STUDY PROTOCOL FOR EVALUATE THE EFFECTIVENESS OF PROGESTIN-PRIMED VERSUS GNRH ANTAGONIST PROTOCOLS IN VIETNAMESE WOMEN UNDERGOING IN-VITRO FERTILIZATION

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BACKGROUND

Controlled Ovarian Stimulation (COS) plays a significant role in the cycle of In Vitro Fertilization (IVF) or Intra-Cytoplasmic Sperm Injection (ICSI). Premature luteinizing hormone (LH) surge is the main reason for cycle cancelation during COS (1). Two principal methods of pituitary suppression are employed to delay the onset of LH peak in the COS cycle: (1) prolonging the administration of a gonadotropin-releasing hormone agonist (GnRH-a) before the implementation of COS in a long-acting agonist regimen (2) immediate pituitary suppression with a gonadotropin-releasing hormone antagonist (GnRH-ant) in a short-acting antagonist regimen (2). Recently, with the successful development of embryo freezing and thawing techniques, a new COS protocol was proposed using progestin as a "primer" (Progestin-Primed Ovarian Stimulation – PPOS) in the act of inhibiting the LH peak and preventing ovulation occurrence. In 2015, Kuang et al. first reported this protocol and demonstrated efficacy in preventing premature luteinization (3). The PPOS protocol is dominant due to its patient-friendly benefits: oral administration, convenience, and lower cost. Several studies have highlighted the potency of PPOS in prohibiting premature LH surge and promoting positive pregnancy outcomes compared to classical COS regimens in infertile women with normal ovarian reserve (NOR) or those with polycystic ovary syndrome (PCOS) (4-11).

An important factor in determining the effect of progesterone (stimulatory or inhibitory) on pre-ovulatory gonadotrophin secretion, or even its synergistic or antagonistic effect with estrogen, is the timing of administration, the rate of administration, and P4/E2 hormone ratio. Previous studies still have not had consensus; some studies show that at the end of the follicular phase, progesterone concentration increases slightly due to the secretion of granulosa cells of pre-ovulatory antral follicles, causing a slight increase in LH concentration, proving the role of progesterone's stimulating role on gonadotropins (12); However, the LH peak only achieve when progesterone is administered after estrogen has previously been primed. On the other hand, high levels of progesterone from early in the menstrual cycle have been shown to suppress LH peaking (13), with its primary role being

to inhibit follicle development and subsequent inhibition of ovulation, which is the contraceptive basis of progestin-only contraceptives. Recently, it has not been possible to use a single hypothesis related only to estradiol, progesterone, gonadotrophin, GnRH, kisspeptin, and the time to start taking progestogen to explain the mechanism of LH peak appearance and ovulation induction. DYG is a potential alternative progestin for the PPOS protocol in ART (4, 10); it has a high selectivity for progesterone receptors with potent progestogenic activity. In contrast to other progestins, DYG has no clinically relevant agonistic or antagonistic action on the androgen, estrogen, and glucocorticoid receptors and only mild antimineralocorticoid features. Unlike natural progesterone, dydrogesterone has good oral bioavailability; therefore, it may reduce the side effects of progestins (14-16). However, few studies compared the PPOS protocol's efficacy with DYG and GnRH-antagonist protocols. In this RCT, we compared the effects of two protocols, PPOS using DYG and GnRH-ant, on the ovarian response and clinical outcomes in patients undergoing IVF or ICSI.

METHODS AND ANALYSIS

Objectives

This trial aims to compare the efficacy and safety of the PPOS protocol to the GnRH antagonist protocol in patients undergoing IVF procedures.

Design of the trial

This was an open-label, randomized controlled trial conducted at the Assisted Reproduction Center of Tam Anh General Hospital between January 2023 and December 2024. The study aimed to estimate the efficacy of the intervention (PPOS protocol) compared to a control group receiving a gonadotropin-releasing hormone (GnRH) antagonist protocol. Inclusion criteria were women aged 20-45 years undergoing IVF. Exclusion criteria included failure to complete ovarian stimulation, systemic diseases like renal failure or lupus, uterine abnormalities, random-start controlled ovarian stimulation cycles, and preimplantation genetic testing for aneuploidies. The study details were explained to all participants, and informed consent was provided. This study was approved

by the Ethics Committee of Hanoi Medicine University, decision number 842, dated July 7, 2023.

Eligibility criteria

Eligible patients must meet all inclusion criteria, and no listed exclusion criteria exist.

Inclusion criteria

Women meeting the sample selection criteria are as follows: (1) Women aged 20 to 45; (2) Infertility due to male factors, fallopian tube factors, or unknown causes; (3) Undergoing IVF in one COS cycle and intended to apply either GnRH antagonist protocol or PPOS protocol; (4) Voluntary participation in research.

Exclusion criteria

Women who met any of the following criteria were excluded: (1) Any contraindications to ovarian stimulation and IVF/ICSI treatment; (2) Hyperprolactinemia or other endocrine diseases; (3) Those who took hormone drugs within the past three months; (4) Suffering from systemic diseases such as kidney failure, lupus erythematosus, depression, etc.; (5) Abnormal structure of the uterine cavity; (6) Patients with endometriosis or cancer; (7) Random-start cycles; (8) Oocyte donation cycles; (9) Perform embryo biopsy.

Recruitment of study participants

Infertile patients come for examination at the Assisted Reproduction Center of Tam Anh General Hospital, Hanoi, and the Assisted Reproduction and Graft Technology Center of Hanoi Medical University. In the basic infertility examination process at the hospital, the clinicians will consider the patient's inclusive and exclusive criteria. The clinicians will contact a research team member if the inclusive criteria are met. A research team member or hospital physician will invite the patient to participate, advise on the study procedure and the benefits and risks of participating, and answer any patient questions (Appendix 2). If the patients agree to participate, they will consent to participate in the trial (Appendix 3). Each participant will be randomly assigned to the treatment group using the PPOS protocol or the control group using the GnRH-ant regimen.

