PRZYCHODNIA LEKARSKA NOVUM

PROTOCOL OF THE THERAPEUTIC EXPERIMENT "Standardization of variable conditions of embryo transfer into the uterine cavity in the procedure of medically assisted procreation in humans"

EMBRYO PASSPORT/ EMBRYOCASE A randomized, prospective, single-center, controlled, single-blind study to evaluate the impact of standardization of variable embryo transfer conditions in a medically assisted procreation procedure in humans on the effectiveness of the procedure, by using: standardization of culture conditions and selection of embryo for transfer through the use of an incubator with a time-lapse observation system and artificial intelligence, an Embryopass applicator - electronically controlled device for controlled transfer, and the Embryocase case maintaining optimal environmental conditions for the embryo outside the incubator during the peri-transfer time.

ACRONYM: EFECT

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Przychodnia Lekarska nOvum

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Therapeutic experiment *- In accordance with the Act on the Professions of Doctor and Dentist (Journal of Laws of 2008 No. 136, item 857), a clinical trial is classified as a medical experiment. The Act distinguishes between two types of medical experiments - a therapeutic experiment and a research experiment, hence the term study or therapeutic experiment will be used interchangeably in the further part of the protocol.*

1 Table of contents

1. ABSTRACT

STUDY TITLE

A randomized, prospective, single-center, controlled, single-blind study to evaluate the impact of standardization of variable embryo transfer conditions in a medically assisted procreation procedure in humans on the effectiveness of the procedure, by using: standardization of culture conditions and selection of embryo for transfer through the use of an incubator with a time-lapse observation system and artificial intelligence, an Embryopass applicator - electronically controlled device for controlled transfer, and the Embryocase case maintaining optimal environmental conditions for the embryo outside the incubator during the peri-transfer time.

ACRONYM:

EFECT

STUDY PRODUCT

1. Embryocase case maintaining optimal environmental conditions for the embryo in which the embryo is placed in the peritransfer time outside the incubator (36.5oC – 37.5oC);
2. Embryopass Applicator - an electronically controlled device for controlled embryo transfer;
3. Protocol for standardization of embryo transfer conditions TL(AI)/EMBRYOCASE/EMBRYOPASS.

STUDY PHASE

NOT APPLICABLE

RESEARCH CENTER

Clinic Lekarska nOvum ul Bociania 13, 02-807 Warsaw, Poland

OBJECTIVES OF THE STUDY

EFFECTIVENESS

- **PRIMARY PURPOSE**

  The key objective of the study is to confirm the positive impact of standardization of the parameters of the embryo transfer procedure variables on its effectiveness

  1. Percentage of biochemical pregnancies

  The indicators confirming the achievement of the primary goal will be:

  a. confirmation of the positive impact of standardization of the environmental conditions in which the embryo is located outside the incubator during the peritransfer time will be an increase in the percentage of pregnancies (βhCG result indicating pregnancy 10-15 days after embryo transfer) in the group of patients in whom ET was performed with the help of the Embryocase case by 10% compared to the control group;

  b. confirmation of the effectiveness of the Embryopass applicator – achieving at least the same percentage of pregnancies (criteria as above) in the group of patients in whom ET was performed with the help of the Embryopass applicator in relation to the control group;

  c. confirmation of the effectiveness of standardization of variable conditions in the ET procedure (environmental changes and the human factor) will be an increase in the percentage of pregnancies (criteria mentioned above) in the group of patients in whom ET was performed with the help of the Embryocase case in combination with the Embryopass applicator compared to the control group by 15%.

  2. Percentage of clinical pregnancies in all groups of patients (visible gestational follicle at 25 - 35 days after ET).

- **SECONDARY PURPOSE**

  The secondary objective of the study is:

  1. **confirmation of the beneficial impact of standardization of parameters and conditions of the ET procedure on its effectiveness** achieved thanks to the use of the Embryocase case and the Embryopass applicator – an electronically controlled device for controlled embryo transfer by: obtaining an increased percentage of implantation

(percentage of implanted embryos) – visible pregnancy follicle in ultrasound 25-35 days after ET in the group of patients in whom ET was performed with the help of the Embryocase case, Embryopass applicator, and Embryocase in combination with Embryopass compared to a control group in which ET was performed according to the standard procedure adopted at the center (manually).

- **SECONDARY OBJECTIVE (AFTER COMPLETION OF THE STUDY)**
  1. **estimating the impact of using the Embryocase case and the Embryopass applicator separately and in combination on** the percentage of live births – LBR (after completion of the study).

SAFETY

- Estimation of the safety profile of the use of the Embryocase case and the Embryopass applicator in the ET procedure (the rate of adverse events during transfer in the study group and control groups).

USABILITY

- Evaluation of the convenience of using the Embryocase case and the Embryopass applicator by an embryologist and a doctor (questionnaire - answer to 5 questions).
- Evaluation of the repeatability of the standardization procedure - the time of embryo collection and the time of embryo administration Embryopass vs. manual transfer.

STUDY PLAN

A randomized, prospective, single-center, controlled, single-blind study evaluating the impact of standardization of variable embryo transfer conditions in a medically assisted procreation procedure in humans on the effectiveness of the procedure, by using: standardization of culture conditions and selection of embryo for transfer by using an incubator with a time-lapse observation system and artificial intelligence, use of an applicator Embryopass - an electronically controlled device for controlled transfer, the use of the Embryocase case maintaining optimal environmental conditions for the embryo outside the incubator during the peritransfer time and the combination of all elements into one innovative embryo transfer protocol is to demonstrate an increase in the percentage of biochemical, clinical and implantation pregnancies in patients undergoing cryotransfer of the embryo at the blastocyst

stage, for whom this will be the first transfer, or the second if The previous one ended with pregnancy and the birth of a child.

Patients who have frozen embryos at the blastocyst stage - at least 1 good quality, 3BB (bl3.1.1) or more, have not had a fresh transfer due to contraindications, this will be their first transfer at all or the first after an effective previous one, embryo culture was carried out in the Time Lapse incubator with AI. Once included in the study, embryos will be thawed in the order consistent with the highest AI rating.

Screening of patients will take place within 60 days prior to randomisation. Study candidates will be assessed by the investigator at the screening visit for compliance with the study inclusion and study exclusion criteria.

Preparation for the transfer of a thawed embryo will take place in the ovulatory cycle - natural or induced letrozole. Once ovulation is confirmed in an ultrasound and/or hormonal examination, a temporarily synchronized embryo thawing will be ordered.

Randomization will take place on the day of CRIO-ET, i.e. on the 5th day after ovulation, counting the day of follicle rupture as day 0, confirmation of ovulation by the occurrence of the LH peak (LH peak day + 6 days), or by the administration of chorionic gonadotropin on the day of confirmation of the presence of the preovulatory follicle 17-22 mm, HCG drug (HCG administration day +7 days). Patients who meet all inclusion criteria and do not meet any of the exclusion criteria will be enrolled in the study (randomised). Randomization will be randomized on a 1:1:1:1 basis, the same number of patients will be assigned to the control group and each of the three study groups.

Embryo transfer of a thawed embryo (CRIO-ET) will be performed on day 5 after ovulation or LH+6, or HCG+7. Only one good quality embryo is allowed to be administered, as indicated by the AI. Key aspects of embryo transfer, both manually and with the use of the Embryocase case and the Embryopass applicator, will be standardized. Soft catheters (Cook symbol K-JETS-7019-ET) will be used for the transfer. Embryo transfer will be performed under ultrasound guidance, and the embryo will be placed 1.5-2 cm from the bottom of the uterus. The time from complete embryo acquisition to the catheter to the completion of the transfer must not exceed 2 minutes. During the transfer, the time of embryo collection and administration from the catheter to the uterine cavity will be subject to additional

evaluation and comparison – manually vs. with the Embryopass applicator. Immediately after embryo administration, the catheter will be withdrawn from the uterus and examined under a microscope. Once it is confirmed that the embryo has not been left in the catheter, the doctor will remove the speculum. After the transfer is complete, the patient will get up and leave the transfer room.

Supplementation of the luteal phase In order to supplement the luteal phase, patients will use progesterone starting from the day of ovulation +1 until the visit (25 - 35 days after ET) Duphaston orally 3x1 tabl. and 10 mg plus Cyclogest 2x400 mg globules vaginally according to the regimen used in the center. On the day of the appointment 25-35 days after CRIO-ET, your doctor will decide whether to continue using progesterone or exclude it.

Confirmation of pregnancy will occur at the βhCG visit 10-15 days after ET by determining the level of βhCG in the blood serum. In the absence of pregnancy (βhCG ≤ 5.3 mIU/ml), the patient will stop taking progesterone and end the test, while in the case of confirmation of pregnancy, the patient will continue to take progesterone continuously until the clinical pregnancy is confirmed.

Confirmation of clinical pregnancy will occur at the visit 25-35 days after ET in all patients who tested positive for βhCG. Confirmation of clinical pregnancy or its absence ends the examination.

Termination data (GEU, miscarriage or childbirth) will be collected from patients in the form of a questionnaire that the patient can send to the center or provide the information contained in it by phone to one of the members of the study team after the patient completes the study.

PLANNED NUMBER OF PATIENTS

The total number of patients enrolled in the study (randomized) is expected to be **160**.

Randomization will be based on a 1:1:1:1 basis. This means that 40 patients will be placed in the control group and each of the three groups of subjects.

