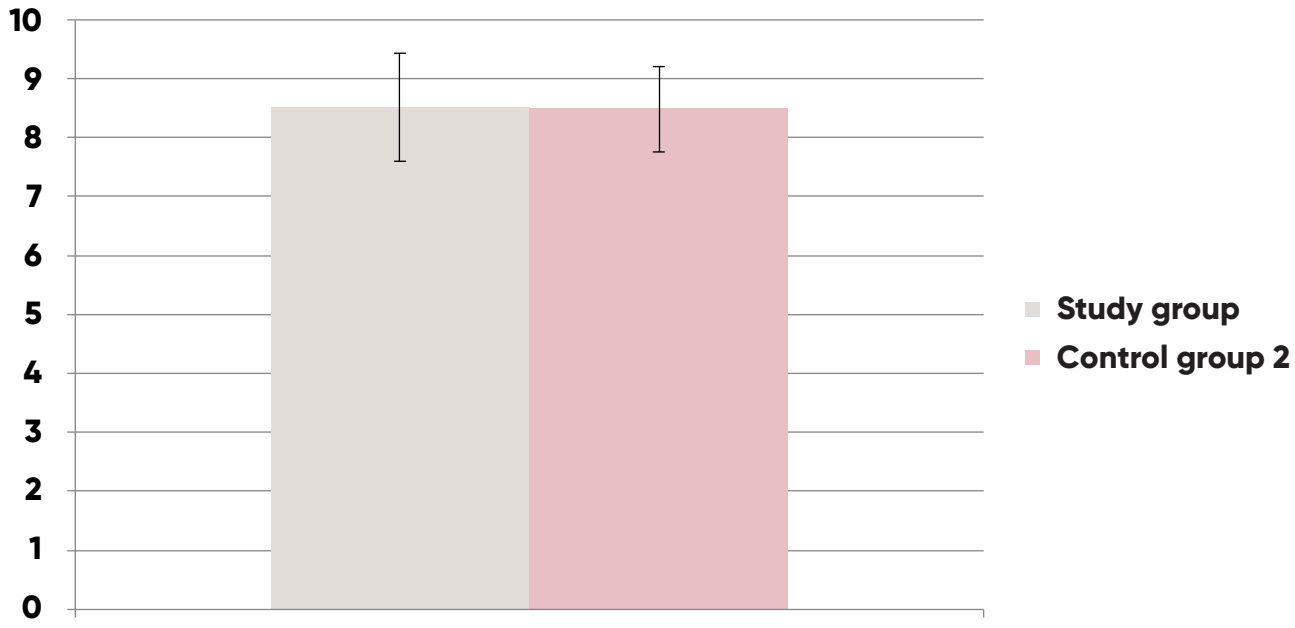
E2 days were measured during the ERA cycle in the study group and during the FET cycle in control group 2. Endometrial thicknesses and P4 levels 24 hours before starting exogenous P4 were also measured in both groups. Additionally, the total hours from P4 initiation to ERA sample collection were evaluated in the study group. The mean values of these parameters are presented in chart 78-80. The difference of these values between the groups was not significant (p>0.05).

Chart 78. The mean E2 days in the groups



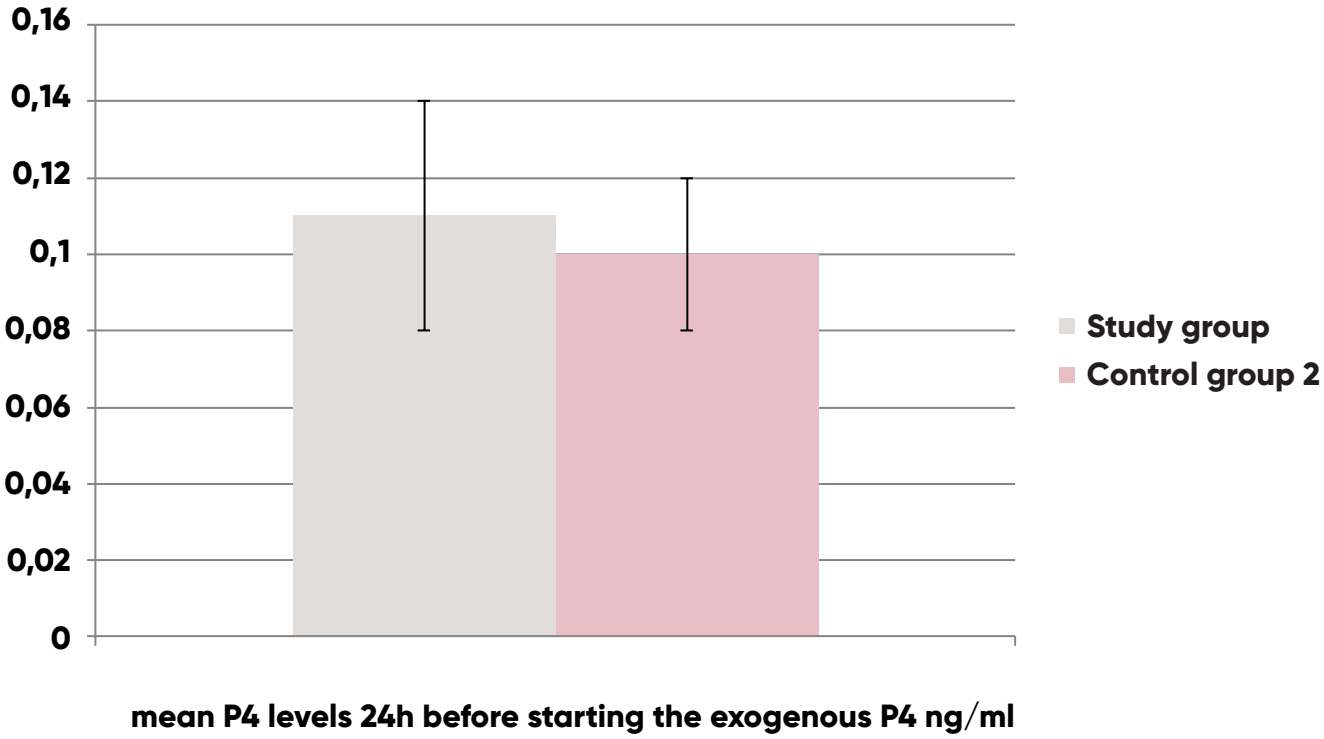
	Study group ERA cycle	Control group 2 ERA cycle
Mean E2 days (SD)	11.4 (0.9)	11.4 (0.8)
Levene's Test for Equality of Variances		
F-test=, p=	0.01, 0.968	
t-test for Equality of Means		
independent t-test=, p=	0.01, 0.969	

Chart 79. The mean endometrial thickness in the groups



	Study group ERA cycle	Control group 2 ERA cycle
Mean endometrial thickness (SD), mm	8.51 (0.91)	8.48 (0.72)
Levene's Test for Equality of Variances		
F-test=, p=	0.29, 0.757	
t-test for Equality of Means		
independent t-test=, p=	0.20, 0.844	

Chart 80. The mean P4 levels 24h before starting the exogenous P4 in the groups

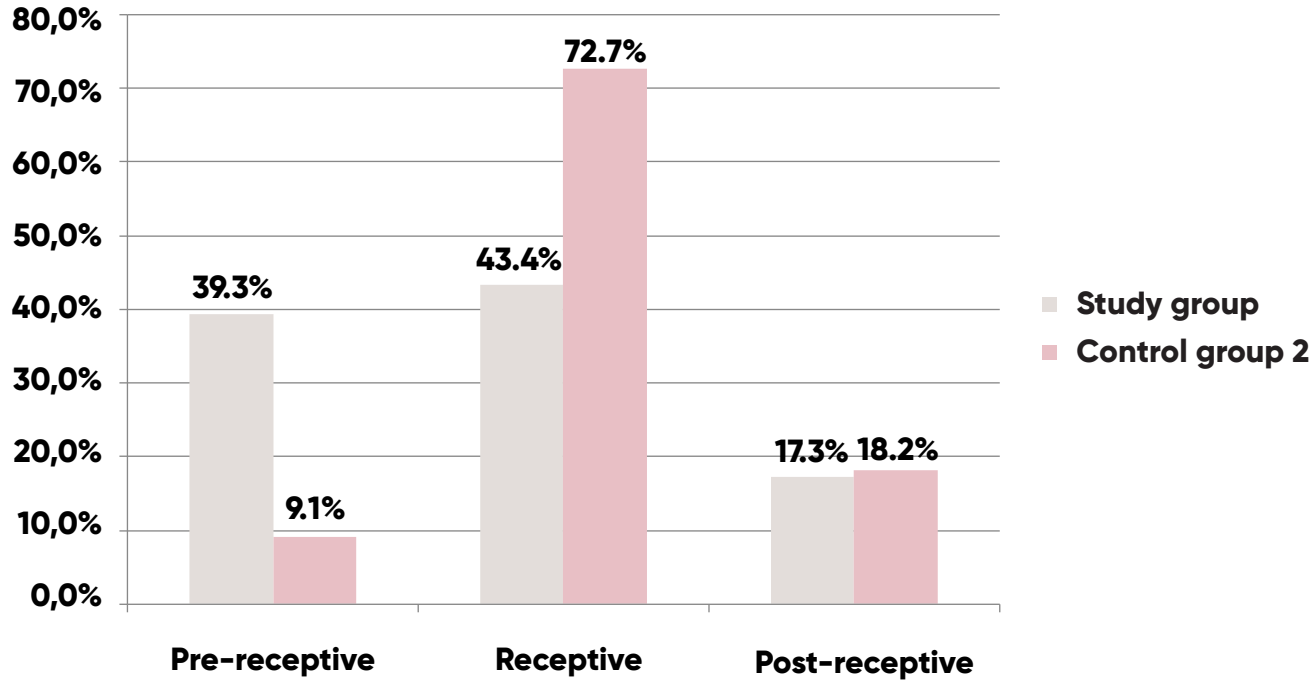


	Study group ERA cycle	Control group 2 ERA cycle
Mean P4 level 24h before starting the exogenous P4 (SD), ng/ml	0.11 (0.03)	0.10 (0.02)
Levene's Test for Equality of Variances		
F-test=, p=	137.55, <0.001	
t-test for Equality of Means		
independent t-test=, p=	2.05, 0.042	

4.3.8 ERA result

The ERA results determined as pre-receptive, receptive and post-receptive in the groups are given in the Chart 81. Pre-receptive and receptive conditions were significantly more prevalent in the study group ($p<0.001$) and receptive conditions were significantly more prevalent in the control group 2 ($p<0.001$).