Sample size estimation

The sample size was estimated to optimize the precision of non-inferiority testing. This statistical inference is based on a Negative Binomial (NBI) regression model, which aims to compare the average number of retrieved oocytes (or MII oocytes) between two distinct groups, as described by Cundill and Alexander (2015) (17). The following formula encapsulates the estimation process:

$$\sqrt{N} = \frac{Z_{1-\alpha}\sqrt{\left(\frac{1}{\mu_0}+\frac{1}{k_0}\right)\left(\frac{1}{Q_1}+\frac{1}{Q_0}\right)}+Z_{1-\beta}\sqrt{\frac{1}{Q_1}\left(\frac{1}{\mu_1}+\frac{1}{k\_1}\right)+\frac{1}{Q_0}\left(\frac{1}{\mu_0}+\frac{1}{k_0}\right)}}{log\ (\mu_0) - log\ (\mu_1) - \delta}$$

Where:

N represents the aggregate sample size, with each unit being a cycle or patient.

μ_0 and μ_1 denote the average outcome values in the control group (GnRH_ant regimen) and treatment group (PPOS regimen), respectively. The outcome might be the count of retrieved oocytes or MII oocytes.

Q_0 and Q_1 are the allocation ratios for the control and treatment groups, respectively. They represent the proportion of the total sample size that should be allocated to each group. In our research, both Q0 and Q1 were set to 0.5.

k_0 and k_1 are the dispersion parameters of the NBI distribution for the control and treatment groups, respectively.

δ is the non-inferiority margin (in log scale), signifying the minimum clinically or practically significant difference.

Z_p is the critical value, or pth percentiles of a standard normal distribution

α is the Type I error, set at 0.05, and β is the Type II error, set at 0.2 (corresponding to a statistical power of 0.8).

From January 1st to October 10th, 2022, at Tam Anh Hospital, 804 patients underwent ovarian stimulation. We observed average retrieved oocyte counts of 11.2 and 11.4 and average mature oocyte counts of 7.62 and 7.54 in the PPOS and GnRH-ant groups, respectively. We engaged in a simulation process with these observations, utilizing the formula to determine potential sample size values. We let $\mu 0$ and $\mu 1$ span an interval from 6 to 12 units, while the difference between the groups ranged from -1.5 to 1.5 units. The non-inferiority margin remained constant at log(1.5) units. The results from this simulation are illustrated in Figure 1. According to this result, we concluded that a sample comprising 200 patients (split equally between the groups) would be required. This size ensures that no difference in the number of retrieved or MII oocytes between the PPOS and control group would exceed 1.5 units.

Randomization Process

We apply a stratified randomization process to ensure unbiased allocation of participants to GnRH-ant and PPOS treatment arms. The process is designed and executed using Python programming language. Initially, each participant will be assigned a unique identification (ID) constructed from a combination of uppercase letters (A-Z) and numbers (0-9), with customizable ID lengths. Concurrently, each participant is categorized based on the "POSEIDON" criterion, a categorical stratification variable with four potential categories: P1, P2, P3, and P4. Participants are allocated to these strata by a predefined probability distribution of 14.86%, 25.39%, 15.99%, and 43.76%, respectively. This distribution was derived from the actual distribution of POSEIDON categories among patients who underwent IVF treatment at Tam Anh Hospital during 2020-2022. Within each "POSEIDON" stratum, participants are randomized equally across the two treatment groups (GnRH-ant or PPOS protocol). The process ensures that if there's an odd number of units in a stratum, the extra unit is randomized within the stratum rather than across the entire dataset.

Treatment method

Ovarian stimulation protocols

Controlled ovarian hyperstimulation commenced on the second day of the menses utilizing recombinant follicle-stimulating hormone (Follitrope, LG Chem, South Korea) at dosages between 150-300 international units (IU) per day. Initial gonadotropin dosages were determined per patient based on baseline parameters, including age, AMH, baseline FSH, body mass index (BMI), antral follicle count (AFC), and titrated subsequently per folliculogenesis response. In the gonadotropin-releasing hormone (GnRH) antagonist protocol group, pituitary suppression began on stimulation day six via daily 0.25 milligram GnRH antagonist administration (ganirelix or cetrorelix). In the progestin-primed ovarian stimulation (PPOS) group, 30mg/day of dydrogesterone was initiated on cycle day 2 through to trigger day. Serum luteinizing hormone (LH) levels were quantified on stimulation days one, six, and eight alongside trigger day using competitive electrochemiluminescence immunoassays via Cobas analyzer. Once dominant follicles reached ≥20 mm diameter or ≥ 3 follicles met 18 mm threshold, final oocyte maturation was induced. Depending on ovarian hyperstimulation risk, either 0.2 mg triptorelin (Diphereline, Ipsen Pharma Biotech, France) alone was administered for high-risk patients or a Dual trigger consisting of 2000 IU hCG (IVF-C, LG Chem, South Korea) combined with 0.2 mg triptorelin (Diphereline, Ipsen Pharma Biotech, France) was used for the remaining patients. Oocyte retrieval via transvaginal aspiration was performed approximately 36 hours later.