TL/AI control group – CRIO-ET manual

research group 1 TL/AI – CRIO-ET manual + EMBRYOCASE

study group 2 TL/AI – CRIO-ET using EMBRYOPASS

research group 3 TL/AI – CRIO-ET using EMBRYOPASS + EMBRYOCASE

CRITERIA FOR THE SELECTION OF PATIENTS FOR THE STUDY

INCLUSION CRITERIA:

1. The patient and her partner gave written, informed consent to participate in the clinical trial.
2. The patient underwent a medically assisted procreation procedure using IVF or ICSI in accordance with the applicable law, did not have a fresh transfer, has all frozen embryos in the blastocyst stage, of which at least 1 is of good quality
3. You and your partner are not currently taking part in or have not participated in any other clinical trial in the last 6 months.
4. The patient's age on the day of screening ranges from ≥18 to ≤38 years.
5. The patient's BMI on the day of screening ranges from ≥18 to < 30.
6. Both partners have normal infectious tests performed 6 months before ET and bacteriological tests performed one month before ET.
7. The patient has had IVF or ICSI embryo culture performed in an incubator with a Time Lapse monitoring system and an Artificial Intelligence system (or if not, the embryo will be placed in the EmbryoScope after thawing) and has not had a fresh transfer (all embryos after stimulation and puncture have been frozen), and after thawing she has at least 1 good quality blastocyst evaluated before freezing by an embryologist as a good quality blastocyst or bl class. 3.2.2. The embryos have also been evaluated by AI prior to freezing and will be thawed in the future in order of the highest AI-confirmed rating.
8. The patient has a normal uterus and endometrium at least 7 mm on ET.
9. The patient must be prepared for cryotransfer in the ovulatory cycle with natural or induced letrozole, and after ovulation in the current cycle, take a progesterone preparation at the doses specified above for luteal phase supplementation.
10. On the day of transfer (5th day after ovulation), the patient must have at least one thawed, developed embryo of good quality – pre-frozen blastocyst 3.2.2 thawed according to the highest AI rating.
11. The patient must be qualified by the doctor for the procedure embryo transfer on day 5 after ovulation.

12. Embryo transfer was performed without general anesthesia or other procedures and/or additional antispasmodic drugs prior to transfer (e.g. oxytocin antagonist) with the exception of routine preparation (drotaverine 1 table and 20 mg p.o. per hour prior to transfer).

EXCLUSION CRITERIA:

1. Failure to meet the inclusion criteria.
2. The patient was found to have abnormalities in the anatomical structure of the uterus and reproductive tract, which, according to the researcher, could reduce the chances of getting pregnant.
3. The patient was diagnosed with endometrial polyp(s).
4. The patient was diagnosed with submucosal or intramural uterine fibroids.
5. The patient was diagnosed with fallopian tube hydromas.
6. The patient was diagnosed with ≥3 endometriosis.
7. The patient or her partner is a carrier of a genetic defect that may have an impact on lowering fertility.
8. The patient or her partner is currently or has been undergoing cancer treatment in the past, which may have had a negative impact on fertility.
9. The patient has had 1 unsuccessful embryo transfer in the past, which means no clinical pregnancy.
10. During the trial transfer in the preceding cycle, difficulties in entering the uterine cavity (the so-called difficult transfer) were found or if the patient had had an embryo transfer in the past and it was described as difficult, even if she became pregnant as a result.
11. Embryos are formed from eggs after PBB (polar body biopsy) of oocytes have been examined or have undergone genetic testing of embryos.
12. The embryos were formed from oocytes after cryopreservation.
13. Semen was obtained for a procedure other than normal ejaculation (retrograde bladder ejaculation, epididymis biopsy, testicular biopsy, M-TESE).
14. The patient did not take progesterone for luteal phase supplementation.
15. Inability to perform embryo transfer on day 5 after ovulation due to medical or random reasons.

16. The patient has indications for general anesthesia or pre-transfer antispasmodic procedure/medication (oxytocin receptor antagonist other than Drotaverine, or others that may affect implantation (intralipid, neupogen, and other significant in the investigator's judgment).
17. The patient is to have an incision made in the AH areola before the ET procedure.
18. The patient wants to have Embryoglue used for transfer.
19. The patient takes heparin, Clexane, Acesan, Relanium drugs on and after embryo transfer.

DATA ANALYTICS AND STATISTICS

The data obtained in the study, which will be subjected to comparative analysis in the control group and each of the study groups:

PRIMARY PURPOSE

1. pregnancy rate - βhCG result indicating pregnancy 10-15 days after ET;
2. percentage of clinical pregnancies - visible pregnancy follicle on ultrasound 25-35 days after ET;

SECONDARY PURPOSE

1. implantation rate (percentage of implanted embryos) – visible pregnancy follicle on ultrasound 25-35 days after ET;

SECONDARY OBJECTIVE (AFTER COMPLETION OF THE STUDY)

1. Percentage of live births (LBR) in the control group and each of the study groups

SAFETY

Estimation of the safety profile of the use of the Embryocase case and the Embryopass applicator in the ET procedure (the rate of adverse events during transfer in the study group and control groups).

STATISTICAL ANALYSIS

Statistical analysis of the results will be carried out using the t-Student test for constant variables and the chi-square test for categorical variables. All data will be presented as mean ± standard deviation. A p value of < 0.05 will be considered statistically significant. It is possible to introduce additional statistical methods by the decision of the statistician.

PLANNED USE OF RESULTS

After obtaining partial results and completing the study, its results will be presented during industry conferences in Poland and abroad in the field of infertility treatment, the key ones being: PTMRiE (Polish Society of Reproductive Medicine and Embryology) Congress, ESHRE Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE); It is planned to publish the results in an industry journal, as well as patent protection for EMBRYOCASE and the TL/EMBRYOPASS/ EMBRYOCASE protocol. Ultimately, the center plans to implement the inventions on the medical market in Poland and abroad, seeing numerous benefits for patients resulting from the standardization of the embryo transfer procedure, as well as for infertility treatment centers (description in the justification for conducting the study).

2. LIST OF TABLES

3. LIST OF DIAGRAMS

4. ABBREVIATIONS USED

AE	*Adverse Event*	Adverse event
AH	*Assisted hatching*	Incision of the transparent sheath of the embryo
AI	*Artificial Intelligence*	Artificial Intelligence
βhCG	*B-human chorionic gonadotropin*	Human chorionic gonadotropin β subunit
BMI	*Body Mass Index*	Body Mass Index
CRIO-ET	*Cryotransfer*	Embryo transfer of a thawed embryo
E2	*Estradiol*	Estradiol
Embryocase	*Embryocase*	A case that maintains optimal environmental conditions for an embryo placed in a catheter outside the incubator during the peritransfer time (nOvum's own invention)

Embryopass	*Embryopass*	Electronically controlled device for controlled embryo transfer - an invention of the nOvum clinic, constructed as part of the RPMA.01.02.00-14-5674/16 project. The applicator was tested on an animal model, obtaining a high pregnancy and birth rates. Industrial design reservation, patent, and CE1434-MDD-089 certificate certifying compliance with the requirements for medical devices in accordance with EEC/93/42 Directive
ET	*Embryo Transfer*	Embriotransfer
ICSI	*Intra-Cytoplasmic Sperm Injection*	Docytoplasmic sperm injection
IVF	*In vitro Fertilization*	The classic method of *in vitro fertilization*
P4	*Progesterone*	Progesterone
UAE	*Serious Adverse Event*	Serious adverse event
TL	*Time Lapse*	Time-lapse imaging system
Ultrasound	*Ultrasound Guide*	Visualization with ultrasound

5. INTRODUCTION

5.1. Literature review

The treatment process using advanced assisted reproduction techniques is multi-stage and complex. It usually consists of the following stages: diagnosis and preparation of patients for the treatment process, controlled hormonal stimulation of the ovaries to obtain more eggs (oocytes) than in the natural cycle, acquisition and preparation of sperm, collection of oocytes (oocytes) through ovarian puncture, in vitro fertilization of egg cells, *in vitro* embryo culture, transfer of embryos into the uterine cavity, freezing of the remaining embryos for future use. Embryo transfer is an integral, final and perhaps decisive element of the entire process of in vitro fertilization treatment. The effectiveness of infertility treatment, determined at the level of 25-35% of pregnancies achieved after embryo transfer, has long aroused the interest of researchers who are

looking for additional elements that can improve it and increase the effectiveness of in vitro fertilization treatment. The main cause of failures is considered to be spontaneous genetic errors occurring both in embryonic development in nature and in *vitro*, preventing the proper development of embryos and its final inhibition; these errors cannot be eliminated. The lack of pregnancy, despite the proper development of embryos, can also be caused by hormonally unprepared endometrium of the uterus or lack of synchronization of embryo development with endometrial development. Individualization of transfers, diagnostics of the so-called "implantation window" or performing transfers of thawed embryos in natural cycles are procedures that can increase the chance of success of the treatment process. Numerous studies conducted over the last decade see the reasons for the limited effectiveness of infertility treatment using the assisted reproduction method, among other things, in the transfer technique itself, and this stage of the procedure of infertility treatment using the assisted reproduction method has become the subject of particular interest of the nOvum medical clinic team. Success in the form of a pregnancy depends on the elimination of errors and the optimization of each of the stages of treatment. Embryo transfer is a stage of treatment that can still be improved and optimized, especially since it is estimated that about 30% of failures result from poor transfer technique.