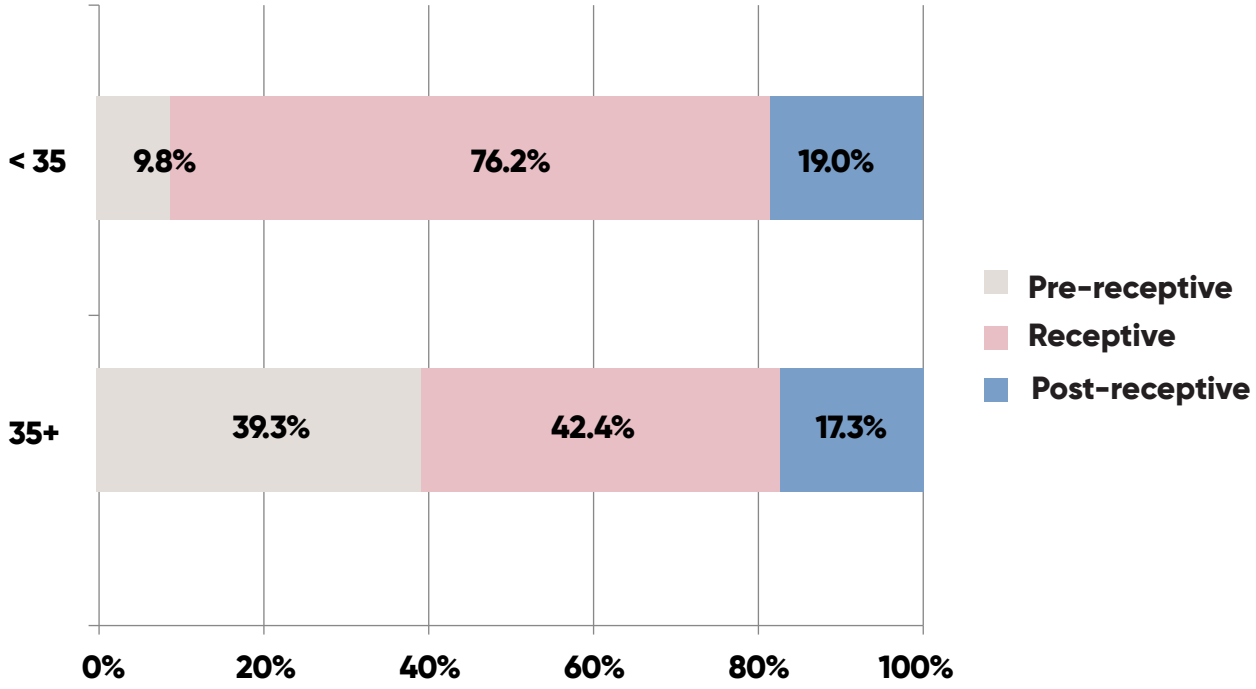
Chart 81. ERA results in study group and control group2 group.



ERA result	Study group	Control group 2	Chi-Square	df	p-value, Asymp. Sig.
Pre-receptive	48 (39.3%)	4 (9.1%)	14.88	2	<0.001
Receptive	53 (43.4%)	32 (72.7%)			
Post-receptive	21 (17.3%)	8 (18.2%)			

The study results reveal that the WOI was modified more frequently in older patients compared to the younger cohort. There was no statistically significant difference in early endometrial receptivity between the two groups. Nonetheless, late endometrial receptivity was more common in older patients **(Figure 7).**

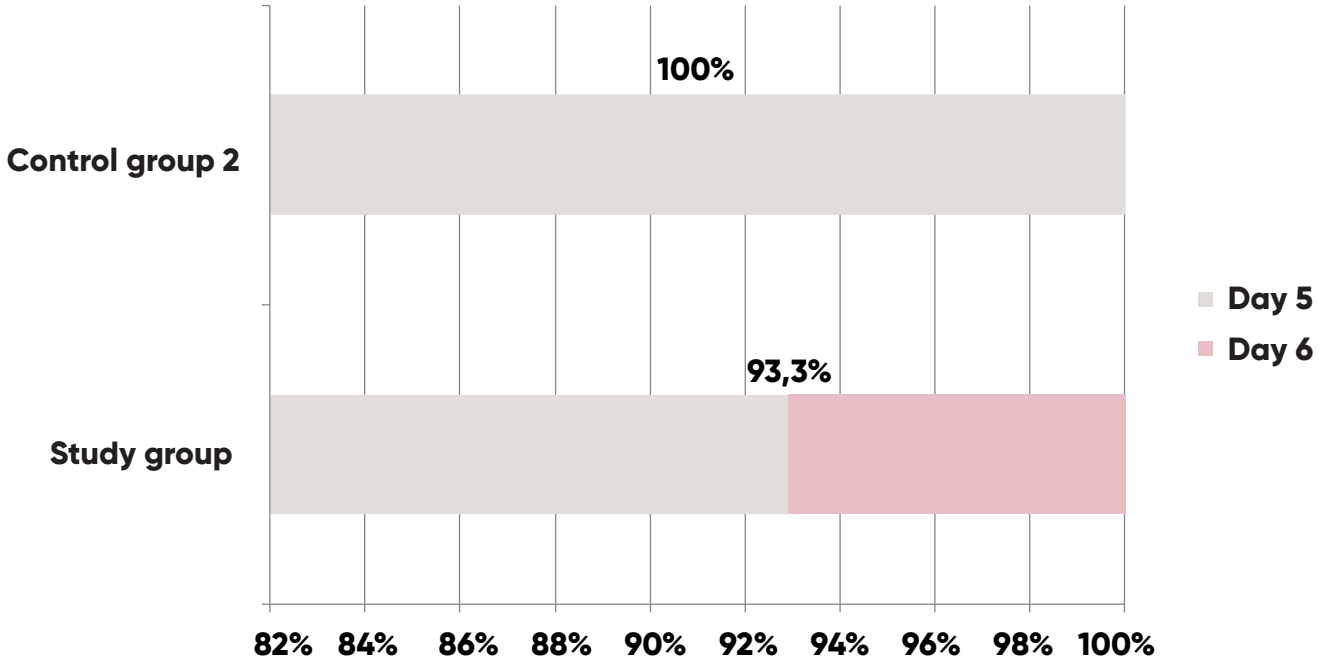
Figure 7| Types of endometrial receptivity in the groups



4.3.9 FET cycle

The total number of developed embryos was 223 in the study group and 72 in control group 2. The data on the day of embryo development at FET are presented in the Chart 82. The distribution of the percentages of embryos developed on days 5 and 6 revealed a significant difference between the study and control groups.

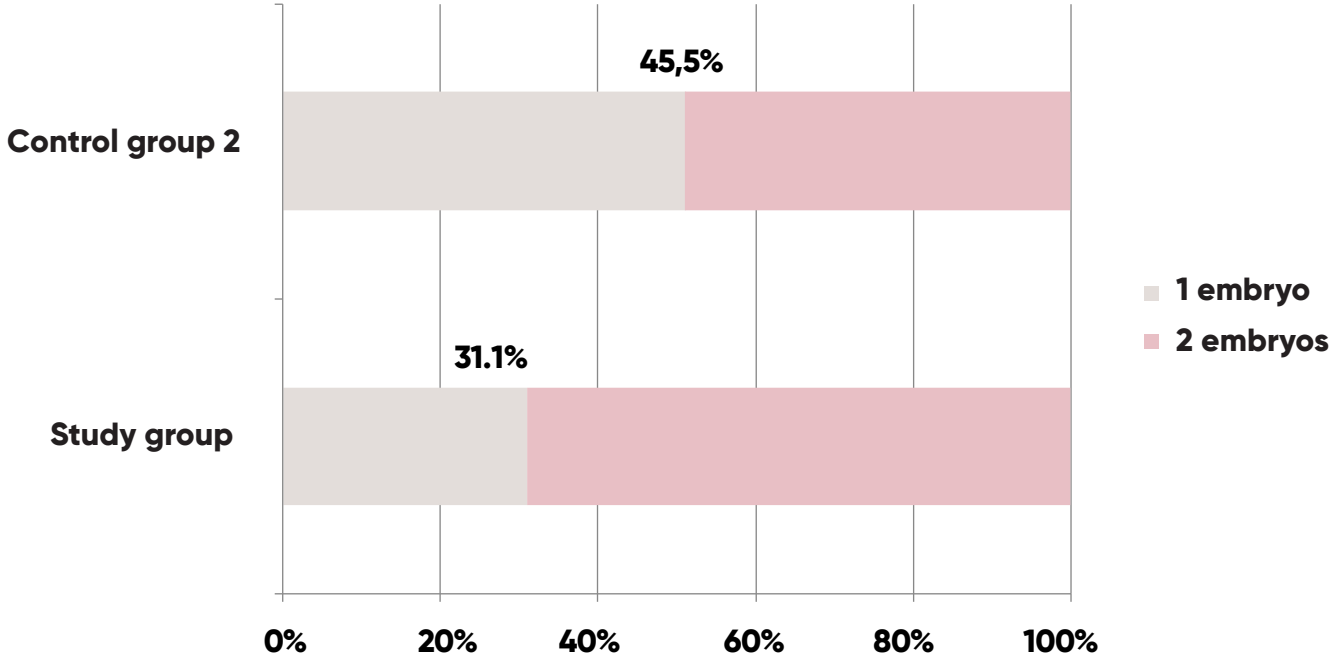
Chart 82. The data of embryo development day at FET in the groups.