Laboratory protocol

Oocyte-cumulus complexes were incubated for 2 hours in G-IVF medium (Vitrolife) using Origio benchtop incubators to complete nuclear maturation. After removing cumulus cells, denuded oocytes were evaluated under an inverted microscope to validate the achievement of metaphase II status, while degenerate, large, or severely dysmorphic oocytes were excluded. Intracytoplasmic sperm injection (ICSI) was executed 3-4 hours post-retrieval by experienced embryologists. Resultant zygotes were cultured in continuous single media

(Fujifilm Irvine Scientific) within tri-gas incubators (37°C, 5% O2, 6% CO2) until day 3. Strict morphological criteria were enforced, only retaining normally fertilized two pronuclei zygotes while eliminating abnormal multinuclear embryos. Cleavage-stage quality was graded at 67-69 hours per Istanbul consensus based on cell number, fragmentation, multinucleation, and uniformity. On post-ICSI day 3, embryologists counseled patients on pursuing blastocyst culture versus cryopreservation. The morphology of the blastocysts was evaluated using the Gardner and Schoolcraft grading system, and embryos meeting the criteria of 3-6 AA/AB/BA blastocysts or 1-2 AA/AB embryos were classified as good quality.

Outcome measurements

Primary outcomes

The primary outcome is the success rates, including maturation, fertilization, and probabilities of achieving Day 3 and Day 5 embryos.

Secondary outcomes

The secondary outcome is the process of the evolution of LH levels in the PPOS group.

We transferred the cryopreserved embryo in the next cycle after the stimulation cycle. Perform quantitative hCG test 10 days or 13 days after S5 or S3 embryo transfer: (1) If there is a positive hCG test (>5 mIU/ml), the pregnancy will continue to be monitored; (2) Negative hCG test (<5 mIU/ml), we determined the outcome to be a failure in the first embryo transfer, and the study ended.

Cancel cycles

The cycle is canceled for COS cycles where no follicles are larger than 12 mm after ten days of ovarian stimulation. The characteristics of the canceled cycle (starting dose, total gonadotropin doses, COS days, LH, E2, and P4 measurements) are still recorded in the data collection table. The patient will have COS performed again in the next cycle.

Safety endpoints

The safety endpoints include the incidence of moderate or severe OHSS, miscarriage rates, ectopic pregnancy, and the complications of oocyte retrieval. Ovarian hyperstimulation syndrome (OHSS) is due to the excessive response of the ovaries to ovarian stimulation. OHSS is an uncommon complication (1 - 5% per cycle) and dangerous during ovarian stimulation. According to the American Society for Reproductive Medicine (ASRM) in 2016, OHSS is commonly classified into mild, moderate, severe, and critical based on symptoms, ovarian size, ascites, and haemoconcentration (Hct)**.** Mild OHSS is diagnosed when presenting abdominal bloating, mild abdominal pain, and ovarian size < 8cm. Moderate OHSS is diagnosed by the ultrasonographic presence of ascites, ovarian size of 8-12 cm, with Hct >41%, and increased white blood cell count (>15 x 10^9/L). Severe OHSS is defined as clinical ascites (occasionally pleural effusions), Oliguria, hematocrit>45%, hypoproteinemia, and ovarian size usually >12 cm. Critical OHSS is diagnosed by tense ascites or large pleural effusions, hematocrit>55%, white blood cell count >25 x 10^9/L, oliguria/anuria, thromboembolism, acute respiratory distress syndrome, and so on. Complications of oocyte retrieval: (1) Vaginal bleeding: After completing the oocyte retrieval, the patient will have a speculum placed to examine the vagina. If bleeding is seen at the needle puncture site, the clinician will use gauze to press on the bleeding site and wait about 5 minutes. After that, the clinician checks again; if there is still bleeding, the clinician will sew the needle puncture site. (2) Bladder hemorrhage: caused by the needle piercing the egg through the bladder, with the primary symptom being red urine. Most cases will not require intervention. Patients should drink plenty of water, and in some cases, hemostatic drugs or antibiotics may be considered. If red urine persists or has blood clots in the bladder, washing the bladder could be regarded, and a cystoscopy could be performed to stop the bleeding. (3) Internal bleeding due to ovarian cyst rupture: After oocyte retrieval, if the patient has symptoms: abdominal pain (lower abdomen, one side or both sides, can spread to the shoulders), abdominal examination shows tenderness and reflexes abdominal wall, pain when touch pouch of Douglas or lethargy, fatigue, pale skin. The patient will be monitored for vital signs (pulse, blood pressure, temperature, SpO2),

and an ultrasound will be performed to check the ovaries, fluids, and abdominal cavity. At the same time, we will take blood to test for red blood cell index, hemoglobin, and hematocrit. If the patient's vital signs are stable, the amount of abdominal fluid is low, and the hematocrit is > 30%, the patient will be using hemostatic medication; the vital signs and hematocrit are monitored to determine whether the bleeding continues. When there are hemodynamic changes, or the hematocrit continues to decrease below 30%, the patient undergoes laparoscopic surgery to find the bleeding site in the ovary and cauterize the bleeding. (4) Infection at the egg retrieval site: infrequent, with symptoms such as fever, lower abdominal pain, pain on one side of the pelvis with increased white blood cells, and increased CRP. The patient will be treated with broad-spectrum antibiotics according to the hospital protocol.

The method of data collection

Personnel: This includes one main researcher and three associates**.**

Tools: Conducted using data collection table (Appendix 1)

Data collection plan: The data collection process corresponding to each step of the research process, shown in detail in Figure 2.3, is expected to occur over 18 months (from January 1, 2023, to June 30, 2024).