Description of the procedure of embryo transfer into the uterine cavity (embryo transfer) performed manually:

Embryo transfer is a multi-stage and complex procedure. To perform it, special catheters (catheters) tightly connected to a syringe are used.

The embryo transfer procedure can be divided into individual stages:

- preparing the patient for embryo transfer through ultrasound examination and performing a trial transfer procedure (with an empty catheter without embryos) to verify that entry through the cervix into the uterine cavity is easy and to assess the length and direction of the uterine cavity as well as the cervix;
- synchronization of the endometrium with the age of embryos;
- preparation of the patient before the transfer, cleaning the cervical canal of cervical mucus to avoid possible blockage of the transfer catheter and preventing the delivery of embryos into the uterine cavity or the penetration of part of the cervical mucus into the cavity and disruption of the embryo implantation process;

- proper collection of embryos into the transfer catheter by the embryologist, which is an extremely important element of the entire procedure. There are several methods – you can collect embryos directly from drops from the medium or transfer them to a transfer dish from which they are taken into a catheter, but studies have not shown any differences in the percentage of pregnancies. The technique of collecting embryos into the catheter may also differ in the volume of fluid collected with the embryo; Scientific studies suggest that too much or too little fluid volume can negatively affect the transfer result, however, data on the volume of the medium are conflicting. Another issue is the collection of air bubbles (markers) into the catheter along with the medium (fluid in which the embryos are grown), which are to help move the embryos in the catheter, and the visualization of this process in ultrasound. However, even in this matter, no significant differences were found in the percentage of pregnancies. The researchers suggest that the density of the transfer medium, the choice of syringe type, the type of catheter, and the speed at which embryos are taken into the catheter may be key factors in the process of embryo uptake into the catheter;
- insertion of a *leading catheter into* the uterine cavity – involves inserting a transfer catheter through the internal cervical outlet into the uterine cavity just behind the internal outlet;
- visualization of the embryo transfer process using an ultrasound machine – this is not an element necessary for the proper conduct of the procedure, but it is very helpful. It allows you to visualize the woman's reproductive organ, allows you to assess both possible anatomical obstacles and the right depth to which the catheter should be inserted into the uterine cavity without causing damage to the mucous membrane or stimulating it to excessive contraction. It allows you to visualize the entire transfer process - the movement of embryos marked with air bubbles from the catheter to the inside of the uterine cavity;
- Proper embryo transfer - performed by a doctor with the assistance of an embryologist, consists in inserting a guide catheter, a catheter containing embryos suspended in the transfer medium, the so-called embryonic catheter, into the previously inserted uterine catheter. This is a key stage of the entire embryo transfer process – if performed incorrectly, it reduces the chances of success. After inserting the guide catheter, the operating doctor places the

embryonic catheter in it and gently moves it inside the uterus. After making sure during the visualization in the ultrasound examination that the embryonic catheter is located in the optimal place of the uterine cavity, the operator presses the plunger of the syringe connected to the embryonic catheter and carefully, by pressing on the plunger, initiates the movement of embryos from the catheter to the inside of the uterine cavity. Once the maximum resistance of the plunger has been reached and the last marking marker (air follicle) remains in the uterine cavity, the doctor retracts the catheters from the cavity. Retracting the embryonic catheter too abruptly or releasing pressure on the plunger can pull the embryos back into the catheter, while administering the embryos too abruptly can cause damage to the embryos. The catheter can be withdrawn from the uterus immediately after embryo administration or with a delay of approx. 30 seconds; research on this issue does not report significant differences in the number of clinical pregnancies achieved;

- Checking the catheters – after the embryos are injected into the uterine cavity, the embryonic catheter is flushed using a transfer medium under a microscope to make sure that there are no embryos in it and that they remain in the uterine cavity.

The most important stages of embryo transfer that have become the subject of interest of the research team of the nOvum Clinic and improvement are the variable elements of the procedure, which may affect its success:

a) selection of embryos for transfer – the stage of the procedure depends on the experience of the embryologist, despite the introduction of embryo assessment standards, the embryo assessment by the embryologist remains subjective,
b) embryo collection into the catheter – a stage of the procedure depending on the training and experience of the embryologist and therefore burdened with variability,
c) transfer of embryos placed in a catheter from the laboratory to the transfer room – at this stage, the embryos are affected by changing environmental conditions, which may affect their development and implantation,
d) Administration of embryos from the catheter into the uterine cavity – another variable stage depending on the human factor, i.e. the experience and training of the doctor.

In recent years, scientific reports have been published on the conditions to which embryos in the catheter are subjected during transfer to the uterine cavity [1,3,4,5,6,7,8].

The above-mentioned reports and own experience confirming the unfavorable conditions that affect the embryos placed inside the catheter during the peritransfer time (the time the embryo remains in the catheter outside the incubator from being taken into the catheter to be administered into the uterine cavity) and during their transfer to the uterine cavity, such as: a sharp drop in temperature, shear stress, speed and pressure differences, prompted our team to work on standardizing the embryo transfer procedure.

The first stage of work was a design on the design of an applicator that would allow for a controlled process of embryo acquisition into the transfer catheter, as well as the transfer of embryos into the uterine cavity at a constant, optimal speed and would eliminate the adverse conditions to which embryos are exposed during this procedure. An electronically controlled embryo transfer device will increase the safety and repeatability of the procedure and thus increase its effectiveness.

Some mentions of the construction of a device to standardize the embryo transfer procedure appeared in the scientific publications of the team of researchers of the Department of Obstetrics and Gynaecology, VU University Medical Centre in Amsterdam, The Netherlands. The subject of particular interest of this group was the proper placement of embryos in the uterine cavity through the correct speed of their injection during the transfer procedure. The team's research resulted in the creation of the "*PRET* (*the pump-regulated embryo transfer device*)". This device connects directly to the transfer catheter. The "*PRET device"* works with the use of an automatic peristaltic pump capable of pumping small volumes of fluid and air. However, according to our current knowledge, this device has not entered the market, nor has a patent description of the device with such a name been found [*2,7*].

The next stage of work on the standardization of the embryo transfer procedure concerns the peritransfer conditions in which the embryo is located outside the incubator during the peritransfer time, i.e. between its collection into the catheter by the embryologist in the embryology laboratory and its administration into the uterine cavity performed by the doctor in the transfer office. Once the embryo is placed in the catheter, the embryologist must transfer the catheter from the laboratory to the transfer room and hand it over to the doctor. Even a short time outside the incubator causes a

change in the environmental conditions in which the embryo placed in the catheter is located (a sharp drop in temperature), which has been described in the available pioneering publication (Nick Macklon, Olga Delikari, Giuseppina Lamanna, Alison Campbell, Simon Fishel, Zaloa Larreategui Laiseca, Marcos Ferrando Serrano, Charlotte Coat, Peter Svalander). Embryos are exposed to a significant drop in temperature during the embryo transfer procedure: a pilot study. Reprod Biomed Online. 2021 Aug; 43(2):193-195. doi: 10.1016/j.rbmo.2021.05.014. Epub 2021 May 25 [8]. In this pilot study, the team of researchers showed a rapid drop in temperature in the catheter outside the incubator during the peritransfer time by up to 10◦C, which, given the current state of knowledge regarding the range of optimal and unfavorable temperatures for embryonic development, which has already been known, indicates that the embryo in the peritransfer period may be subjected to unfavorable and stress-inducing environmental factors, which may result in impaired development - aneuploidy, Mosiacism and reduced embryonic implantation potential (Munné, S., Alikani, M., Ribustello, L., Colls, P., Martínez-Ortiz, Pedro A. Referring Physician Group., McCulloh, D.H. Euploidy rates in donor egg cycles significantly differ between fertility centers. Human Reproduction 2017; 32: 743–749) [9].

In order to refer to the literature report in this regard and to gain new knowledge about the environmental conditions prevailing in the transfer catheter, and thus affecting the embryo placed in it in the peritransfer time, the nOvum team conducted their own experiment on the way between the embryology laboratory and the transfer room (a parameter planned for standardization), which confirmed the described temperature changes in the catheter. Maintaining optimal environmental conditions in which the embryo is located outside the incubator during the peritransfer time may therefore turn out to be crucial and is the subject of the study. The new knowledge gained will be used to develop a prototype of a device that minimizes environmental changes in the catheter by maintaining a constant and optimal temperature during the peritransfer time – the EMBRYOCASE case.

5.2 Subject of the study

5.2.1 Embryopass – electronically controlled device for controlled embryo transfer

The subject of the study is an electronically controlled applicator enabling controlled embryo transfer into the uterine cavity, which is one of the most important elements of the medically assisted procreation procedure. The embryopass allows the transfer of

embryos into the uterine cavity in controlled conditions and increases the safety of the procedure.

Features of the Embryopass applicator:

- ✓ the cycle of taking the charge into the catheter, as well as its administration from the catheter into the uterine cavity, ensures repeatability of the procedure thanks to pre-programmed constant values of volume, time, speed;
- ✓ the volume of the administered charge (i.e. the fluid with the administered embryo) is electronically controlled. After the injection of the entire volume of charge, it is impossible to retract the delivery system, which significantly increases the safety of the procedure of administering the embryo into the uterine cavity and eliminates the risk of retracting the embryo back into the catheter;
- ✓ the ability to detect the phenomenon of occlusion (catheter obstruction).