Embryo development day at FET	Study group	Control group 2
Day 5, n(%)	208 (93.3%)	72 (100.0%)
Day 6, n(%)	15 (6.7%)	0 (0.0%)

Chi-square tests	Value	df	P - Asymp. Sig. (2- tailed)	P - Exact Sig. (2- tailed)
Pearson Chi- Square	5.08	1	0.022	0.024
Likelihood Ratio	5.12	1	0.023	
Fisher's Exact Test Continuity Correction	4.98	1	0.027	

Chart 83. The data of transferred embryos at FET in the groups.



Transferred embryos perpatient at FET		Study group		Control group 2
1 embryo, n(%)		38 (31.1%)		20 (45.5%)
2 embryos, n(%)		84 (68.9%)		24 (54.5%)
Chi-square tests	**Value**	**df**	**P - Asymp. Sig. (2- tailed)**	**P - Exact Sig. (2- tailed)**
Pearson Chi- Square	2.86	1	0.087	0.089
Likelihood Ratio	2.92	1	0.086	
Fisher's Exact Test Continuity Correction	2.25	1	0.115	

4.3.10 ART outcome

Pregnancy rates defined by beta-hCG value in the study and control groups are given in Chart 84. Odds ratio of the pregnancy in the study group vs. control group 2 was OR=1.54 (95%CI - 0.69-3.43). This value was not significant (p=0.293).

Implantation rate in the study and control groups are given in Chart 85. Number of implanted embryos was 114 (54.1%) in the study group, and 36 – in the control group 2 (50.0%). Odds ratio of the successful implantation in the study group vs. control group 2 was OR=1.05 (95%CI - 0.61-1.78). This value was not significant (p=0.869).

Chart 84. The pregnancy rates in the groups.

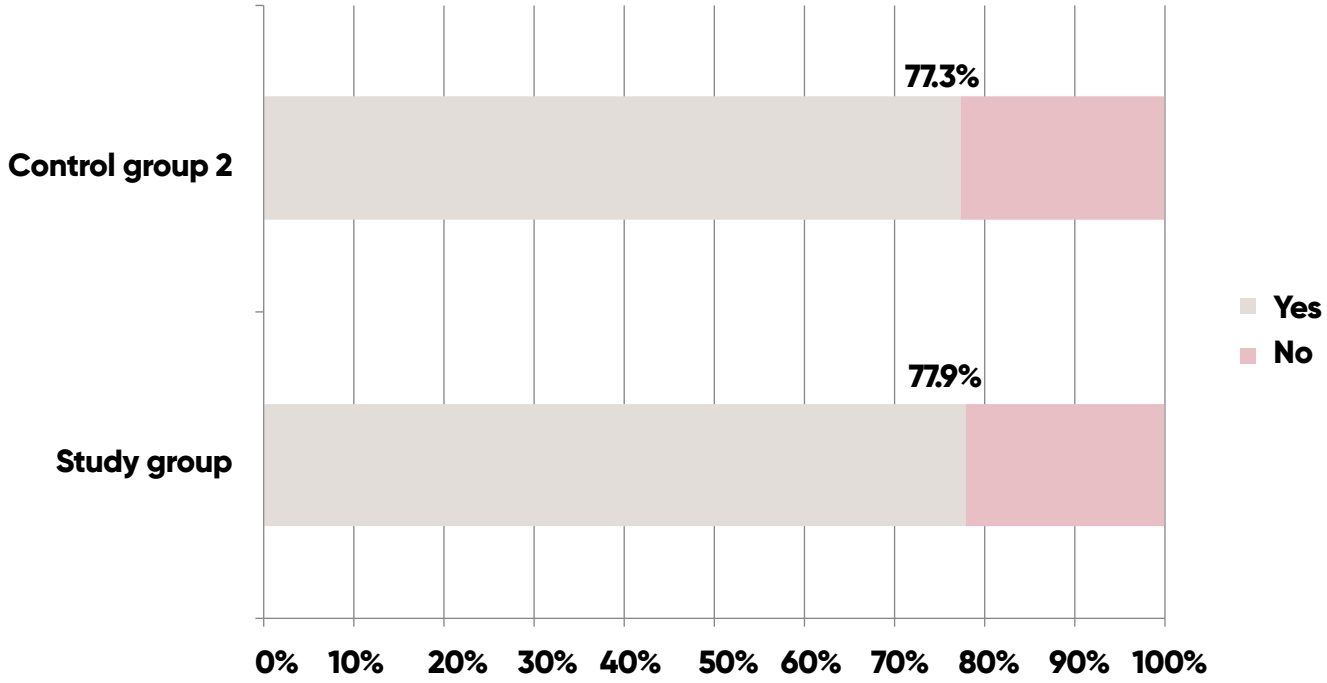
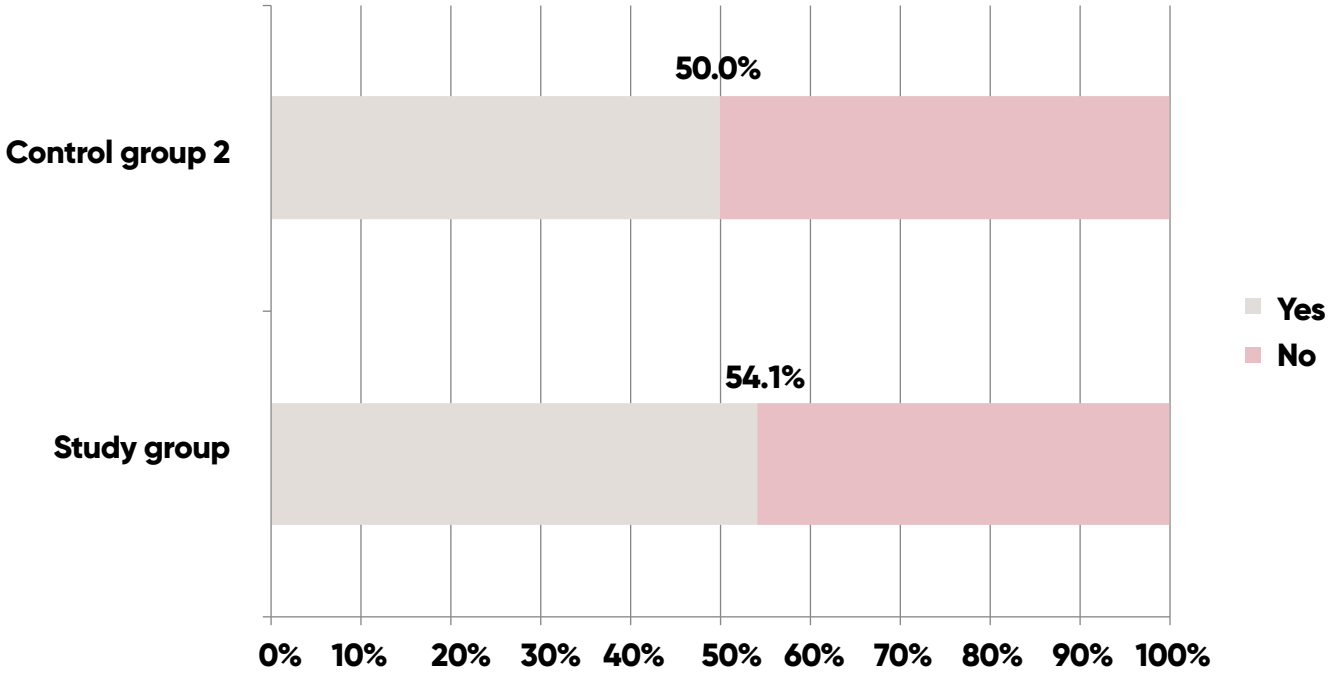
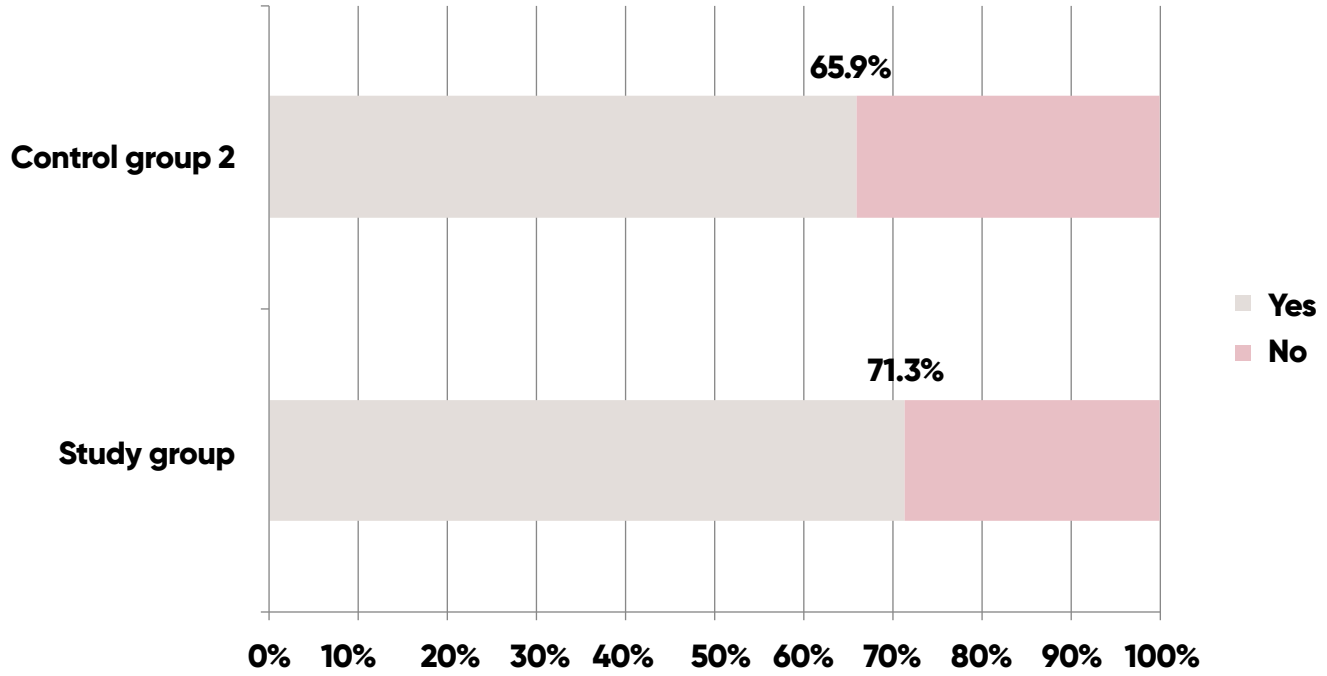


Chart 85. The implantation rates in the groups.