Research variables

Independent variable

Table 1 Independent variables

Variable name	Define	Type	Scale/encoding	Unit	How to collect
Treatment grouping	One of 2 groups: Intervention group: using PPOS protocol. Control group: using GnRH-ant regimen	Qualitative, binary	0 = control group (GnRH-ant regimen) 1 = Intervention group (PPOS regimen).		According to medical records
Time	Time point for LH quantification	Discrete qualitative	1 = S6 2 = S8 3 = ovulation day		According to medical records
Sampling location	Center/hospital where samples were collected. There is significant clustering of the data and may contribute to random effects on the observed results.	Discrete qualitative	1,2,3...: number corresponding to each hospital/reproduction center		

Covariations

Table 2 Covariates

Variable name	Define	Type	Scale/encoding	Unit	How to collect
A. Characteristics of the study population					
Age	Time is calculated in calendar years from the date of birth on the wife's ID card to the date of participation in the study	Continuous variables	[20 – 45]	Years old	Administrative information from medical records
Height	Height at the time of selection for the study, accurate to 0.5 cm	Continuous dosing	[150, 165.5, … 172]	cm	Direct measurement
Weight	Body weight at the time of selection for the study	Continuous variables	[45, ... 55... 60]	kg	Direct measurement

Variable name	Define	Type	Scale/encoding	Unit	How to collect
BMI	The patient's body mass index, according to the formula: BMI=Weight (kg)Height (m)2	Continuous variables	[19.1 … 23.4 …]	kg/m^2	Secondary data, calculated from height and weight
Infertility period	Time from when you wanted to have a baby and did not use contraceptives until you were selected for the study	Continuous variables	[twelfth …]	month	Interview and record in medical records
Causes of infertility	Is the cause of the patient's infertility	Identification	0 = Male factor 1 = Fallopian tube factor		Based on diagnosis recorded in

Variable name	Define	Type	Scale/encoding	Unit	How to collect
			2 = POR 3 = Ovulatory disorders 4 = Unknown cause		medical records
AFC	Number of ovarian follicles measuring 2 - 10mm on transvaginal ultrasound on day 2 of the menstrual cycle	Discrete quantification	[2 … 4 …]	ovarian follicles	Test results/medical records
AMH	Patient's AMH concentration before IVF (up to 3 months)	Quantitative, continuous	[0.5,1,2, …]	ng/ml	Test results/medical records

Variable name	Define	Type	Scale/encoding	Unit	How to collect
B. Characteristics of the in vitro fertilization cycle					
Gonadotropin starting dose	The first dose of gonadotropin (FSH + hMG) is administered during the ovarian stimulation cycle	Identification	0. 150 1. 225 2. 300 3. 375	IU	Take notes from medical records
Total gonadotropin dose	Total number of FSH + hMG drug units injected for ovarian stimulation	Quantitative, continuous	[1800, …]	IU	Take notes from medical records
COS time	Number of days of FSH + hMG injection	Quantitative, continuous	[8, …]	day	Take notes from medical records

Variable name	Define	Type	Scale/encoding	Unit	How to collect
Number of days using GnRH-ant	Actual number of days of GnRH-ant use in the GnRH-ant subgroup.	Quantitative, continuous	[4, …]	day	Take notes from medical records

Primary outcomes of the study (dependent variable)

Table 3 Dependent variables in the study

Variable name	Define	Type	Scale/encoding	Unit	How to collect
A. Ovarian stimulation results					
Expected number of oocytes	Expected number of oocytes to be collected: number of oocytes with size ≥ 12 mm on the day of ovulation.	Quantitative, discrete	[0,1,2,3…]	oocytes	Recorded from medical records
Number of oocytes obtained	Number of oocytes obtained	Quantitative, discrete	[0,1,2,3…]	oocytes	Recorded from

Variable name	Define	Type	Scale/encoding	Unit	How to collect
	after aspiration under the guidance of transvaginal ultrasound.				medical records
Number of MII oocytes	Number of ovarian follicles at metaphase II (MII) stage obtained after aspiration under the guidance of a transvaginal ultrasound probe	Quantitative, discrete	[0,1,2,3…]	oocytes	Recorded from medical records
Number of fertilized oocytes	Number of oocytes with 2 pronuclei appearing after about 18-20 hours after performing ICSI	Quantitative, discrete	[0,1,2,3…]	oocytes	Recorded from medical records

Variable name	Define	Type	Scale/encoding	Unit	How to collect
Number of embryos N3	Number of N3 embryos obtained after fertilization	Quantitative, discrete	[0,1,2,3…]	Embryo	Recorded from medical records
Number of embryos N5	Number of N5 embryos obtained after fertilization	Quantitative, discrete	[0,1,2,3…]	Embryo	Recorded from medical records
No embryos	The condition of eggs not being able to create embryos after IVF/ICSI	Binary	0 = No 1 = Yes		Recorded from medical records
Cancel cycle	The COS cycle is canceled according to the criteria in section 2.5.3	Binary	0 = No 1 = Yes		Recorded from medical records
Ovarian hyperstimulation (OHSS)	Is a treatment-induced complication of	Binary	0 = No 1 = Yes		Monitor and record from

Variable name	Define	Type	Scale/encoding	Unit	How to collect
	physiological ovarian hyperstimulation, characterized by symptoms of varying degrees as described in section 2.6.				medical records
OHSS level	The severity of OHSS is classified according to the standards in section 2.6	Qualitative	0 = Mild 1 = Average 2 = Severe 3 = Critical		Monitor and record from medical records
B. Describe the progression of LH hormone					
Average LH concentration	LH concentration was measured repeatedly on days S6, S8 and the day to initiate oocyte	Quantitative, continuous	[… 1,1 …]	IU/L	Recorded from medical records

Variable name	Define	Type	Scale/encoding	Unit	How to collect
	maturation and induce ovulation.				

Note: From these primary variables, secondary outcomes can be determined, for example: ratio of MII oocytes / total number of aspirated oocytes; ratio of retrieved oocytes/expected total number of oocytes; ratio of fertilized oocytes/total number of oocytes collected, etc.