The Embryopass applicator - an electronically controlled device for controlled embryo transfer is adapted to UltraCruz 3 – Part Soft-Ject Disposable Syringes, 1 ml Tuberculin. The built-in power supply system is powered by an electronic microprocessor motion controller, which is controlled by a control system. The control system consists of buttons to control the process of charge picking and injection (air/medium/embryo) and an LED display. A strain gauge force sensor that controls the phenomenon of occlusion is activated in the first phase of injection of the load into the uterine cavity. Exceeding the programmed force causes the injection to be stopped and the compression system returns to its initial conditions before the injection begins. The microprocessor motion controller built into the electronic applicator calculates the rotational speed of the drive system with high accuracy according to the programmed parameters of the transfer catheter charging and embryo transfer to the uterine cavity. The electronic microprocessor motion controller in the applicator also takes care of the precise measurement of time, speed and volume of the load/discharge, as well as displays the relevant information and messages using light signals of different colors and in different sequences, as well as controls the battery charge status. The Embryopass has a positioning of the correct attachment of the syringe plunger in the syringe attachment holder to protect against the syringe slipping out of the syringe attachment holder or accidental pushing out of the syringe plunger.

The applicator for controlled embryo transfer was designed to work in the conditions prevailing in the embryology laboratory. The applicator does not come into direct

contact with biological material or the patient's body and does not need to be adapted to work in sterile conditions. It is a lightweight, easy-to-use device, enabling safe transfer conditions with constant, repeatable parameters.

To perform the transfer procedure using the Embryopass device, you need to:

• syringe connected to the catheter, flush the medium according to the procedure adopted, then press the plunger of the syringe, gently pushing out the medium so that the tip of the catheter is completely filled with the medium;

• the syringe must be fixed in the syringe holder in such a way that the end of the syringe plunger is positioned inside the delivery system, the correct fitting of the syringe in the syringe holder is confirmed by a clicking sound;

• Once you have inserted the syringe and made sure that it is inserted correctly, you can start working with the applicator.

Embryo collection into a transfer catheter using the Embryopass applicator:

• The procedure of embryo collection by an embryologist is multi-stage. Each of these steps is performed by pressing the appropriate button in the control system. The end of each stage is signaled by appropriate sequences and colors of light signals visible on the LED display located in the control system.

Procedure for injecting embryos from a catheter into the inside of the uterine cavity:

• It is a one-step procedure performed by a doctor. By pressing the appropriate button in the control system, the doctor starts this procedure. The end is signaled by an appropriate light signal visible on the LED display located in the control system.

Occlusion detection:

• the Embryopass applicator is equipped with a strain gauge force sensor used to detect occlusion (obstruction) of the catheter. If during the procedure of embryo delivery into the uterine cavity there is a catheter obstruction that prevents the embryo from being pushed out, the applicator detects occlusion (an increase in pressure in the catheter) and prevents further embryo administration. The applicator signals the detection of occlusion by means of a light signal visible on the LED display located in the control system. The discharge system is automatically retracted to the beginning of the load ejection phase, eliminating pressure in the catheter and preventing the load from being ejected.

The electronically controlled device for controlled embryo transfer – Embryopass is an in-house invention of the nOvum clinic, constructed by the clinic as part of the

RPMA.01.02.00-14-5674/16 project. The applicator was tested on an animal model (sheep), obtaining a high percentage of pregnancies and births. The final result of the project is a prototype model of the EMBRYOPASS applicator, meeting the assumed design conditions and functionalities, launched and tested in laboratory and clinical conditions (animal model). **Industrial design claim, patent, and CE1434-MDD-089 certificate certifying compliance with the requirements for medical devices in accordance with the EEC/93/42 directive have been obtained.**

5.2.2 Case EMBRYOCASE

In order to gain new knowledge about the environmental conditions prevailing in the transfer catheter, and thus affecting the embryo placed in it in the peritransfer time, on the way between the embryological laboratory and the transfer office (a parameter planned to be standardized in the innovative embryo transfer protocol) and to refer to the literature report in this area (Nick Macklon, Olga Delikari, Giuseppina Lamanna, Alison Campbell, Simon Fishel, A Zaloa Larreategui Laiseca, Marcos Ferrando Serrano, Charlotte Coat, Peter Svalander *"Embryos are exposed to a significant drop in temperature during the embryo transfer procedure: a pilot study" Reproductive BioMedicine Online Volume 43, Issue 2, August 2021, Pages 193-195* The nOvum clinic team conducted its own study in this area, the results of which are described in the *paper* ***"Protocol for the study of environmental changes in peritransfer time: documentation of temperature changes in the embryonic catheter in the peritransfer time defined as the time from the removal of the catheter from the incubator through the stage of embryo acquisition with the medium to the catheter, the path between the laboratory and the transfer room, to the time of insertion of the embryonic catheter into the uterine cavity during the embryo transfer procedure"*** (Appendix No. 2 to the study protocol).

In a pilot study [8], a team of researchers showed a sharp drop in temperature in the catheter outside the incubator during the peritransfer time by up to 10◦C, which, given the current state of knowledge regarding the range of optimal and unfavorable temperatures for embryonic development, which has already been known, indicates that the embryo may be subjected to unfavorable and stress-inducing environmental factors in the peritransfer period, which may result in a reduced implantation potential of the embryo [9].

The report presented in the publication [8] was verified by the research team of the nOvum Medical Clinic as part of its own research experiment. The new knowledge

gained will be used to develop a prototype device that minimizes the occurrence of environmental changes in the catheter by maintaining a constant and optimal temperature in the peritransfer time – Embryocase. The subject of the research – the EMBRYOCASE case, like the previously patented EMBRYOPASS applicator, is by definition a safe, easy to use, safe and reliable device. It will not have direct contact with the embryo or the woman's body. Such features and functionalities of the product make it possible to find the right niche for them in the medical market. The implementation of the invention will enable the standardization of the embryo transfer procedure, increase the effectiveness of assisted reproduction (ART) treatment, and increase the quality of patient service.

5.2.3 Innovative protocol for standardization of the embryo transfer procedure time-lapse (AI)/ Embryopass/Embryocase

Combining three innovative solutions into one optimal system: it is supposed to improve the effectiveness of infertility treatment:a) optimization of embryo selection through the use of a device for culture and monitoring equipped with an embryo culture system in an incubator with continuous monitoring of embryo development using cameras (time-lapse) and artificial intelligence
b) implementation of an innovative, patented invention of the centre's own – the EMBRYOPASS applicator – into the embryo transfer (ET) procedure, c) development and implementation of a case stabilizing the conditions of the embryo's environment in the peri-transfer period Embryocase.

5.3 Justification for conducting the study

The market of products dedicated to assisted reproduction techniques lacks electronic devices to standardize the procedure of embryo transfer into the uterine cavity. On the other hand, the interest in this topic indicates a high awareness of the importance of the embryo transfer procedure into the uterine cavity in the community of researchers involved in the treatment of infertility. If the effectiveness of the Embryopass, Embryocase and TL(AI)/Embryopass/Embryocase protocol is supported by an increase in the rate of pregnancies during the embryo transfer procedure to the uterus, it will be a significant breakthrough in this field. The protocol for standardizing embryo transfer conditions, which the nOvum Clinic would like to implement, containing two inventions that eliminate the influence of external factors (human, environmental) on the success of the ET procedure, significantly exceeds the procedure used so far. It

will improve and increase the efficiency of the embryo transfer procedure used so far in everyday practice, which will be measured by the estimated percentage of pregnancies, as well as fill the existing gap in this area. This will bring great benefits to patients, shortening the time to pregnancy and will increase the safety of the embryo transfer procedure by optimizing the conditions to which embryos are subjected during the procedure.

Both Embryopass, Embryocase and the innovative Time-Lapse (AI)/ EMBRYOPASS/ EMBRYOCASE standardization protocol for embryo transfer are solutions that have not yet been introduced on the market in Poland and abroad. An analysis of the range of products and services offered to patients by competing centres through the presented websites indicates that there are no service providers, manufacturers or entities in the country that use such a solution in clinical practice.

The introduction of the Time-Lapse (AI)/ EMBRYOPASS/ EMBRYOCASE protocol also improves the activities related to the performance of the embryo transfer procedure in medically assisted procreation centers. A positive effect at the center level will be: automation of the procedure in the center, optimization of daily procedures, reduction of risk, increased productivity, reduction of costs, improvement of service quality, better patient service.

5.4 Risks and benefits

The benefits of using the Embryopass applicator and the Embryocase in the embryo transfer procedure are, first of all, the possibility of standardizing the procedure, which may lead to an increase in the percentage of pregnancies and implantations obtained as a result of the assisted reproduction procedure. These benefits are possible to achieve through the use of the Embryopass applicator because:

- the negative impact of factors affecting embryos during transfer to the uterine cavity has been eliminated thanks to a constant, optimal rate of embryo uptake into the transfer catheter,
- the negative impact of factors affecting embryos during transfer to the uterine cavity has been eliminated thanks to a constant, optimal volume of charge taken into the transfer catheter,
- the negative impact of factors affecting embryos during transfer was eliminated thanks to a constant, optimal injection rate of embryos from the transfer catheter into the uterine cavity,

- the negative impact of factors affecting embryos during transfer was eliminated by determining and programming the optimal duration of embryo injection from the transfer catheter into the uterine cavity,
- the negative impact of the human factor on the quality of the embryo transfer procedure into the uterine cavity has been eliminated by eliminating pressure spikes in the catheter (the pressure force on the plunger is constant) both during the collection of the load into the catheter and during injection into the uterine cavity,
- the safety of the embryo transfer procedure into the uterine cavity has been increased by introducing a system for detecting occlusion (obstruction) of the transfer catheter,
- The safety of the embryo transfer procedure has been increased by eliminating the possibility of retracting the plunger and pulling the embryo back into the catheter, during or after its injection into the uterine cavity.