Live birth rate defined by the successful delivery in the study and control groups are given in Chart 86. Odds ratio of the Live birth rate in the study group vs. control group 1 was OR=1.29 (0.62-2.69). This value was not significant (p=0.500).

Chart 86. The live birth rates in the groups.



The summarized data of reproductive outcomes in the groups are given in Table 18.

The pregnancy and obstetrical complications in the groups are given in Table 19.

Table 18. Reproductive Outcomes at First ET during 1-year Follow-up: Intention-to-treat Analysis in the study and control Groups.

Outcome	Study group	Control group 2	study vs. control	
			Odds Ratio (95%CI)	P-value
Transfers, n	122	44		
Pregnancy rate, n (%)	95 (77.9%)	34 (77.3%)	1.03 (0.45-2.36)	0.94
Implantation rate, n (%)	114/223 (54.1%)	36/72 (50.0%)	1.05 (0.61-1.78)	0.87
Live birth rate n (%)	87 (71.3%)	29 (65.9%)	1.29 (0.62-2.69)	0.50
Singleton	63 (72.4%)	24 (82.8%)	1.17 (0.55-2.48)	0.69
Multiple (all twins)	24 (27.6%)	5 (17.2%)	0.55 (0.19-1.60)	0.27
Clinical miscarriages, n (%)	3/95 (3.2%)	2/29 (6.9%)	1.83 (0.63-5.34)	0.27
Biochemical pregnancies, n (%)	5/95 (5.3%)	0/29 (0.0%)	N/A	N/A

Table 19. Pregnancy and Obstetrical complications at First ET during 1-year Follow-up: Intention-to-treat Analysis in the study and control Groups.

Outcome	Study group	Control group 2	study vs. control	
			Odds Ratio (95%CI)	
Ectopic pregnancies, n (%)	0/95 (0.0%)	0/29 (0.0%)	N/A	N/A
Neonatal mortality, n (%)	0/95 (0.0%)	0/29 (0.0%)	N/A	N/A
Preeclampsia, n (%)	6/95 (6.3%)	0/29 (0.0%)	N/A	N/A
HBP, n (%)	0/95 (0.0%)	0/29 (0.0%)	N/A	N/A
Hyperemesis	0/95 (0.0%)	0/29 (0.0%)	N/A	N/A
IUGR	0/95 (0.0%)	0/29 (0.0%)	N/A	N/A
Metabolic Disorders	0/95 (0.0%)	0/29 (0.0%)	N/A	N/A
Infections	0/95 (0.0%)	0/29 (0.0%)	N/A	N/A
Gestational Diabetes	0/95 (0.0%)	0/29 (0.0%)	N/A	N/A
1st trimester Bleeding	36/95 (37.9%)	0/29 (0.0%)	N/A	N/A
3rd trimester Bleeding	0/95 (0.0%)	0/29 (0.0%)	N/A	N/A

4.4 Multiple Regression Analysis

Multiple Regression Analysis

Model 1.

Outcome variable Y1- Implantation Rate
Independent Variables (X):

X2 - Age
X3 - BMI
X4 - Personal History - Allergy
X5 – Personal History - Surgery
X6 - Personal History - Tobacco
X7 – Personal History - Exercise
X8 - Family history - Diabetes
X9 – Family history - Cardiovascular Diseases
X10 - Family history - Oncology
X11 – Family history - Male Infertility
X12 – ART history – Previous Pregnancies
X13 – ART history – Previous Term Pregnancies
X14 – ART history – Previous Miscarriages
X15 – ART history – Previous Live Births
X16 – ART history – Previous Curretages
X17 – ART history – Implantation Failure
X18 – ART history – Implantation Failure with PGT-A
X19 – ART history – Infertility Duration
X20 – Menstrual Cycle Regularity
X21 – ART indication – Male Factor
X22 – ART indication – Advance Maternal Age
X23– ART indication – Endometriosis
X24 – ART indication – Ovarian Factor
X25 – ART indication – Tubal Factor
X26 – ART indication – Diminished Ovarian Reserve
X27 – ART indication – RIF (>2 IVF cycles)
X28 – ART indication – RPL Failure (>2)
X29 – Uterine Pathology – Adenomyosis
X30 – Uterine Pathology – Myoma
X31 – Donor Egg / Patient Oocytes basal FSH
X32– Donor Egg / Patient Oocytes basal AMH
X33 – Sperm concentration
X34 – Donor Egg / Patient Oocytes AFC
X35 – Donor Egg / Patient MII Oocytes

X36 – Fertilized Oocytes
X37 – Fertilization Rate
X38 – PGTA Analyzed
X39 – Vitrified Embryos
X40 – Blastocyst formation rate
X41 – FET cycle E2 days
X42– FET cycle Endometrial Thickness
X43– FET cycle - measured during the previous 24th starting the exogenous P4
X44– ERA Result

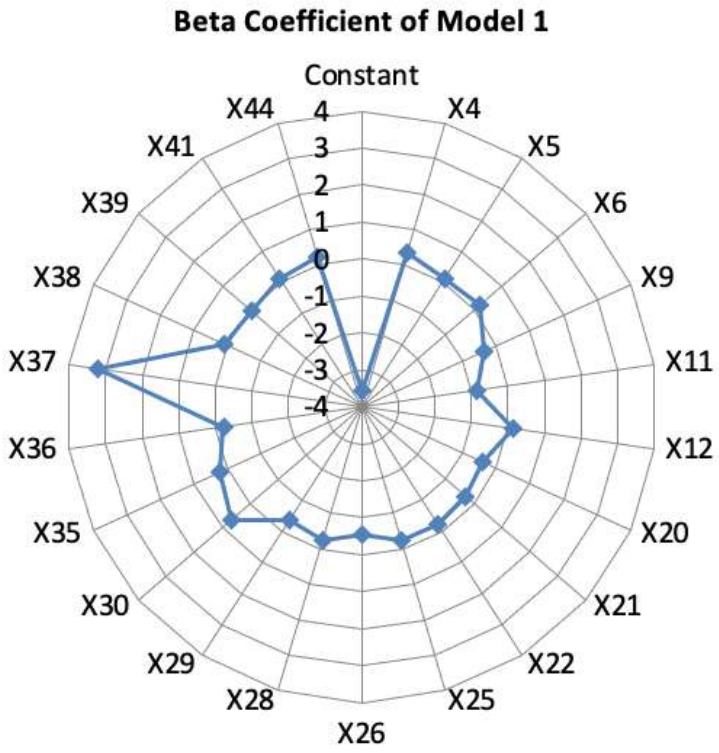
Variables	Beta Coefficient	t-test	Sig.
Constant	-3.04	-2.13	.035
X2 - Age	.01	.79	.434
X3 - BMI	-.03	-1.48	.143
X4 - Personal History - Allergy	.79	1.75	.082
X5 – Personal History - Surgery	.25	2.49	.014
X6 - Personal History - Tobacco	.44	2.57	.011
X7 – Personal History - Exercise	-.62	-2.47	.015
X8 - Family history - Diabetes	.29	1.37	.173
X9 – Family history - Cardiovascular Diseases	-.31	-2.54	.012
X10 - Family history - Oncology	-.93	-1.45	.151
X11 – Family history - Male Infertility	-.65	-2.67	.009
X12 – ART history – Previous Pregnancies	.14	.94	.350
X13 – ART history – Previous Term Pregnancies	-.06	-.46	.650
X14 – ART history – Previous Miscarriages	.06	.60	.548
X15 – ART history – Previous Live Births	.00	2.50	.014
X16 – ART history – Previous Curretages	.12	1.12	.267
X17 – ART history – Implantation Failure	-.08	-1.64	.104
X18 – ART history – Implantation Failure with PGT-A	.07	.82	.413
X19 – ART history – Infertility Duration	-.01	-.85	.399
X20 – Menstrual Cycle Regularity	-.64	-3.87	.000
X21 – ART indication – Male Factor	-.22	-2.13	.035
X22 – ART indication – Advance Maternal Age	-.26	-1.89	.061
X23– ART indication – Endometriosis	-.09	-.43	.669
X24 – ART indication – Ovarian Factor	-.24	-.99	.324
X25 – ART indication – Tubal Factor	-.47	-3.34	.001
X26 – ART indication – Diminished Ovarian Reserve	-.44	-2.16	.033
X27 – ART indication – RIF (>2 IVF cycles)	.25	1.71	.090
X28 – ART indication – RPL Failure (>2)	-.38	-2.12	.036
X29 – Uterine Pathology – Adenomyosis	-.18	-1.23	.222
X30 – Uterine Pathology – Myoma	.88	3.17	.002
X31 – Donor Egg / Patient Oocytes basal FSH	.01	.42	.677
X32– Donor Egg / Patient Oocytes basal AMH	-.07	-1.72	.088
X33 – Sperm concentration	.00	1.75	.084
X34 – Donor Egg / Patient Oocytes AFC	.01	1.12	.267
X35 – Donor Egg / Patient MII Oocytes	.09	1.07	.287
X36 – Fertilized Oocytes	-.08	-.83	.409
X37 – Fertilization Rate	1.78	1.25	.213
X38 – PGTA Analyzed	.12	2.60	.010
X39 – Vitrified Embryos	-.07	-1.14	.256
X40 – Blastocyst formation rate	-.48	-.65	.519
X41 – FET cycle E2 days	.13	2.52	.013
X42– FET cycle Endometrial Thickness	.06	1.12	.267
X43– FET cycle - measured during the previous 24th starting the exogenous P4	3.75	1.07	.288
X44– ERA Result	.28	5.28	.000

Variables in rose cells have been excluded from the model.

Backward Stepwise Exclusion

A sequence of excluded variables – X31, X15, X23, X13, X40, X19, X18, X42, X8, X43, X17, X3, X2, X34, X33, X14, X24, X7, X10, X27, X32.