Statistical analysis plan

Data analysis and visualization were done using the R programming language (ver 4.3.1). The following analytic procedure was applied to evaluate multiple outcomes across successive stages of a controlled ovarian stimulation procedure: (1) For the count data outcomes, such as the number of retrieved oocytes and number of maturated (MII) oocytes, their distribution was described using median and 5th to 95th percentiles. The adjusted effect of the PPOS regimen on these outcomes will be evaluated by a Negative Binomial (NBI) regression analysis; (2) The success rates, including maturation rate, fertilization rate, probabilities of achieving Day 3 and Day 5 embryos, as well as their transferability, were also described as median and 5th to 95th percentiles. The effectiveness of the PPOS protocol compared to GnRH-ant on the probability of success was evaluated as adjusted risk difference (aRD) and odds ratio (aOR) via a binomial regression model; (3) To achieve a precise estimation of the PPOS protocol's effect, adjustment for multiple baseline and procedural factors such as maternal age, AFC, baseline levels of AMH, LH, trigger method,

total FSH dose and length of stimulation was applied to regression analysis. The plausibility of each adjusted factor was confirmed based on data visualization and statistical analysis of the relationship between that factor and the targeted outcome. NBI and binomial regression models were fitted using the GAMLSS package (18). Confidence intervals for the marginal effects were determined by the delta method (19). Statistical inferences were based on null hypothesis testing at the significance level 0.05.

ETHICS IN RESEARCH

During IVF/ICSI, preventing premature luteinization is an essential part of COS ovarian stimulation. Among the current COS regimens, the antagonist COS regimen is the most commonly used regimen because it helps shorten ovarian stimulation time and is patient-friendly by reducing the amount of exogenous gonadotropin drugs required. injection and reduce treatment costs. However, the effectiveness in preventing LH surge is still quite variable. Recently, the PPOS regimen was born with the purpose of inhibiting the LH peak and preventing ovulation from occurring. Still, it has proven to be advantageous when the progestin route of administration is oral, meeting patient friendliness because of the reduction in the worry of having to inject too much medicine during the COS cycle. At the same time, progestin is also much cheaper than subcutaneous GnRH-ant. It should be noted that, until now, no regimen is optimal in effectiveness for all subjects. This also contributes to ensuring fairness and ethics in research. When participating in the study, patients are fully counseled about ovarian stimulation drugs, the IVF procedure, and the risks of complications that may occur during IVF, such as bleeding. vagina at the oocyte retrieval site, internal bleeding due to ovarian cyst rupture or infection at the aspiration site, ovarian hyperstimulation syndrome, possibility of cycle cancellation, fertilization rate, clinical pregnancy, and study progress (0. Patients also know which regimen they will use when undergoing ovarian stimulation (open-label). When agreeing to participate in the study, the patient will sign a consent form to participate in the study (04). All patient information is encrypted and confidential, used only for research purposes, not for any other purpose.

Patient and public involvement

Patients and the public are not involved in the trial process.

SUMMARY OF THE RESEARCH IMPLEMENTATION PROCESS

Table 4 Summary of the research implementation process

Time	Research stages						
	Sample selection	**Random grouping**	**After randomization**				
	After examination	Third day of menstruation	Ovarian stimulation	Oocyte aspiration	Fertilization and embryo culture	Embryo transfer	Monitor
Sample selection							
Receiving patients	x						
Examination	x						
Basic testing	x						
Sign consent	x						
Random grouping		x					
Intervention							
PPOS regimen			⧗ ⟷				
GnRH-anta regimen			⧗ ⟷				
Sampling and outcome assessment							

Time	Research stages						
	Sample selection	**Random grouping**	**After randomization**				
	After examination	Third day of menstruation	Ovarian stimulation	Oocyte aspiration	Fertilization and embryo culture	Embryo transfer	Monitor
Anthropometry and physiological status	x						
Characteristics of KTBT			x				
Main outcome							
Pregnancy progresses							x
Secondary outcomes							
Expected number of oocytes, number of oocytes recovered, number of MII oocytes, cycle cancellation				x			
Number of fertilized ovules, N3, N5 embryos, no embryos					x		
OHSS				x			

Time	Research stages						
	Sample selection	**Random grouping**	**After randomization**				
	After examination	Third day of menstruation	Ovarian stimulation	Oocyte aspiration	Fertilization and embryo culture	Embryo transfer	Monitor
Average LH concentration			x				