The introduction of the Embryocase case into the Embryo Transfer procedure will eliminate the negative impact of environmental factors (rapid temperature drop) affecting the embryo placed in the transfer catheter outside the incubator during the peritransfer time.

Risks of using Embryopass and Embryocase in the embryo transfer procedure:

- The assumption of the test is to demonstrate the complete safety of the use of an electronic device for controlled embryo transfer during the procedure. The embryopass does not come into contact with the embryo or the patient's body,
- The assumption of the test is to demonstrate the complete safety of using the Embryocase case maintaining the optimal temperature for embryos during the embryo transfer procedure. Embryocase has no contact with the embryo or the patient's body.

6. OBJECTIVES OF THE STUDY

6.1 Effectiveness

In order to estimate the impact of standardization of ET conditions on its efficacy, data on the percentage of biochemical pregnancies, implantation and clinical pregnancies obtained (and after the study also live birth data) in the groups of patients in whom the embryo transfer procedure was performed using Embryopass, Embryocase and both devices together and compared with the results in the control group will be analyzed. Factors such as:

1. embryo class (including degree of expansion, germ node quality, trophoblast quality);
2. type of procedure.

6.2 Safety

The safety profile of the Embryopass applicator and the Embryocase will be presented as a percentage of adverse events.

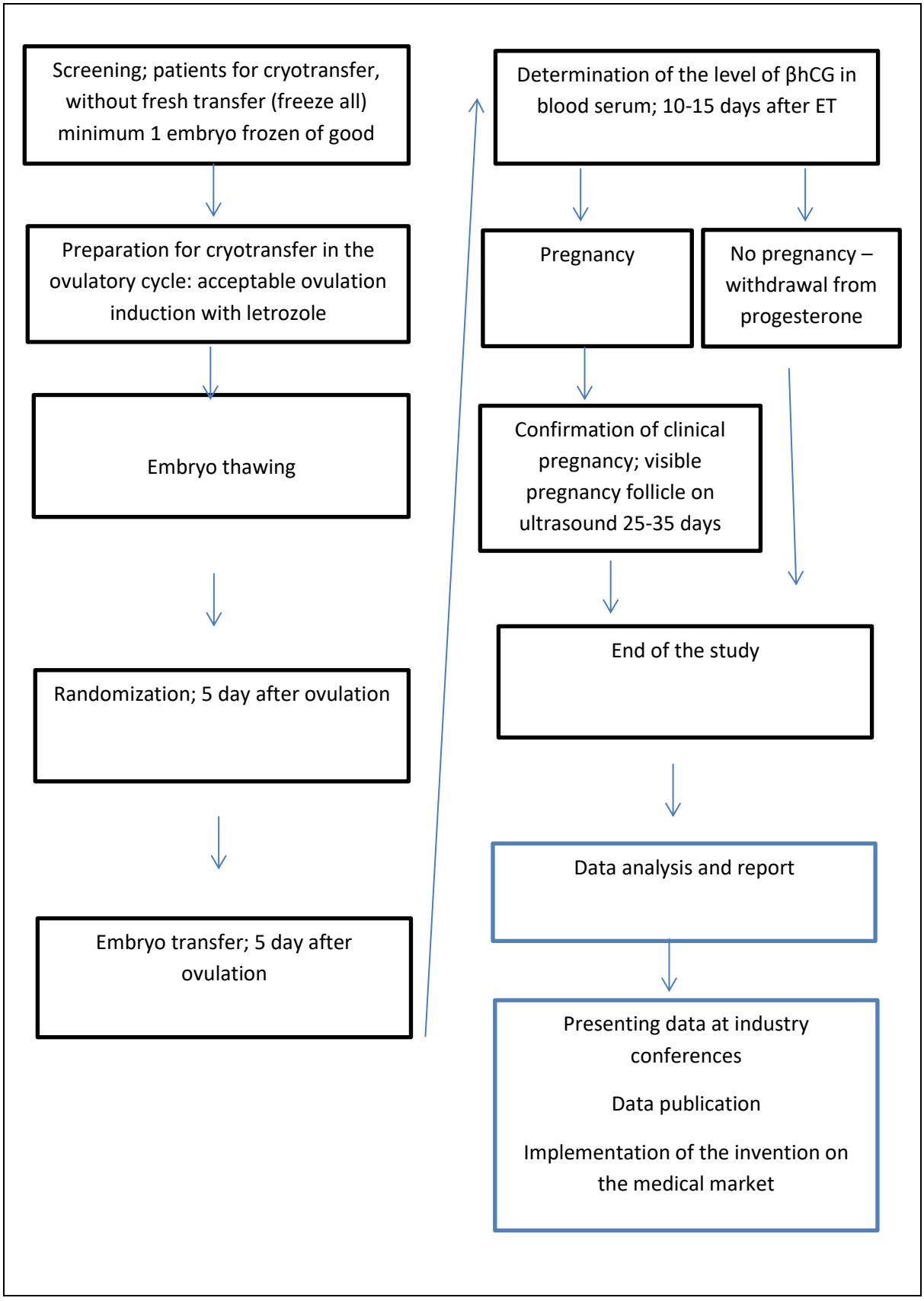
6.3 Usability

The following will be evaluated:

- convenience of using the Embryocase case and the Embryopass applicator by the embryologist and doctor,
- repeatability of the procedure - the time of embryo collection into the catheter and the time of embryo delivery into the uterine cavity.

7. STUDY DESIGN

Diagram 1 – study design



8. GROUP EXAMINED

8.1 Female patients

The study will involve patients whose embryos have been created and subjected to cryopreservation at the "nOvum" Medical Clinic. Patients will be informed about the possibility of taking part in the study by the attending physician and additionally in the form of an internal announcement (registration, leaflets in the waiting room), on the website and, if necessary, on the Social Media of the nOvum Clinic.

8.1.1 Description of the population

The study population will consist of patients aged ≥18 to ≤38 years who have undergone a controlled ovarian stimulation and puncture procedure, and then the collected oocytes have been fertilized by IVF or ICSI. The embryos were cultured in an incubator with the Time-Lapse system, the embryos were evaluated by an embryologist and an Artificial Intelligence system, and then all were cryopserved. Currently, patients are preparing for their first cryotransfer. On the day of thawing, the patient will be randomized to be randomized to either a control group or one of three study groups. The embryos will be transferred to the uterine cavity on day 5 after ovulation. According to the randomized group procedure.

The development of embryos before freezing in the following days of development will be subject to evaluation by an embryologist according to the criteria routinely used in the center (PTMRiE criteria; ESHRE – see Table 1, Table 2, and by artificial intelligence (according to the capabilities of AI in day 3 and day 5). Ratings will be compared.

In the case of morphological assessment of blastocysts, TQ (top quality) embryos include blastocysts at the stage of development of min. 3 with an additional TE and ICM rating of at least 1 or 2. Other embryos that do not meet these criteria are referred to as weak – NTQ (non-top quality). NTQ (non top quality) embryos include embryos with a different number of cells than recommended on a given day of observation and a morphotic element rating scale of 3.

All embryos will be frozen and described according to the AI assessment, on which the order of thawing will depend (the thawing of the embryos will occur in the order of the best rated by the AI).

Table 1

Classification of embryos on day 3 according to PTMRiE *In the description of an embryo at this stage of development, the symbol of the dividing embryo should be given: cl (cleavage), then the number of blastomeres and its quality class.*	
Embryo Quality Class	***Morphology***
1- Good	<10% fragmentation, blastomere size adequate to the number, no multinucleation,
2- Medium	10-25% fragmentation, the size of blastomeres adequate to the number, no multinucleation,
3 – weak	>25% fragmentation, blastomere size highly varied, present multinucleation,

Table 2

Classification of embryos on day 5 according to PTMRiE *In the description of the embryo at this stage of development, the symbol of the embryo in the blastocyst stage should be given: bl, followed by the degree of expansion and its class of germ node and trophoblast.*	
Degree of expansion	***Morphology***
1	Early blastocyst. Blastocyst cavity less than half the volume of the embryo.
2	A blastocyst cavity greater than or equal to half the volume of the embryo.
3	A full blastocyst, the cavity of the blastocyst fills the embryo completely.
4	Expanding blastocyst, with a volume larger than the embryo at an earlier stage of development, the transparent sheath becomes thinner.
5	Blastocyst hatching. The trophoblast comes out of the envelope.
6	A hatched blastocyst. The embryo is entirely outside the envelope.

Germ node (ICM)	***The quality of the germ node.***
1	Lots of cells, tightly packed.
2	Few cells, loosely grouped.
3	Very few cells.
Trofoblast (TE)	***The quality of the trophoblast.***
1	A lot of cells forming a tight shell.
2	Few cells forming a loose coating.
3	Very few large cells.