Variables	***Beta Coefficient***	***t-test***	***Sig.***
Constant	*-3.58*	*-3.51*	*.001*
X4 - Personal history - Allergy	*.35*	*2.57*	*.011*
X5 - Personal history - Surgery	*.13*	*2.11*	*.037*
X6 - Personal history - Tobacco	*.22*	*2.61*	*.010*
X9 - Family history - Cardiovascular Diseases	*-.36*	*-4.38*	*.000*
X11 - Family history - Male Infertility	*-.86*	*-5.90*	*.000*
X12 - ART history – Previous Pregnancies	*.14*	*2.72*	*.007*
X20 - Menstrual Cycle Regularity	*-.42*	*-4.34*	*.000*
X21 - ART indication – Male Factor	*-.28*	*-4.25*	*.000*
X22 - ART indication – Advance Maternal Age	*-.20*	*-2.26*	*.026*
X25 - ART indication – Tubal Factor	*-.23*	*-2.55*	*.012*
X26 - ART indication – Diminished Ovarian Reserve	*-.54*	*-4.77*	*.000*
X28 - ART indication – RPL Failure (>2)	*-.22*	*-2.00*	*.048*
X29 - Uterine Pathology – Adenomyosis	*-.36*	*-5.58*	*.000*
X30 - Uterine Pathology – Myoma	*.68*	*5.24*	*.000*
X35 - Donor Egg / Patient MII Oocytes	*.22*	*3.80*	*.000*
X36 - Fertilized Oocytes	*-.22*	*-3.51*	*.001*
X37 - Fertilization Rate	*3.20*	*3.20*	*.002*
X38 - PGTA Analyzed	*.10*	*4.58*	*.000*
X39 - Vitrified Embryos	*-.06*	*-2.79*	*.000*
X41 - FET cycle E2 days	*.11*	*3.82*	*.006*
X44 - ERA Result	*.24*	*6.02*	*.000*



Reporting results in APA style

Results of the multiple linear regression indicated that there was a strong collective significant effect between the variables - X4, X5, X6, X9, X11, X12, X20, X21, X22, X25,X26, X28, X29, X30, X35, X36, X37, X38, X39, X41, X44 and Outcome variable Y1 - Implantation Rate [$F(21, 138) = 11.00$, $p < .001$, $R2 = 0.63$, $R2adj = 0.57$].

ANOVA table

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Regression	21	15.46	.74	11.00	.000
Residual	138	9.24	.07		
Total	159	24.69			

Model Summary (Impl_Rate)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.79	.63	.57	.26

Source: SPSS 23.0 (IBM, Chicago, IL, USA).

Multiple Regression Analysis
Model 2.

Outcome variable Y1- Pregnancy Rate
Independent Variables (X):

X2 - Age
X3 - BMI
X4 - Personal History - Allergy
X5 – Personal History - Surgery
X6 - Personal History - Tobacco
X7 – Personal History - Exercise
X8 - Family history - Diabetes
X9 – Family history - Cardiovascular Diseases
X10 - Family history - Oncology
X11 – Family history - Male Infertility
X12 – ART history – Previous Pregnancies
X13 – ART history – Previous Term Pregnancies
X14 – ART history – Previous Miscarriages
X15 – ART history – Previous Live Births
X16 – ART history – Previous Curettages
X17 – ART history – Implantation Failure
X18 – ART history – Implantation Failure with PGT-A
X19 – ART history – Infertility Duration
X20 – Menstrual Cycle Regularity
X21 – ART indication – Male Factor
X22 – ART indication – Advance Maternal Age
X23– ART indication – Endometriosis
X24 – ART indication – Ovarian Factor
X25 – ART indication – Tubal Factor
X26 – ART indication – Diminished Ovarian Reserve
X27 – ART indication – RIF (>2 IVF cycles)
X28 – ART indication – RPL Failure (>2)
X29 – Uterine Pathology – Adenomyosis
X30 – Uterine Pathology – Myoma
X31 – Donor Egg / Patient Oocytes basal FSH
X32– Donor Egg / Patient Oocytes basal AMH
X33 – Sperm concentration
X34 – Donor Egg / Patient Oocytes AFC
X35 – Donor Egg / Patient MII Oocytes
X36 – Fertilized Oocytes
X37 – Fertilization Rate
X38 – PGTA Analyzed
X39 – Vitrified Embryos
X40 – Blastocyst formation rate
X41 – FET cycle E2 days
X42– FET cycle Endometrial Thickness
X43– FET cycle - measured during the previous 24th starting the exogenous P4
X44– ERA Result

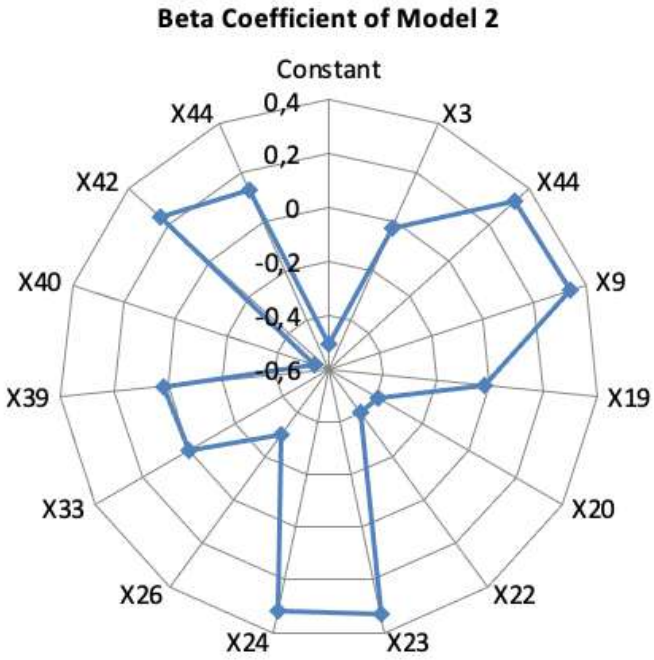
Variables	Beta Coefficient	t-test	Sig.
Constant	-1.57	-.98	.329
X2 - Age	.05	2.38	.019
X3 - BMI	-.07	-2.62	.010
X4 - Personal History - Allergy	1.86	3.68	.000
X5 – Personal History - Surgery	.09	.83	.410
X6 - Personal History - Tobacco	.27	1.40	.163
X7 – Personal History - Exercise	-.87	-3.11	.002
X8 - Family history - Diabetes	.25	1.07	.285
X9 – Family history - Cardiovascular Diseases	.28	2.04	.044
X10 - Family history - Oncology	-2.29	-3.19	.002
X11 – Family history - Male Infertility	-.53	-1.94	.055
X12 – ART history – Previous Pregnancies	-.11	-.65	.518
X13 – ART history – Previous Term Pregnancies	.06	.39	.697
X14 – ART history – Previous Miscarriages	.14	1.31	.192
X15 – ART history – Previous Live Births	.00	-.47	.638
X16 – ART history – Previous Curretages	.43	3.69	.000
X17 – ART history – Implantation Failure	-.06	-1.07	.287
X18 – ART history – Implantation Failure with PGT-A	-.08	-.77	.441
X19 – ART history – Infertility Duration	-.02	-1.34	.182
X20 – Menstrual Cycle Regularity	-.69	-3.71	.000
X21 – ART indication – Male Factor	.12	1.05	.296
X22 – ART indication – Advance Maternal Age	-.49	-3.20	.002
X23– ART indication – Endometriosis	.14	.57	.569
X24 – ART indication – Ovarian Factor	.26	.96	.339
X25 – ART indication – Tubal Factor	-.02	-.14	.890
X26 – ART indication – Diminished Ovarian Reserve	-.43	-1.84	.068
X27 – ART indication – RIF (>2 IVF cycles)	-.09	-.53	.597
X28 – ART indication – RPL Failure (>2)	-.26	-1.28	.202
X29 – Uterine Pathology – Adenomyosis	-.03	-.20	.843
X30 – Uterine Pathology – Myoma	.87	2.78	.006
X31 – Donor Egg / Patient Oocytes basal FSH	.02	.78	.436
X32– Donor Egg / Patient Oocytes basal AMH	-.16	-3.53	.001
X33 – Sperm concentration	.00	3.38	.001
X34 – Donor Egg / Patient Oocytes AFC	.02	2.22	.029
X35 – Donor Egg / Patient MII Oocytes	-.08	-.82	.412
X36 – Fertilized Oocytes	.11	1.00	.317
X37 – Fertilization Rate	-.71	-.44	.659
X38 – PGTA Analyzed	.03	.60	.552
X39 – Vitrified Embryos	-.03	-.40	.689
X40 – Blastocyst formation rate	-1.29	-1.54	.127
X41 – FET cycle E2 days	.10	1.65	.101
X42– FET cycle Endometrial Thickness	.19	3.34	.001
X43– FET cycle - measured during the previous 24th starting the exogenous P4	8.23	2.09	.039
X44– ERA Result	.18	3.13	.002

Variables in rose cells have been excluded from the model.

Backward Stepwise Exclusion

A sequence of excluded variables – X25, X29, X37, X16, X38, X13, X21, X12, X18, X5, X35, X14, X36, X15, X43, X7, X30, X28, X6, X31, X27, X8, X17, X41, X2, X11, X34, X32, X10.

Variables	*Beta Coefficient*	*t-test*	*Sig.*
Constant	*-.51*	*-2.53*	*.014*
X3 - BMI	*-.03*	*-3.20*	*.002*
X4 - Personal History - Allergy	*.33*	*2.12*	*.035*
X9 – Family history - Cardiovascular Diseases	*.34*	*3.79*	*.000*
X19 – ART history – Infertility Duration	*-.02*	*-2.35*	*.020*
X20 – Menstrual Cycle Regularity	*-.39*	*-4.34*	*.000*
X22 – ART indication – Advance Maternal Age	*-.40*	*-4.39*	*.000*
X23– ART indication – Endometriosis	*.33*	*3.29*	*.001*
X24 – ART indication – Ovarian Factor	*.32*	*2.40*	*.017*
X26 – ART indication – Diminished Ovarian Reserve	*-.30*	*-2.32*	*.022*
X33 – Sperm concentration	*.00*	*3.92*	*.000*
X39 – Vitrified Embryos	*.02*	*2.30*	*.023*
X40 – Blastocyst formation rate	*-.54*	*-2.72*	*.007*
X42– FET cycle Endometrial Thickness	*.24*	*7.45*	*.000*
X44– ERA Result	*.13*	*3.04*	*.003*



Reporting results in APA style

Results of the multiple linear regression indicated that there was a strong collective significant effect between the variables – X3, X4, X9, X19, X20, X22, X23, X24, X26, X33, X39, X40, X42, X44 and Outcome variable Y1 - Pregnancy Rate [F(14, 151) = 9.73, p < .001, R2 = 0.47, R2adj = 0.43].