FIGURE

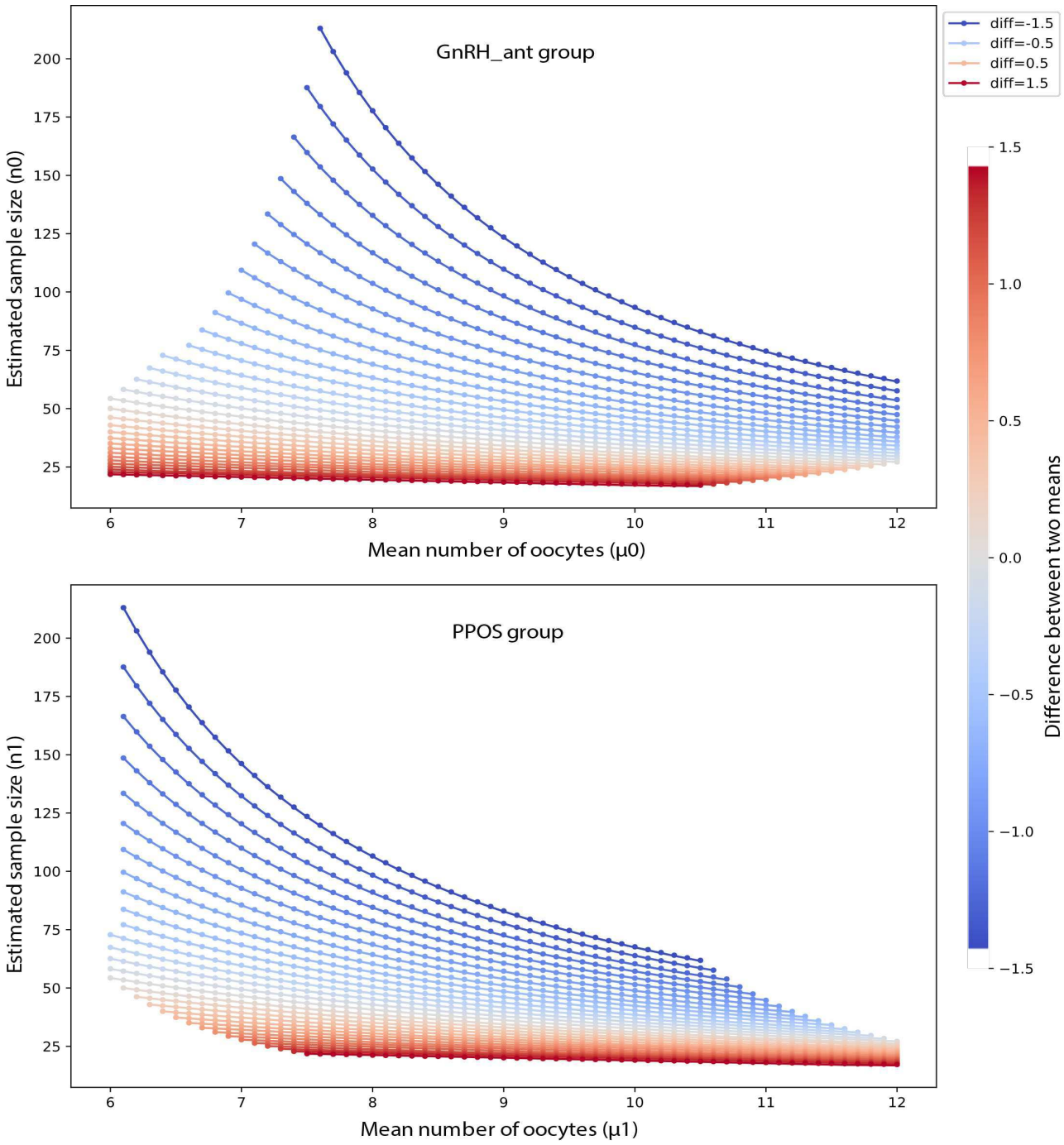

Figure 1 Results from the simulation estimate the sample size.

Note: According to this result, a sample comprising 200 patients (split equally between the groups) would be required. This size ensures that no difference in the number of retrieved or MII oocytes between the PPOS and control group would exceed 1.5 units.

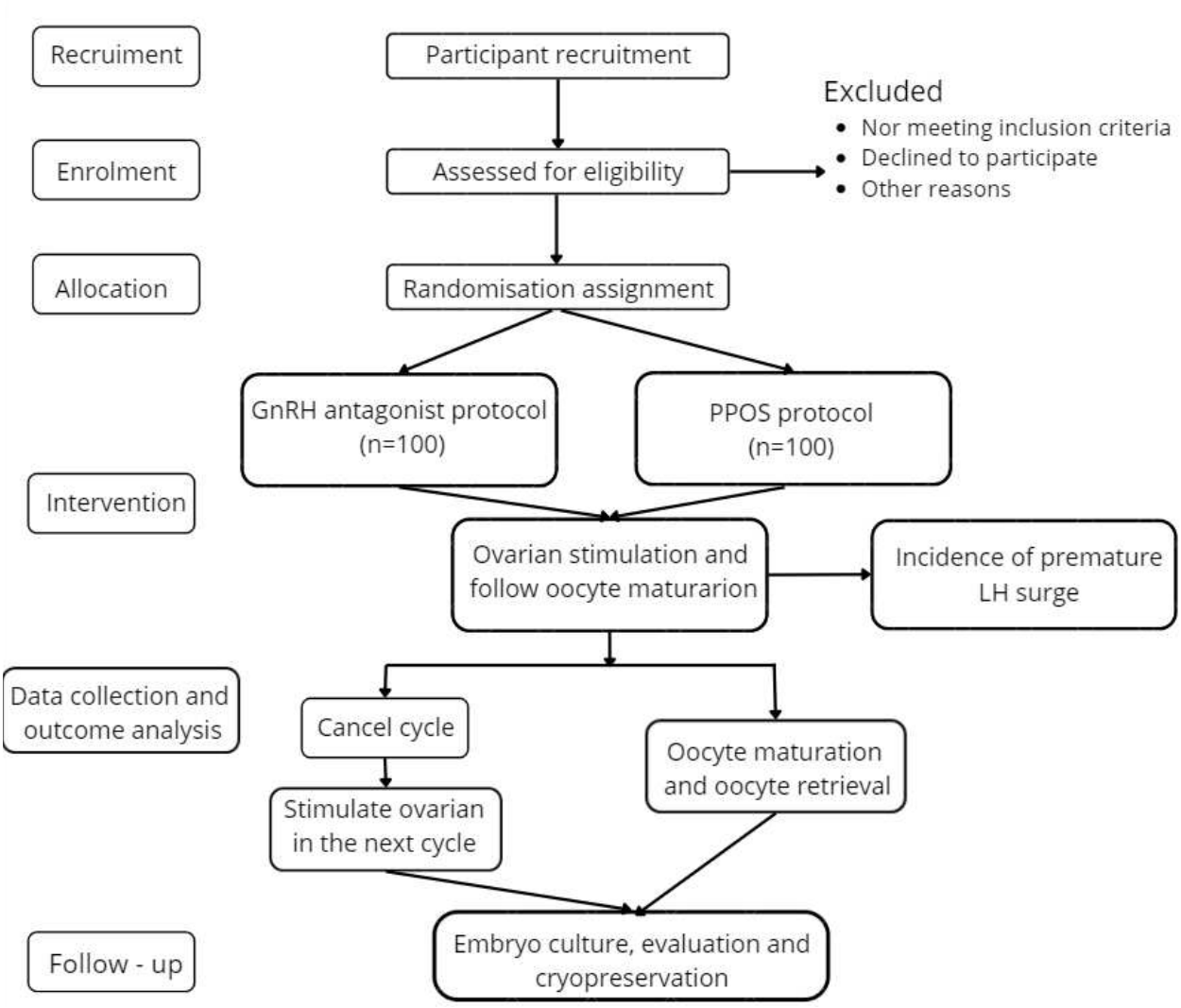

Figure 2 Flowchart of the participant allocation

APPENDIX

APPENDIX 1

DATA COLLECTION TABLE

STUDY

COMPARISON OF FERTILISATION RESULTS BETWEEN TWO OVARIAN STIMULATION PROTOCOLS WITH PROGESTIN AND ANTAGONIST IN VIETNAM POPULATION