8.1.2 Number of patients

The total planned number of randomized patients is 160. In this pool, patients will be equally divided between the study group and the three control groups, Due to the risk of not being qualified for transfer on the 5th day after ovulation for various reasons (lack of a good quality embryo, lack of adequate endometrial width or other reasons important in the investigator's assessment in all patients), it is estimated that the percentage of the so-called " *screen failure"* i.e. patients who do not meet the criteria for inclusion in the study on the day of randomization will be about 20%, which is why the assumed number of patients who will undergo screening is 200. In addition, it is estimated that about 1% of patients will lose contact with the center, as a result of which it will not obtain complete data.

8.1.3. Study Region

Single-center study. Patients will be recruited in one center – the nOvum Medical Clinic at 13 Bociania Street in Warsaw.

8.2 Criteria for the selection of patients for the study

Described in Abstract

9. TEST PROCEDURES (METHODOLOGY)

9.1 Informed consent of patients to participate in the study

Before proceeding with any medical procedures, the patient and her partner must, after obtaining all information about the clinical trial from the investigator: sign the **"INFORMED CONSENT FORM - PATIENT" and the "INFORMED CONSENT FORM - PATIENT" in the presence of the investigator.**The informed consent document will include the patient's identification number in the clinical trial (the so-called **screening number**) – the next number from EEZ4412_001 to EEZ4412_200. If a patient withdraws consent to the study or is excluded from the study at any time, the identification number is not passed on to the next patient.

The "Informed Consent Form" must be signed by the Patient in duplicate. Each copy will bear the signature of the patient and the researcher, respectively. One copy of each of the consents will remain in the documentation of the examination at the "nOvum" Medical Clinic, the next will be handed over to the patient and her partner, respectively.

Patients must be informed that their participation in the study is voluntary and they can withdraw their consent to participate in the clinical trial at any time. The withdrawal of consent must be made in writing in the presence of the investigator.

From the moment of signing the informed consent until the end of the patient's participation in the clinical trial, all information about the patient covered by the subject of the study will be collected in the form of paper medical documentation, in electronic form in the electronic database of the nOvum Medical Clinic "bmedica2" and the database dedicated to clinical trials - eCRF

9.2 Blinding/Unblinding the Test

The study will be partially blinded. Patients will not be informed until the end of the study whether they will be placed in the control group or in one of the 3 groups of subjects. Staff participating in the study: doctors, embryologists and nurses, due to the specificity of performing the embryo transfer procedure to the uterus (no possibility of blinding), will know which group the patient has been assigned to, but they will undertake to maintain secrecy and not to provide each other with information orally during the procedure that could blind the patient. Information on how to perform the transfer (control group, one of the three groups of respondents) will be included in the electronic eCRF. During the transfer, the husband will not be able to be present, about which the patients will be informed at the screening stage. Statisticians analyzing the

results of the survey will be blinded. The results on the effectiveness of transfers in all groups will be the first to be statistically analysed. At this stage, blinded data will be provided to the statistics to guarantee the objectivity of the analysis. Blinding will occur after analyzing the efficacy and AEs and SAEs of all groups. The test will be unblinded after the test report has been prepared. Patients will be informed by the investigator about their membership in groups only at their explicit request.

9.2.1 Emergency Blinding

Premature blinding for an individual patient may occur in the event of AE or SAE, the occurrence of which, in the opinion of the investigator, may be related to the use of Embryocase/ Embryopass. If it is necessary to unblind the patient, it is not required to exclude her from the examination. Each case of unblinding must be documented by the investigator in the study documentation, along with a description of the circumstances and arguments in favor of unblinding.

9.3 Screening

Screening for the study must be preceded by the signing of informed consent by the patient and her partner. Screening must be performed no more than 60 days prior to the estimated randomization.

During screening, all of the information listed below must be collected:

- initial eligibility criteria and non-emission;
- Demographics: date of birth, race;
- medical history: karyotypes or other genetic tests if performed, evaluation of laboratory tests normally required in an IVF/ICSI treatment center, cause and duration of infertility, concomitant diseases, oncological treatment (if any), partner/donor sperm test confirming the presence of sperm in the ejaculate from the day of embryo formation, assessment of the menstrual cycle (must be regular – i.e. in the doctor's description "ovulatory 21-35 days"), obstetric history;
- medications taken by both partners within 3 months before screening and currently;
- gynecological ultrasound examination (performed within the last 3 months at most);
- hormonal stimulation of the ovaries in the cycle in which the embryos were formed and the course of the in vitro fertilization procedure;
- class, quality and procedures to which the embryos were subjected;

- A description of the previous transfer or trial transfer.

During screening, all of the above procedures must be performed:

- measurement of height, weight, BMI,
- measurement of pressure, heart rate and temperature,
- physical examination,
- gynecological examination,
- gynecological ultrasound examination (if the patient has not provided an examination performed in the last 3 months),
- A trial transfer, if not done before the puncture, in the following days of monitoring after good bacteriology.

  Trial transfer procedure

  Preparing the patient for embryo transfer by performing a trial transfer procedure (with an empty catheter without embryos) in order to assess the length and direction of the uterine cavity and cervix, as well as diagnosing possible obstacles (a procedure performed in a cycle other than the one being tested, to prevent possible mechanical damage to the mucosa) will only be performed if there is no information in the medical records about how the previous transfer took place.

9.4 Synchronization of the endometrium with embryonic age

Endometrial synchronization will take place in the natural cycle if the cycles are ovulatory or induced by Letrozole. In order to supplement the luteal phase starting from the day of ovulation +1 until the visit 25 - 35 days after ET, the Patient will take the progesterone preparation vaginally and orally according to the routine regimen used in the nOvum clinic: Duphaston orally 3x1 tabl. 10 mg plus Cyclogest 2x400 mg globules vaginally. On the day of the appointment 25 - 35 days after CRIO-ET, your doctor will decide whether to continue using progesterone or turn it off.

9.5 Defrosting embryos

Doctor - The doctor's order to thaw the embryos and make an appointment for a transfer visit will take place in accordance with the routine practice adopted at the center.

Embryologist - Embryo thawing will occur according to the highest rating indicated by AI:

(a) on the day preceding CRIO-ET (for blastocysts with a degree of expansion rated as 3 before freezing);

b) on the day of CRIO-ET (in the case of blastocysts with a degree of expansion assessed at least 4, at least 2 hours before embryo transfer to the uterine cavity.

Defrosting will be carried out in accordance with the routine procedure adopted at the "nOvum" Medical Clinic.

9.6 Evaluation of embryos after thawing

Evaluation of embryos after thawing will be performed by an embryologist and on its basis the embryo will be qualified for ET.

9.7 Randomization

Randomization will take place on the day of CRIO-ET, i.e. on the 5th day after ovulation, counting the day of follicle rupture as day 0, confirmation of ovulation by the occurrence of the LH peak (LH peak day + 6 days), or by the administration of chorionic gonadotropin on the day of confirmation of the presence of the preovulatory follicle 17-22 mm, HCG drug (HCG administration day +7 days).

Randomization will be randomized. Patients who meet all inclusion criteria and do not meet any of the exclusion criteria may be randomised. The patient's assignment to the control group or the study group will take place by drawing envelopes by an independent person (an administrative employee of the embryology laboratory), who is not part of the research team.

The randomization number will be determined by a string of 6 characters: from EE_001 to EE_200. The numbers will be located in unmarked envelopes – each number will correspond to membership in a specific research group. The number will only be known to embryologists who are members of the research team. After randomly selecting the envelope, the administrative employee will open it and hand it over to the embryologist, who is a member of the research team. Both of them will sign the number drawn for the patient with the date of the draw. The number of numbers will be selected so that the same number of patients will be assigned to the control group and three groups of subjects.

9.8 Transfer visit

The transfer visit will be divided into two stages:

Stage I: The following procedures will be performed prior to randomization to ensure that the patient is eligible for the study:

- measurement of pressure, heart rate and temperature,
- gynecological ultrasound,

- the medications taken by the patient will be evaluated, and they will receive further recommendations on the use of the medication,
- AEs will be evaluated,
- The doctor will communicate with the laboratory and confirm whether the embryo is eligible for transfer after thawing and whether the patient meets the criteria for inclusion in the study.

Stage II: after randomisation, the patient enrolled in the clinical trial will undergo embryo transfer of a thawed embryo using a method consistent with the randomisation group in which she was placed.

To perform the embryo transfer procedure, i.e. the administration of embryos into the uterine cavity, soft catheters will be used - Cook Medical Guardia™ Access Embryo Transfer Catheter K-JETS 7019, tightly connected to the UltraCruz 3 syringe – Part Soft-Ject Disposable Syringes, 1 ml Tuberculin. In the absence of the indicated medical devices on the market, the use of certified substitutes is allowed. In addition, in the test group, the catheter/syringe transfer kit will be accompanied by an Embryopass applicator, an Embryocase case or both devices at the same time. Common to the control group and all research groups will be the cultivation of embryos in the TLS system and the evaluation and selection of embryos for transfer by artificial intelligence (AI).

The embryo transfer procedure will be divided into stages:

a. Preparation of the patient prior to transfer, as described in section 5.1 – literature review

   In all groups, the control group and the three subjects, patients will be prepared for embryo transfer in the same way.

b. The insertion of a catheter leading to the uterine cavity consists in inserting, with great delicacy, the catheter through the internal opening of the cervix into the interior of the uterine cavity just behind the internal outlet.