ANOVA table

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Regression	14	13.63	.97	9.73	.000
Residual	151	15.12	.10		
Total	165	28.75			

Model Summary (Pregnancy Rate)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.69	.47	.43	.32

Source: SPSS 23.0 (IBM, Chicago, IL, USA).

Multiple Regression Analysis
Model 3.
Outcome variable Y1- Livebirth Rate
Independent Variables (X):

X2 - Age
X3 - BMI
X4 - Personal History - Allergy
X5 – Personal History - Surgery
X6 - Personal History - Tobacco
X7 – Personal History - Exercise
X8 - Family history - Diabetes
X9 – Family history - Cardiovascular Diseases
X10 - Family history - Oncology
X11 – Family history - Male Infertility
X12 – ART history – Previous Pregnancies
X13 – ART history – Previous Term Pregnancies
X14 – ART history – Previous Miscarriages
X15 – ART history – Previous Live Births
X16 – ART history – Previous Curretages
X17 – ART history – Implantation Failure
X18 – ART history – Implantation Failure with PGT-A
X19 – ART history – Infertility Duration
X20 – Menstrual Cycle Regularity
X21 – ART indication – Male Factor
X22 – ART indication – Advance Maternal Age
X23– ART indication – Endometriosis
X24 – ART indication – Ovarian Factor
X25 – ART indication – Tubal Factor
X26 – ART indication – Diminished Ovarian Reserve
X27 – ART indication – RIF (>2 IVF cycles)
X28 – ART indication – RPL Failure (>2)
X29 – Uterine Pathology – Adenomyosis
X30 – Uterine Pathology – Myoma
X31 – Donor Egg / Patient Oocytes basal FSH
X32– Donor Egg / Patient Oocytes basal AMH
X33 – Sperm concentration
X34 – Donor Egg / Patient Oocytes AFC
X35 – Donor Egg / Patient MII Oocytes
X36 – Fertilized Oocytes
X37 – Fertilization Rate
X38 – PGTA Analyzed
X39 – Vitrified Embryos
X40 – Blastocyst formation rate
X41 – FET cycle E2 days
X42– FET cycle Endometrial Thickness
X43– FET cycle - measured during the previous 24th starting the exogenous P4
X44– ERA Result

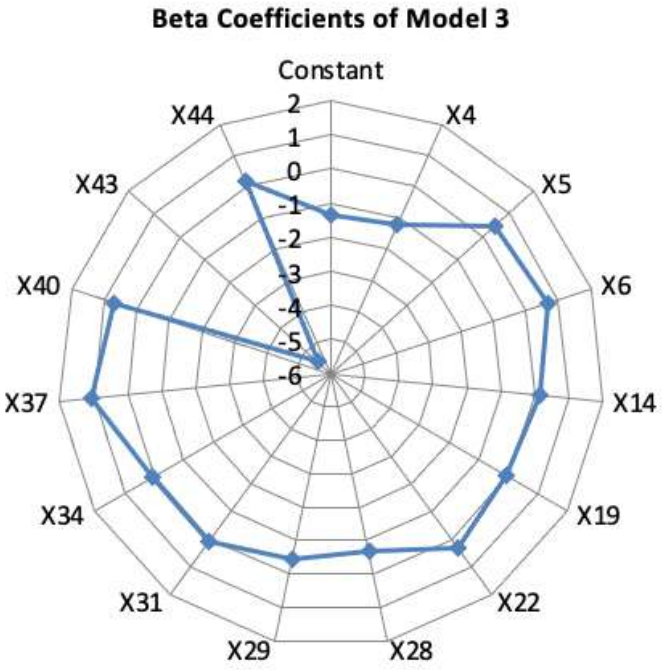
Variables	Beta Coefficient	t-test	Sig.
Constant	18.14	15.00	.000
X2 - Age	.08	3.64	.000
X3 - BMI	.04	1.37	.175
X4 - Personal History - Allergy	-2.21	-2.43	.017
X5 – Personal History - Surgery	.90	5.76	.000
X6 - Personal History - Tobacco	1.23	5.99	.000
X7 – Personal History - Exercise	.19	.37	.715
X8 - Family history - Diabetes	-.20	-.68	.496
X9 – Family history - Cardiovascular Diseases	-1.33	-9.76	.000
X10 - Family history - Oncology	-.25	-.26	.792
X11 – Family history - Male Infertility	.05	.19	.848
X12 – ART history – Previous Pregnancies	1.29	5.68	.000
X13 – ART history – Previous Term Pregnancies	.00	.00	1.000
X14 – ART history – Previous Miscarriages	-.01	-.10	.920
X15 – ART history – Previous Live Births	.00	.00	1.000
X16 – ART history – Previous Curretages	-.57	-2.85	.005
X17 – ART history – Implantation Failure	-.23	-3.68	.000
X18 – ART history – Implantation Failure with PGT-A	.29	2.35	.021
X19 – ART history – Infertility Duration	-.08	-5.42	.000
X20 – Menstrual Cycle Regularity	.43	1.89	.062
X21 – ART indication – Male Factor	-.62	-5.95	.000
X22 – ART indication – Advance Maternal Age	.53	2.59	.011
X23– ART indication – Endometriosis	-.69	-2.67	.009
X24 – ART indication – Ovarian Factor	-.67	-2.45	.017
X25 – ART indication – Tubal Factor	-.23	-1.17	.244
X26 – ART indication – Diminished Ovarian Reserve	-.35	-1.21	.230
X27 – ART indication – RIF (>2 IVF cycles)	-.21	-1.21	.230
X28 – ART indication – RPL Failure (>2)	-1.60	-6.61	.000
X29 – Uterine Pathology – Adenomyosis	-.77	-2.78	.007
X30 – Uterine Pathology – Myoma	-.25	-.39	.697
X31 – Donor Egg / Patient Oocytes basal FSH	.19	4.28	.000
X32– Donor Egg / Patient Oocytes basal AMH	.07	1.48	.142
X33 – Sperm concentration	-.01	-4.78	.000
X34 – Donor Egg / Patient Oocytes AFC	.06	5.03	.000
X35 – Donor Egg / Patient MII Oocytes	.07	.44	.663
X36 – Fertilized Oocytes	-.07	-.43	.665
X37 – Fertilization Rate	2.25	.89	.378
X38 – PGTA Analyzed	.16	2.30	.024
X39 – Vitrified Embryos	-.20	-1.97	.052
X40 – Blastocyst formation rate	2.50	2.36	.021
X41 – FET cycle E2 days	.10	1.56	.123
X42– FET cycle Endometrial Thickness	-.43	-5.49	.000

X43– FET cycle - measured during the previous 24th starting the exogenous P4	*-16.43*	*-2.42*	*.018*
X44– ERA Result	*.41*	*4.18*	*.000*

Variables in rose cells have been excluded from the model.

Backward Stepwise Exclusion
A sequence of excluded variables – X13, X15, X12, X39, X11, X24, X38, X8, X17, X27, X25, X3, X10, X9, X33, X20, X7, X18, X32, X35, X42, X41, X2, X23, X21, X26, X36, X30, X16

Variables	***Beta Coefficient***	***t-test***	***Sig.***
Constant	*-1.33*	*-2.61*	*0.10*
X4 - Personal History - Allergy	*-1.23*	*-5.03*	*.000*
X5 – Personal History - Surgery	*.45*	*4.63*	*.000*
X6 - Personal History - Tobacco	*.67*	*5.29*	*.000*
X14 – ART history – Previous Miscarriages	*.17*	*2.11*	*.037*
X19 – ART history – Infertility Duration	*-.06*	*-4.36*	*.000*
X22 – ART indication – Advance Maternal Age	*.34*	*3.83*	*.000*
X28 – ART indication – RPL Failure (>2)	*-.67*	*-3.49*	*.001*
X29 – Uterine Pathology – Adenomyosis	*-.43*	*-3.87*	*.000*
X31 – Donor Egg / Patient Oocytes basal FSH	*.10*	*3.10*	*.002*
X34 – Donor Egg / Patient Oocytes AFC	*.03*	*5.65*	*.000*
X37 – Fertilization Rate	*1.08*	*2.41*	*.018*
X40 – Blastocyst formation rate	*.69*	*3.38*	*.001*
X43– FET cycle - measured during the previous 24th starting the exogenous P4	*-5.47*	*-3.78*	*.000*
X44– ERA Result	*.18*	*3.24*	*.002*



Reporting results in APA style

Results of the multiple linear regression indicated that there was a strong collective significant effect between the variables – X4, X5, X6, X14, X19, X22, X28, X29, X31, X34, X37, X40, X43, X44 and Outcome variable Y1 - Livebirth Rate [$F(14, 121) = 11.20$, $p < .001$, $R2 = 0.56$, $R2adj = 0.51$].

ANOVA table

Source	DF	Sum of Square	Mean Square	F Statistic	P-value
Regression	14	24.03	1.72	11.20	.000
Residual	121	18.53	.15		
Total	135	42.56			

Model Summary (Pregnancy Rate)

R	R Square	Adjusted R Square	Std. Error of the Estimate
.75	.56	.51	.39

Source: SPSS 23.0 (IBM, Chicago, IL, USA).

DISCUSSION

Successful implantation arises from complex interactions between the decidualized endometrium and the implanting embryo. Improving ART outcomes in elderly patients and those with RIF represents a significant barrier in reproductive treatment, and our objective was to discover diagnostic methods to enhance these outcomes.