DATA COLLECTION TABLE

I. PATIENT INFORMATION

Hospital:

File number:

First and last name:

Date of birth: [ ][ ] / [ ][ ] / [ ][ ][ ][ ] (Day Month Year)

Phone number:

Phone number (husband or other relative):

PROTOCOL used: [ ] PPOS [ ] GnRH-ant

II. DATA COLLECTION

A. BACKGROUND INFORMATION

Code	Question	Reply	Note
A1	Date of birth	[ ][ ] / [ ][ ] / [ ][ ][ ][ ]	

Code	Question	Reply	Note
		Day month Year	
A2	Height	[ ][ ][ ] cm	
A3	Weight	[ ][ ],[ ]kg	
A4	Infertility period	[ ][ ][ ] month	
A5	Causes of infertility[1]	[ ] 0. Male factor [ ] 1. Fallopian tube factor [ ] 2. POR [ ] 3. PCOS [ ] 4. Unknown cause	
Day 2 - 3 of menstrual cycle			
A6	AMH	[ ][ ],[ ] ng/ml	
A7	AFC[2]	[ ][ ]	

B. OVARIAN STIMULATION PROCESS

(S1 start date: [ ][ ] / [ ][ ] / [ ][ ][ ][ ])

Code	Question	Reply		Note
B1	First dose of gonadotropins	[ ] 150 IU [ ] 225 IU [ ] 300 IU [ ] 375 IU		
B2	***Day***	***E2 concentration (pg/ml)***	***P4 concentration (ng/ml)***	***LH concentration (IU/L)***

[1]If multiple causes coexist, choose the most dominant cause

[2]Ovarian follicle size 2 - 10 mm

Code	Question	Reply		Note
	[ ][ ] / [ ][ ] (Day S6)	[ ][ ][ ][ ],[ ][ ]	[ ][ ],[ ][ ]	[ ][ ],[ ][ ]
	[ ][ ] / [ ][ ] (Day S8)	[ ][ ][ ][ ],[ ][ ]	[ ][ ],[ ][ ]	[ ][ ],[ ][ ]
	[ ][ ] / [ ][ ] (Trigger)	[ ][ ][ ][ ],[ ][ ]	[ ][ ],[ ][ ]	[ ][ ],[ ][ ]
B3	Total gonadotropin dose	[ ][ ][ ][ ] IU		
B4	COS time	[ ][ ] day		
B5	Number of days using GnRH-ant[3]	[ ][ ] day		
B6	Early LH peak	[ ] 0. No [ ] 1. Yes (on date)		
B7	Number of cancellation cycles	[ ][ ]		

B'. OVARIAN STIMULATION PROCESS

(S1 start date: [ ][ ] / [ ][ ] / [ ][ ][ ][ ])

(For canceled cycles)

[3]For the GnRH-ant protocol group

Code	Question	Reply	Note
B'1	First dose of gonadotropins	[ ] 150 IU [ ] 225 IU [ ] 300 IU [ ] 375 IU	

	Day	***E2 concentration (pg/ml)***	***P4 concentration (ng/ml)***	***LH concentration (IU/L)***
B'2	[ ][ ] / [ ][ ] (Day S6)	[ ][ ][ ][ ],[ ][ ]	[ ][ ],[ ][ ]	[ ][ ],[ ][ ]
	[ ][ ] / [ ][ ] (Day S8)	[ ][ ][ ][ ],[ ][ ]	[ ][ ],[ ][ ]	[ ][ ],[ ][ ]
	[ ][ ] / [ ][ ] (Trigger)	[ ][ ][ ][ ],[ ][ ]	[ ][ ],[ ][ ]	[ ][ ],[ ][ ]

B'3	Total gonadotropin dose	[ ][ ][ ][ ] IU	
B'4	COS time	[ ][ ] day	
B'5	Number of days using GnRH-ant[4]	[ ][ ] day	
B'6	Early LH peak	[ ] 0. No [ ] 1. Yes (on date)	

C. RESULTS OF EMBRYORA AVOLUTION AND INFERTILATION

(Date of oocyte retrieval [ ][ ] / [ ][ ] / [ ][ ][ ][ ])

[4]For the GnRH-ant regimen group

Code	Question	Reply	Note
C1	Expected number of oocytes	[ ][ ]	
C2	Number of oocytes obtained	[ ][ ]	
C3	Number of MII oocytes	[ ][ ]	
C4	Number of fertilized ovules	[ ][ ]	
C5	Number of blanks	[ ][ ]	
C6	Number of embryos N3	[ ][ ]	
C7	Number of embryos N5	[ ][ ]	

APPENDIX 2

RESEARCH INFORMATION SHEET FOR PATIENTS

I. INFORMATION ABOUT RESEARCH

Study name: " COMPARISON OF FERTILISATION RESULTS BETWEEN TWO OVARIAN STIMULATION PROTOCOLS WITH PROGESTIN AND ANTAGONIST IN VIETNAM POPULATION."