   After the catheter leading to the inside of the uterine cavity is placed, the nurse assisting the doctor will inform the embryologist that it is ready for transfer. The embryologist will then begin the procedure of collecting embryos into the catheter.

   In all groups, this stage of the embryo transfer procedure will look the same.

c. Proper embryo acquisition into the transfer catheter. The embryos will be taken into the catheter directly from the medium (medium) intended for embryo transfer from the transfer dish, and the whole operation should not take more than 2 minutes, according to the scheme: air (marker) - medium with embryo-air (marker)-medium.

This step of the procedure will vary from group to group:

in the control group (TL/AI – ET manual and study group no. 1 (TL/AI – ET manual + EMBRYOCASE)

The embryologist will collect embryos into the transfer catheter in a manual manner, according to the above scheme adopted at the center. The time from the start of the 1st air bubble intake to the completion of the media intake will be measured by a stopwatch.

in study groups 2 (TL/AI – ET using EMBRYOPASS) and 3 (TL/AI – ET using EMBRYOPASS + EMBRYOCASE), the embryologist will collect embryos into the transfer catheter with the help of the Embryopass applicator, in accordance with its instructions for use. The embryologist will be trained in the use of the applicator by its designer, which will be confirmed by a training certificate (the Embryopass user manual will be available in the laboratory - attachment to the investigator's brochure). The embryo acquisition time will also be measured.

d. After collecting the embryos into the catheter, the embryologist will transfer the catheter to the transfer room without additional protection in the control group and study group 2, or after placing the catheter with the embryo in the Embryocase case (groups 1 and 3).

e. Visualization of the embryo transfer process using an ultrasound machine allows you to visualize the entire process of embryo transfer from the catheter to the inside of the uterine cavity.

In all groups, transfers will take place under ultrasound control.

f. Administration of embryos into the uterine cavity (embryo transfer). The proper administration of embryos into the uterine cavity consists in the insertion by the doctor of a guide catheter, a catheter containing embryos suspended in the transfer medium, the so-called embryonic catheter, into the previously inserted into the uterine cavity. This is a key stage of the entire embryo transfer process.

This step of the procedure will vary from group to group:

- ✓ In the control group and the 1 study group, the operating physician, after inserting the guide catheter, places the embryonic catheter in it and gently moves it inside the uterus. After making sure that the embryonic catheter is in the optimal place of the uterine cavity (1.5-2 cm from the bottom of the uterus), the operator presses the plunger of the syringe connected to the embryonic catheter with his finger and carefully causes the embryos to move from the catheter to the inside of the uterus by pressing on the plunger. Once the maximum resistance of the plunger has been reached and observing whether the last marking marker remains in the uterine cavity, the doctor retracts the catheters from the cavity. To measure the time it takes for the embryos to be placed in the uterine cavity, the doctor will inform the embryologist about the start and end of the procedure, and its duration will be measured with a stopwatch;
- ✓ in study groups 2 and 3, the doctor will administer embryos from the transfer catheter into the uterine cavity using the Embryopass applicator, according to its instructions for use. The doctor will be trained in the operation of the applicator by its designer, which will be certified by a training certificate. Instead of pressing the plunger of the syringe, the doctor will press the appropriate button on the Embryopass applicator, which will trigger the movement of the syringe plunger.

As the time of embryo administration with the Embryopass applicator is a constant value, there is no need to control the time at this stage. The catheter will be withdrawn from the uterine cavity immediately after the embryos are administered.

g. Checking the catheters. Once the embryos have been administered into the uterine cavity, the embryonic catheter will be flushed using a transfer medium under a microscope to make sure that the embryo remains in the uterine cavity. It is allowed to re-administer the embryo if it remains in the catheter.
h. The course of the procedure and any adverse events that will occur during the embryo transfer procedure will be recorded in the documentation.
i. Immediately after the transfer, the patient will get up from the chair and leave the transfer room.

9.9 βhCG Visit

Confirmation of pregnancy will occur at the visit 10-15 days after ET. During this assessment visit, the following procedures will be carried out

- measurement of pressure, heart rate and temperature;
- determination of βhCG concentration in blood serum. A concentration ≤ 5.3 mIU/ml will be considered negative (no pregnancy), and a result above this value will be considered positive (pregnancy). In the event of a doubtful βhCG result, subsequent determinations will be carried out in accordance with the routine practice of the center;
- the medications you are taking will be evaluated and you will receive further recommendations on medication use.
- AE/SAE will be evaluated.

It is possible for the patient to carry out the pregnancy outside the research center at the place of residence, provided that medical data concerning individual stages of pregnancy development (βhCG, ultrasound, etc. tests will be made available to the doctor of the "nOvum" clinic in the form of documents, e.g. scans, photocopies or consulted with the doctor through any form of consultation, e.g. televisits, video consultation).

In the absence of pregnancy, the patient ends the test and stops taking progesterone.

9.10 Visit – confirmation of clinical pregnancy

- measurement of pressure, heart rate and temperature;
- physical examination;
- examination in speculums;
- ultrasound to assess the presence of a pregnancy follicle in the uterine cavity, 25 – 35 days after ET;
- the medications you are taking will be evaluated and you will receive further recommendations on medication use.
- AE/SAE will be evaluated.

It is possible for the patient to carry out the pregnancy outside the research center at the place of residence, provided that medical data concerning individual stages of pregnancy development (βhCG, ultrasound, etc. tests will be made available to the doctor of the "nOvum" clinic in the form of documents, e.g. scans, photocopies or consulted with the doctor through any form of consultation, e.g. televisits, video consultation).

The examination is completed.

9.11 Termination of pregnancy

• Data on the termination of pregnancy (data on childbirth, miscarriage or ectopic pregnancy) will be collected from patients using a questionnaire developed by the investigators.

• The patient can send the questionnaire to the center or provide the information contained in it by phone to one of the members of the research team.

• The questionnaire is attached as Annex No. 1 to the survey protocol.

9.12 Diagram of procedures/visits

Type / Appointments/Treatments / Type Procedures	Informed consent of patients to participate in the study.	Screening	Synchronization of the endometrium with the age of embryos.	Embryo thawing	Randomization.	Embriotransfer	βhCG Visit	Visit – confirmation of clinical pregnancy.	Termination of pregnancy
Informed consent	X								
Inclusion Criteria		X			X				
Exclusion Criteria		X			X				
Gynecological examination		X	X						
Ultrasound examination		X	X			X		X	
Trial transfer		X							
Ordering the tests required for CRYO-ET		X	X						
Medication regimen		X	X			X	X	X	X
Embryo thawing order			X						

Embryo thawing				X					
Embryo culture after thawing				X					
Embriotransfer						X			
Serum βhCG levels							X		
AE	X	X	X	X	X	X	X	X	X
SAE	X	X	X	X	X	X	X	X	X

10. PARAMETERS TO BE ANALYSED IN THE STUDY

10.1 Demographics

Demographic information collected in the study:

- Age
- Race
- Ethnicity

The information collected from the patient will be recorded in the documentation.

10.2 Body Measurements

During the screening, body measurements will be evaluated:

- Measuring body weight;
- Height measurement
- BMI.

10.3 Medical history

All relevant medical events will be described during screening, indicating whether they occurred in the past or are ongoing in the present. The information collected from the patient will be recorded in the documentation.

10.4 Cause of infertility

During screening, the history of infertility will be described:

- cause of infertility,
- duration,
- Treatment history (previous IVF attempts, if any. Patients who have already undergone in vitro fertilization (IVF) may be included in the study only if the previous transfer ended in pregnancy and childbirth and meet the other criteria).

The information collected from the patient will be recorded in the documentation.

10.5 Evaluation of the menstrual cycle

During the screening, information about the patient's menstrual cycle will be collected:

- Average cycle length
- Cycle regularity

The information collected from the patient will be recorded in the documentation.

10.6 Obstetric Interview

During the screening, information about the patient's obstetric history will be collected:

- number of biochemical and clinical pregnancies,
- miscarriages ≥ 2 consecutive should be a criterion for exclusion,
- number of births.

The information collected from the patient will be recorded in the documentation.

10.7 Readiness to receive embryos after thawing

- During the preparation of the patient for transfer, the width of the endometrium will be assessed during each stimulation visit in accordance with the routine practice of the center (ultrasound examination). The result of the measurement will be recorded in the documentation.
- The patient's ovulation will also be evaluated.

10.8 Embryo Quality

- The quality of embryos and their development will be assessed in accordance with ESHRE and PTMRiE guidelines and by artificial intelligence. Observations will be recorded in the patient's medical records and electronic documentation of the examination.

10.9 Transfer Procedure

The course of the transfer and any difficulties related to the transfer will be recorded in the medical documentation.

10.10 Concomitant medications

10.10.1 Permitted medications/therapies

- Letrozole
- Progesterone (Duphaston, Cyclogest) to supplement the luteal phase,
- Ovulation induction medications, if used,
- other medicines/supplements that the investigator considers have no effect on fertility or the outcome of the embryo transfer procedure,
- Bokmål

10.10.2 Prohibited medications/therapies

- preparations other than hCG used for the purpose of liberation, which prevent the embryo transfer procedure from being performed,
- drugs with proven harmful effects on fertility,
- drugs used in oncological therapy with a proven harmful effect on fertility,
- drugs that have a relaxing and antispasmodic effect on the uterus – atosiban, relanium scopolan (hyoscine butylbromide) or other hyoscine derivatives,
- heparin drugs,
- acetylsalicylic acid.