We identified three cohorts for this research. The study group and control group 2 evaluated both embryo and endometrial factors, while control group 1 tested only the embryo factor. All patients in this investigation had varied reproductive histories, and those in both the study group and control group 1 were of advanced maternal age.

Since age is one of the crucial factors in infertility and ART outcomes, we compared results within the same age groups using different methods, as well as across various age groups. We aimed to determine how egg donation, PGT-A, with or without ERA contributes to achieving high success rates in advanced-age patients.

When comparing the baseline epidemiological characteristics, including BMI, diabetes, metabolic disorders, coronary heart disease, neurological disorders, psychiatric conditions, allergic conditions, cancer, lifestyle factors (including smoking, alcohol use, and physical activity), previous surgical treatments, and family history, no statistically significant differences were found between the groups.

Similarly, regarding the reproductive history, factors such as male infertility, menstrual cycle disorders, structural abnormalities of the uterus, endometriosis, leiomyoma, recurrent miscarriages, previous curettage procedures, prior pregnancies, and live births showed no significant differences among groups. In egg donor cycles, parameters including AFC, AMH levels, basal FSH levels, oocyte quantity, fertilization rates, and blastocyst formation rates were comparable across the study group and control group 1. No statistically significant differences were found in follicular phase duration and endometrial thickness in ERA and FET cycles.

As part of the routine infertility work-up, all patients underwent office hysteroscopy (if not previously performed), along with assessments for insulin resistance and thyroid profile. Thrombophilia screening, which included testing for lupus anticoagulant and anticardiolipin antibodies (IgG and IgM), was also conducted, with all findings within normal ranges.

To minimize bias, all transferred embryos were of high quality and confirmed to be euploid.

Our research reveals statistically significant results, namely 95 pregnancies from 122 pETs (77.9%) in the study group, in contrast to 76 pregnancies from 132 ETs (57.6%) in control group 1. This unequivocally demonstrates the benefits of pET as directed by the ERA. The implantation rates were 54.1% in the study group and 35.5% in control group 1.

The live birth rates were 71.3% for the PGT-A+ERA group and 39.4% for the PGT-A group. These results demonstrate that ERA facilitates successful implantation and promotes normal implantation and decidualization, resulting in reduced pregnancy problems and a decreased clinical miscarriage rate in the PGT-A+ERA cohorts.

The standard histological criteria for dating the luteal phase endometrium are based on predictable events in the glandular and stromal compartments during the menstrual cycle. Key indicators include glandular mitosis (early luteal phase), secretory changes (mid-luteal phase), stromal mitosis, and pseudo-decidualization (late luteal phase). An experienced pathologist can deduce the cycle day from these histological features, with asynchrony of more than two days between histological and actual cycle dating considered pathological — a condition known as "luteal phase defect", historically managed with progesterone supplementation.

Histological dating has largely been replaced by molecular expression-based assays such as Receptiva, the Endometrial Function Test, and the ERA, with the ERA being the most widely used. Notably, while histological dating can vary depending on the biopsy site within the uterus, ERA results are consistent across different locations within the endometrium. This consistency underscores the advantages of molecular assays like ERA in accurately assessing endometrial receptivity, regardless of biopsy site variability, which has been a limitation of traditional histological methods (Place et al., 2023).

For numerous decades, the correlation between the characterisation of WOI and outcomes in ART has been inconsistent. Recent advancements in transcriptome profiling have facilitated a more accurate analysis of the window of implantation (WOI), resulting in the creation of various transcriptomic tests for endometrial receptivity (TER). The ERA test developed by Igenomix is among the most commonly utilized. Evaluating the efficacy of TER-guided tailored pET in ART is essential. The synthesis evidence was utilized to estimate critical clinical outcomes, yielding reliable and precise statistics to facilitate clinical decision-making in reproductive medicine (Glujovsky et al., 2023).

Considering this experience, in our study we selected the ERA as the diagnostic tool for evaluating endometrial receptivity. This decision was guided by the ERA's proven consistency, ensuring a more accurate and standardized assessment of endometrial receptivity in our patient cohort.

In a multicenter, double-blind, parallel-group RCT, no significant difference in LBR was observed between euploid frozen embryo transfers guided by endometrial receptivity testing and those with standard timing. Secondary outcomes, including biochemical and clinical pregnancy rates, also showed no significant differences (Doyle et al., 2022). In contrast to the findings reported in the referenced study, our investigation extends prior research by examining a more complex patient population, including older reproductive-age individuals and those with challenging reproductive histories. Notably, the reference study was limited to a good prognosis cohort, with the exclusion criteria including recurrent pregnancy loss, recurrent implantation failure, surgically aspirated sperm, and donor egg(s).

Our research demonstrated a statistically significant improvement in reproductive outcomes—specifically in IR, PR, and LBR—within the patient group utilizing the ERA. Our research included the study group and control group 1, where patients underwent egg donation and PGT-A, with the ERA test additionally applied in the study group. Remarkably, despite the higher-risk profile of this cohort, our findings revealed significantly enhanced reproductive outcomes in the combined egg donation, PGT-A, and ERA group, achieving success rates comparable to patients of younger reproductive-age. These results underscore the potential of the ERA test to not only improve implantation success but also to support normal implantation and effective decidualization, thereby reducing pregnancy complications and lowering clinical miscarriage rates in the PGT-A + ERA group.

The robustness of these findings is further strengthened by the study's low risk of bias and confounding, attributable to its randomized design, which enhances the internal validity and reliability of the observed outcomes. Collectively, these results advocate for the incorporation of ERA testing in fertility treatment protocols, particularly for patients with complex reproductive backgrounds. Nonetheless, they emphasize the need for additional large-scale studies to further validate and expand upon these promising outcomes.

The international, multicenter RCT was designed to evaluate the impact of assessing endometrial receptivity using the ERA test, applied clinically to pET. The inclusion criteria were carefully crafted to select patients aged 37 years or younger to mitigate the influence of advanced maternal age, which is associated with higher rates of embryo aneuploidy and could introduce confounding variables. Additionally, participants were required to have an antral follicle count of 8 or higher and FSH levels below 8 IU/ml to exclude poor responders and ensure an overall good prognosis cohort.

Within this carefully selected population, intention-to-treat (ITT) analysis did not reveal statistically significant differences in clinical outcomes among the three study arms—pET, FET, and fresh embryo transfer—with respect to first embryo transfer outcomes and cumulative rates up to 12 months. However, CPR was significantly higher in the pET group compared to the FET and fresh embryo transfer groups. Conversely, clinical miscarriage rates were notably higher in the pET group compared to the fresh embryo transfer group.

The primary objective of this RCT was to establish proof-of-principle evidence for the potential benefit of incorporating a personalized endometrial factor diagnosis in the initial infertility work-up. While ITT analysis showed limited benefits of the ERA test aside from a statistically significant increase in CPR compared to FET and fresh embryo transfers, per-protocol analysis yielded a different perspective. It demonstrated significant improvements in first-attempt pregnancy rates, cumulative pregnancy rates over a 12-month period, and implantation rates on the initial transfer attempt, indicating that the ERA test may be useful in diagnosing and managing endometrial factors in infertility treatments (Simón et al., 2020).

The current study builds upon these findings by examining a patient population with a more complex reproductive history. Unlike the prior study, which focused on a good prognosis cohort, our study included older patients and those with a history of reproductive complications. In both the study group and control group 1, patients underwent egg donation, PGT-A and also ERA in the study group. Despite the inclusion of these higher-risk patients, our study demonstrated significantly improved reproductive outcomes in the combined egg donation, PGT-A, and ERA group, showing comparable success to younger reproductive-age patients.

These findings highlight the ERA's capacity to improve implantation success, facilitate normal implantation, and promote effective decidualization, resulting in diminished pregnancy problems and a lowered clinical miscarriage rate in the PGT-A + ERA cohort. Our findings robustly advocate for the incorporation of ERA testing in fertility treatment protocols, especially for patients with intricate reproductive histories, and underscore the necessity for additional large-scale research to validate and elaborate on these results.

For several decades, it has been postulated that glandular secretions play a critical role in sustaining the conceptus prior to implantation across various species. However, their significance in post-implantation growth and the development of the embryo and placenta received limited attention. Recent accumulating evidence from studies in mice and humans over the past decade substantiates the pivotal roles of uterine glands and their secretions in establishing uterine receptivity, facilitating blastocyst implantation, and supporting fetal and placental development post-implantation. Notably, during the first trimester of human pregnancy, the implantation site remains rich in functional glands capable of interacting with diverse cell types, including the decidua, vasculature, immune cells, and trophoblast. Uterine gland dysfunction has been implicated in pregnancy losses and complications such as miscarriage, preeclampsia, and fetal growth restriction. Consequently, advancing our understanding

of uterine gland biology may yield valuable diagnostic and prognostic markers for assessing endometrial function and predicting pregnancy complications, which could benefit both natural conception and assisted reproductive technologies (Kelleher et al., 2018). This concept is aligned with the reduced incidence of pregnancy complications observed in our study within the ERA groups, as ERA optimally identifies the precise window for embryo transfer, thereby enhancing subsequent implantation and promoting normal decidualization.

The influence of advancing age on uterine receptivity remains a critical concern within the medical community. Egg donation serves as an ideal model for examining this relationship, as it allows for the isolation of uterine factors from oocyte quality. However, the existing literature on this topic is limited by methodological challenges, including small sample sizes and inadequate control of key variables, particularly embryo quality. These limitations underscore the need for more robust studies to accurately assess the impact of age on uterine receptivity (Soares et al., 2005).

Investigation of the phenomenon of endometrial aging is aimed to elucidate its underlying mechanisms, identify its onset, and assess its potential impact on endometrial function. Transcriptome profiles from endometrial biopsies obtained during the WOI period were compared between women of younger and advanced reproductive ages. Unlike previous studies on endometrial aging, this analysis was conducted on participants who adhered to a standardized endometrial preparation protocol, reducing variability related to hormonal status and thereby minimizing inter-individual differences. Findings were further validated using samples from participants with natural cycles (NC). Given that HRT is commonly administered to AMA patients in preparation for FET, this study provides compelling evidence of age-related changes in endometrial tissue development, as well as shifts in its molecular profile and cellular composition in women undergoing ART (Loid et al., 2024).