10.11 Endometrial thickness

When preparing the patient for CRIO-ET, endometrial width will be assessed at each visit according to the center's routine practice (ultrasound). The result of the measurement will be recorded in the documentation. A result below 7 mm on the day of ovulation will disqualify from embryo transfer.

10.12 Transfer Procedure

The course of the transfer and any difficulties related to the transfer will be recorded in the medical documentation.

The data to be analysed are:

- type of catheter,
- the degree of difficulty of the transfer,
- ET time: Time of embryo collection into the catheter, time of administration by the doctor in the manual method, total ET time from collection to administration.

11. END OF THE STUDY

11.1 Study Completion

It should be assumed that the patient has completed the clinical trial if:

- She completed all scheduled procedures and visits in accordance with the protocol.
- has not become pregnant as a result of a medically assisted procreation procedure (completion of the study occurs during a βhCG visit 10-15 days after ET),
- positive blood serum βhCG result was found at the visit 10-15 days after ET, ends the study at the clinical pregnancy confirmation visit 25-35 days after ET,

- A questionnaire confirming the termination of pregnancy (childbirth/miscarriage) will allow you to achieve additional goals in the study.

11.2 Withdrawal of consent

The patient and her partner may withdraw their consent to participate in the clinical trial in writing at any time. If one of the partners withdraws consent to a clinical trial during the course of the trial, the trial is considered to have been discontinued. Patients do not need to provide reasons for withdrawing consent, but the investigator must accurately document the circumstances of the withdrawal of consent in the medical records.

After the withdrawal of consent by the patient, no additional treatment data will be collected, but any medical data collected until the withdrawal of consent may be analysed, used for statistical purposes, scientific publications, etc.

11.3 Discontinuation

During the clinical trial, the patient may be excluded from participation in the study by the investigator due to:

- failure to meet the inclusion or exclusion criteria at each stage of the study;
- an adverse event that, in the opinion of the investigator, prevents the patient from further participation in the study,
- withdrawal of consent by one or both partners,
- a material breach of the protocol – failure to comply with the rules of the protocol, as a result of which the test results could be falsified,
- inability to contact the patient.

In place of a patient excluded from the pre-randomisation trial, the centre may enrol another patient in the study, assigning her another screening number.

If a patient has been excluded from a randomized trial, the center will not enroll another patient.

11.4 Reasons for discontinuation of a clinical trial by a facility

The reasons for the discontinuation of a clinical trial by the center may result from various factors. The Centre may decide to discontinue the examination in the event of:

1. Patient safety issues:
   a. the occurrence of unexpected, serious side effects that outweigh the benefit of the treatment,

b. the occurrence of unacceptable risks, e.g. the discovery of new information that suggests that the risks associated with the therapy outweigh the potential benefits.

2. Lack of treatment efficacy:
   a. periodic analyses will show that the subject of the study is not effective in achieving the assumed goals,
   b. The data will indicate that the continuation of the study will not bring positive clinical results.
3. Ethical violations: the study may be terminated if ethical principles are violated.
4. Organizational or financial problems:
   a. lack of sufficient financial resources to continue the study,
   b. too low a level of recruitment of participants, which makes it impossible to obtain representative data.

12. PARAMETERS TO BE ANALYSED OUTSIDE THE STUDY

After the study is completed, data on births will be collected.

13. ADVERSE EVENTS

13.1 Definition

Adverse event – any medical event that deviates from the norm.

An adverse event should be treated as:

- ✓ abnormal laboratory test results,
- ✓ symptoms and diseases that may exclude the patient from the clinical trial,
- ✓ applicator dysfunctions found during its operation,
- ✓ difficult transfer (criteria according to routine procedures of the center)

13.2 Scope and method of collecting data on adverse events

Adverse events will be reported in the medical history from the 1st stimulation visit to the study end visit. Medical events that were noted during the screening visit will not be treated as adverse events, but medical history.

When describing an adverse event, the investigator will note in the medical records:

- the start and end dates of the adverse event,
- diagnosis (name of the adverse event),
- clinical significance,
  - ✓ CS- the event is medically significant,
  - ✓ NCS – no clinical significance,

- relationship with the subject under study,
  - ✓ Yes – the occurrence of an adverse event is related to the use of the study product
  - ✓ no - the occurrence of the adverse event is not related to the use of the study product
- intensity, where:
  - ✓ weak – means no impact on the patient's daily activity,
  - ✓ medium – the patient feels discomfort affecting her daily activity,
  - ✓ serious – the adverse event significantly limits the patient's functioning or poses a threat to her life),
- Actions taken in relation to the subject in relation to the occurrence of an adverse event
  - ✓ No action
  - ✓ resignation from the use of the test object
- actions taken to correct the adverse event

14. SERIOUS ADVERSE EVENTS

14.1 Definition

A serious adverse event is a sudden and unforeseen medical event:

- resulting in the death of the patient,
- posing a threat to the patient's life,
- requiring hospitalization of the patient for more than 24 hours (excluding the patient's planned stay in hospital),
- an adverse event (AE) whose intensity has been defined as "serious" and for which medical action must be taken to avoid hospitalization, threat to life or death of the patient.

14.2 Scope and method of collecting data on serious adverse events

Serious adverse events (SAEs) will be reported in the medical history starting at the 1st stimulation visit.

When describing an adverse event, the investigator will note in the medical records:

- the start and end dates of the serious adverse event,
- diagnosis (name of the adverse event),
- clinical significance,
  - ✓ CS - the event is medically significant,

- ✓ NCS - no clinical significance ,

- relationship with the subject under study,
  - ✓ the occurrence of an adverse event is related to the use of the study product
  - ✓ the occurrence of an adverse event is not related to the use of the study product
- Actions taken in relation to the subject in relation to the occurrence of an adverse event
  - ✓ No action
  - ✓ resignation from the use of the test object
- actions taken to correct the adverse event,

15. METHOD OF DATA COLLECTION AND PROCESSING

15.1 Source data

The source data will be medical documentation in the form of: patients' health and illness history, embryological protocols, electronic documentation in the bmedica system2.

15.2 Electronic Database - eCRF

The information collected in the medical records will be systematically entered into the eCRF system within 14 days of each visit. The database will be closed when all required data has been completed and any doubts and deficiencies have been clarified.

16. DEVIATIONS FROM THE PROTOCOL

Deviations from the protocol are prohibited. Any deviation from the protocol is treated as a protocol deviation. They must be documented and justified by the investigator in the medical records.

17. MONITORING

The planned therapeutic experiment **will not be subject to external monitoring**. This is the center's own study, non-commercial. The lack of external monitoring is justified as the entire research team has a solid GCP background and research procedures and ethical standards are properly implemented at the site, ensuring a high level of internal supervision and patient safety.

Knowledge and experience of the research team at GCP: the entire research team has been trained and is fully aware of the principles of GCP, which are the international ethical and scientific standard for the conduct of clinical trials. Thanks to this, the team

has adequate knowledge of the obligations that arise from the GCP, which minimizes the risk of errors and ethical violations.

High quality of data and procedures: GCP principles ensure that a clinical trial is conducted appropriately and that the data obtained during the experiment is reliable, reliable and accurate. The team knows the rules for reporting and documenting results, which reduces the need for external monitoring.

Responsibility of the research team: each member of the research team is aware of their role and responsibility in the experiment. All employees are trained to comply with ethical principles, which allows for effective risk management and ensures patient safety.

Transparency and compliance with the study protocol: The study will be conducted in accordance with an approved protocol that precisely defines all procedures, including how data is collected and actions to ensure data integrity. In the event of any deviations or issues, the team will act in accordance with GCP policies, allowing for ongoing identification and resolution of issues without the need for formal monitoring.

18. LEGAL AND ETHICAL REGULATIONS

The research center applied to the Bioethics Committee for permission to conduct this study.

19. REPORTING AND PUBLICATIONS

It is planned to prepare a report on the results of the research and publish an article in the scientific press on the standardization of the embryo transfer procedure and the effectiveness of the Embryopass applicator, the Embryocase case and the TL(AI)/ Embryopass/ Embryocase protocol.

20. PROTECTION OF PERSONAL DATA

The administrator of the Patients' personal data is the Medical Clinic "nOvum" Katarzyna Kozioł Piotr Lewandowski Sp. j., 13 Bociania Street, 02-807 Warsaw.

The processing of Patients' personal data is carried out on the basis of Article 6(1)(a) and Article 9(2)(a) of Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (GDPR) and on the basis of the provisions of applicable law.

21. LITERATURE

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9. Munné, S., Alikani, M., Ribustello, L., Colls, P., Martínez-Ortiz, Pedro A. Referring Physician Group., McCulloh, D.H. Euploidy rates in donor egg cycles significantly differ between fertilty centers. Human Reproduction 2017; 32: 743–749.9.

22. ANNEXES

1. Patient Survey on Pregnancy and Childbirth
2. "Protocol for the study of environmental changes in peritransfer time: documentation of temperature changes in the embryonic catheter in peritransfer time defined as the time from the removal of the catheter from the incubator through the stage of embryo acquisition with the medium to the catheter, the path between the laboratory and the transfer room, to the time of insertion of the embryonic catheter into the uterine cavity during the embryo transfer procedure"