Advanced maternal age is recognized as a significant risk factor for impaired cellular senescence and defective endometrial receptivity, both of which may contribute to infertility. Studies using animal models have highlighted potential age-related risks, including tissue fibrosis, alterations in tissue histology, dysregulation of decidualization, and imbalances in immune and inflammatory responses, all of which degrade endometrial receptivity. However, the limited number of publications on this topic suggests that the effects of advancing age on human endometrial function have been underestimated. Over the past two decades, significant progress has been made in elucidating the molecular mechanisms and complex network of biological pathways underlying endometrial receptivity, with a primary focus on the development of diagnostic tools to assess and monitor these critical biomarkers (Ruiz-Alonso et al., 2021a).

As the trend of delaying childbearing to older reproductive ages continues, understanding age-related changes in the endometrium has become increasingly important, particularly in efforts to enhance reproductive outcomes in advanced maternal age. Identifying effective strategies to improve endometrial receptivity in this population is crucial. The methodologies employed in the current study show considerable

promise for optimizing ART outcomes in women of advanced reproductive age, offering potential solutions to address the challenges posed by age-related endometrial changes.

Our findings indicated similar success rates between the study group and control group 2, with significantly elevated live birth rates in the >35 PGT-A+ERA cohort. The relationship between advanced maternal age and endometrial receptivity is intricate, encompassing hormonal, cellular, and molecular factors; however, the observed results indicate that customized interventions, such as PGT-A and ERA, may alleviate certain age-related effects on endometrial receptivity. These findings highlight the potential of individualized strategies to enhance reproductive outcomes in populations of advanced maternal age.

Subsequent examination of the ERA findings indicated that in older patients, the WOI was more often modified than in younger patients, with late receptivity being more common in this group (Gruninger et al., 1998). These findings highlight the clinical significance of ERA in customizing ART protocols to individual endometrial profiles, hence enhancing the probability of successful pregnancies. Furthermore, the comparison between the study group and control group 2, which comprised younger patients, revealed no significant disparity in reproductive outcomes, indicating that ERA-guided pET can enhance the reproductive success rates of advanced-age patients to levels akin to those of younger patients. The data indicates that the primary determinant of effective outcomes is the alignment of ET with the individual's WOI, rather than the patient's chronological age. Nonetheless, it is important to emphasize that study designs such to ours require more extensive documentation in the scientific literature, and the applicability of these findings to diverse populations in various nations warrants further investigation.

Molecular, cellular, and histological changes in the aging endometrium indicate that increasing age negatively affects endometrial biology and may hinder endometrial receptivity. Advanced maternal age affects cellular senescence, a crucial element in the early stages of implantation, posing a substantial obstacle to the development of effective senolytic medicines aimed at alleviating age-related reductions in endometrial receptivity (Pathare et al., 2023). Nonetheless, prior research has indicated divergent results, implying that endometrial receptivity may remain consistent with age, especially when utilizing high-quality embryos (Paulson, 2011). Our findings indicated misplaced receptivity rates of 23.8% in patients under 35 years and 56.6% in those over 35 years, highlighting the significance of ERA in advanced reproductive age. The ERA is demonstrated to be essential for optimizing transfer timing and shows no significant variations in clinical outcomes between these age cohorts.

Adenomyosis, an estrogen-responsive disorder, is closely associated with infertility and implantation failure in IVF. Conflicting reports on reproductive outcomes in patients with adenomyosis undergoing ART have intensified efforts to identify reliable molecular and clinical markers of estrogen receptor activity. Impaired estrogen receptor function (Campo et al., 2011) and defective decidualization (Tomassetti et al., 2013) are

recognized as significant contributing factors to the pathophysiology of adenomyosis. Oocyte donor-recipient cycles are frequently utilized as an optimal model to distinguish between endometrial and embryonic factors contributing to negative IVF outcomes. Studies indicate a reduced live birth rate and a doubled miscarriage rate in recipients with adenomyosis, suggesting that the early stages of embryo invasion and placentation may be adversely affected (Martínez-Conejero et al., 2011). Transcriptomic analyses of isolated human eutopic endometrium have revealed substantial gene expression alterations in the proliferative phase in women with adenomyosis compared to controls. Specifically, 140 genes were upregulated, and 884 genes were downregulated. Among the most differentially expressed genes were those involved in apoptosis regulation, steroid hormone responsiveness, and extracellular matrix remodeling, highlighting potential molecular mechanisms underlying adenomyosis pathology (Herndon et al., 2016). The WOI is significantly displaced in patients with adenomyosis (47.2%) compared to the control group (21.6%), with a statistically significant difference ($P < 0.001$). Findings indicate that the likelihood of a non-receptive ERA, indicating a displaced WOI, is approximately twice as high in adenomyosis patients as in controls, with a risk ratio of 2:1. This displacement may partially account for the reduced pregnancy and implantation rates observed in adenomyosis (Mahajan et al., 2018). In contrast, our study identified no statistically significant differences in endometrial receptivity displacement between patients with and without adenomyosis. Additionally, there were no discernible differences in fertility outcomes, including PR, IR, or LBR.

While endometrial preparation is a standard component of infertility treatment, the intricacies of optimizing endometrial receptivity remain unresolved. The molecular mechanisms underlying implantation appear to be highly complex. In contrast to the significant advancements in embryo culture, progress in understanding endometrial physiology has been relatively limited. However, we may be on the cusp of a breakthrough in deciphering embryo-endometrial signaling and the mechanisms driving embryo-endometrial synchrony, which could transform our approach to enhancing implantation success (Paulson, 2019).

An additional understanding of uterine anomalies that could impede embryo implantation is required to enhance treatment results. This review provides a thorough examination of the effects of both organic and non-organic uterine diseases on endometrial receptivity. The results demonstrate that many uterine diseases can hinder embryo implantation via diverse pathways. Furthermore, endometrial receptivity analysis (ERA) has proven effective in identifying dysregulation during the implantation window, serving as a crucial instrument for recognizing potential impediments to successful implantation (Hiraoka et al., 2023). The ERA represents a notable progression in improving IVF results by determining the Window of Implantation and customizing the timing of embryo transfer. This test has exhibited significant accuracy and reproducibility. Furthermore, sophisticated molecular methods such as the ERA have demonstrated the potential to enhance outcomes for patients with RIF. These instruments provide the precise identification of the Window of Implantation (WOI) and align the embryo transfer (ET) with the endometrium's receptive condition, hence improving the likelihood of successful implantation and pregnancy (Gajjar et al., 2024). Our research indicates that pET, directed by ERA, markedly enhances ART results in elderly individuals. The rate of pregnancy, the implantation rate and live birth rate were dramatically

elevated in the study group compared to control group 1, underscoring the effectiveness of ERA in enhancing reproductive outcomes. The live birth rate in the research group was nearly twice that of control group 1, signifying a significant enhancement in outcomes via tailored approaches.

It is essential to consider and integrate all available evidence that could optimize the outcome of a patient's first IVF cycle, as this may represent their only opportunity for a successful pregnancy. Prioritizing optimal care from the outset also addresses economic considerations by potentially avoiding multiple costly, unsuccessful cycles. In this context, any novel, evidence-based intervention that demonstrates a ≥10% increase in LBR compared to standard IVF, at a cost not exceeding 10% of a single IVF cycle, should be rigorously evaluated and discussed with the patient as part of their treatment plan.

The cost-effectiveness of the ERA and pET has emerged as a significant issue in reproductive medicine. The ERA aims to determine the ideal timing for embryo transfer through the analysis of gene expression in endometrial tissue, intending to improve implantation and live birth rates. While research suggests that ERA-guided pET may enhance these results, the elevated procedural expenses pose obstacles to its overall cost-effectiveness. Research indicates that fresh embryo transfer (ET) provides the lowest cost per live birth, while pre-implantation embryo transfer (pET) necessitates a greater live birth rate to attain cost-effectiveness relative to conventional techniques. Considering these factors, it is essential to reconcile clinical efficacy with cost feasibility when integrating ERA-guided pET into therapy protocols (Ruiz-Alonso et al., 2021b).

A potential weakness of our study is the recruitment of individuals for randomized controlled trials (RCTs). The elevated expense of ART, which lacks insurance coverage, poses significant obstacles in recruiting adequate participants. This financial obstacle may affect the generalizability and scalability of our results. Additional randomized controlled trials would provide greater insight by increasing the patient population under 35 and by comprehensively comparing the results of PGT-A tested embryo transfers with the traditional method against personalized embryo transfer based on the endometrial receptivity analysis. Nonetheless, issues such as deferring fertility due to late reproductive age and delayed consultations with fertility specialists persist as substantial impediments.

In conclusion, our research underscores the pivotal function of pET directed by ERA in enhancing ART results for elderly patients. By synchronizing ET with the patient's specific WOI, physicians can improve implantation success rates, resulting in increased pregnancy and live birth rates. Future research ought to concentrate on enhancing these individualized strategies and investigating supplementary factors affecting endometrial receptivity, including the microbiota, to enhance reproductive success for all ART patients.

CONCLUSIONS

C1. In patients with a history of RIF, pET based on endometrial receptivity assessment with euploid embryos significantly improves the outcomes of ART, such as pregnancy rates, implantation rates, and live birth rates. decrease the rates of obstetric complications, including miscarriage rates, ectopic pregnancy rates, preterm delivery, preeclampsia, and intrauterine growth retardation.

C2. In patients with a history of RIF, pET based on endometrial receptivity assessment with euploid embryos significantly decrease the rates of obstetric complications, including miscarriage rates, ectopic pregnancy rates, preterm delivery, preeclampsia, intrauterine growth retardation.

C3. The frequency of endometrial WOI displacement in infertile women with RIF is significantly higher in those of advanced reproductive age (>35 years old) compared to younger women (<35 years old).

C4. In advanced-age women, endometrial WOI displacements more frequently manifest as late receptivity, whereas in younger women are more commonly present as early receptivity WOI shifts.

C5. Integrating molecular diagnostics such as ERA into clinical practice and developing recommendations for pET based on obtained data will enhance the effectiveness of ART programs, ensuring higher success rates and better reproductive health outcomes for patients of varying ages.

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