Researcher and contact information

Main implementer: Doctor. Than Trong Thach

Contact phone: +84908.40.00.40

Email: thachthan007@gmail.com

Research sponsor: This research was not sponsored by any individual or entity. The person conducting the research pays for the necessary expenses of the research themselves.

II. RESEARCH PURPOSES

Progestin-primed ovarian stimulation regimen (using Dydrogesterone as a "primer" for the COS cycle) compared to a conventional ovarian stimulation regimen (GnRH-ant and gonadotropin) in Vietnamese women. performed in vitro fertilization.

III. REASON FOR INVITING TO PARTICIPATE IN THE STUDY

You were invited to participate in this study because you were treated with in vitro fertilization and met the above study inclusion criteria. You need to carefully read and understand the content provided in this information sheet. Before you decide to

participate in this study, you will be carefully explained about the research objectives and procedures, and you can also ask questions if necessary. After you are satisfied and understand this study and want to participate, you will sign a voluntary commitment to participate.

IV. RESEARCH PROCESS

Step 1: Invite to participate in the study

When you come for a check-up at the Artificial Reproductive Technology Center of Tam Anh General Hospital, Hanoi, the doctor will review the standards after performing basic surveys of the infertility examination process. receive and eliminate her. If you meet the sample selection criteria, your doctor will invite you to participate, advise you on the research process, the benefits and risks of participating, and answer your questions. If you agree to participate, you will sign consent to participate in the study.

Step 2: Randomize groups

After signing consent to participate in the study, she will be randomly assigned to 1 of 2 study subgroups (PPOS regimen group or GnRH-ant regimen group). You will know which group you are in. The doctor will collect some of your information into a data collection table.

Step 3: Stimulate the ovaries and monitor follicle development

First, you will be injected with ovarian stimulation medication starting on the 2nd or 3rd day of your menstrual cycle (called stimulation day S1). From day 5 onwards, the doctor will adjust or maintain the drug dose according to the response of the ovaries until ovulation is stimulated. The doctor will monitor the response of the ovaries by transvaginal ultrasound to measure the size of ovarian follicles, and the concentration of estradiol (E2), LH, and progesterone (P4) in the serum will be measured on the morning

of S5, S8, and ovulation induction day. To prevent a premature LH peak, for **the GnRH-anta protocol**, you will receive a subcutaneous injection of 0.25 mg of Cetrorelix (Cetrotide; Merck Serono) once daily from S5, along with ovarian stimulation medication until the day before. when ovulation is triggered. For **the PPOS protocol**, she will receive Dydrogesterone (DYG) 10mg, 1 pill x 3 times/day from S1 and continuously every day until the day of oocyte retrieval. When there are more than 2 ovarian follicles with a diameter greater than 17 mm, she will inject oocyte maturation medication with Triptoreline 0.2mg (Diphereline; Ipsen) or hCG 10,000 IU (IVF C 5000; LG) or recombinant hCG DNA 250 mcg (Ovitrelle; Merck Serono), and oocyte retrieval was performed 36 – 38 hours later. For stimulation cycles where no follicles are larger than 15 mm after 7 days of ovarian stimulation, the cycle is canceled. She will still have ovarian stimulation done again in the next cycle.

Step 4: Oocyte retrieval, fertilization, and embryo culture

The doctor will perform transvaginal oocyte retrieval 36 - 38 hours after the injection of medication to trigger oocyte maturation. After aspiration, the eggs will be fertilized by intracytoplasmic sperm injection (ICSI). Embryos will be cultured until day 3 or day 5 (depending on the number of embryos and the clinician's decision) and then completely frozen.

Step 5: Monitor the patient after oocyte retrieval

After aspiration, you will be monitored and treated for the following complications. These are complications that can occur with any IVF process.

- Vaginal bleeding: After the oocyte retrieval is completed, a speculum will be placed to examine the vagina. If bleeding is seen at the needle puncture site, the doctor will use gauze to press on the bleeding site and wait for a while. 5 minutes. After that,

the doctor checks again, and if there is still bleeding, he can consider injecting hemostatic medicine.

- Bladder hemorrhage rarely occurs due to the needle piercing the egg through the bladder, with the main symptom being red urine. Most cases will not require intervention. You will be told to drink plenty of water, and in some cases, hemostatic medication or antibiotics may be considered. If red urine persists or there are blood clots in the bladder, bladder irrigation, and cystoscopy can be performed to stop the bleeding.
- Internal bleeding due to ovarian cyst rupture: After oocyte retrieval, if there are symptoms of abdominal pain (lower abdomen, one side or both sides, can spread to the shoulders), lethargy, or fatigue, you will be examined and Perform tests to diagnose. Subsequent treatment will be performed according to the hospital's protocol.
- Infection at the egg retrieval site: very rare, with symptoms such as fever, lower abdominal pain, and pain on one side of the pelvis, and blood tests are performed to determine infection. After that, she will be treated with broad-spectrum antibiotics according to the hospital protocol.
- Ovarian hyperstimulation syndrome is due to excessive response of the ovaries to ovarian stimulation. This is an uncommon complication (1 - 5% in one cycle) but is a dangerous complication during ovarian stimulation. You may have symptoms such as abdominal pain, nausea, vomiting, difficulty breathing, rapid weight gain, and decreased urination. Depending on the level of symptoms and tests, it can be divided into levels: mild, moderate, severe and critical; and she will be monitored and treated according to the hospital's protocol.

Step 6: Embryo transfer and confirmation of pregnancy

You will have frozen embryos transferred in the next cycle and a quantitative hCG test will be performed 10 days or 13 days after N5 or N3 embryo transfer: (1) If the hCG test is positive (> 5 mIU/ml), the pregnancy will continue to be monitored; (2) If the hCG

test is negative (< 5mIU/ml), the outcome is determined as failure in the first embryo transfer and the study ends. She will still continue to undergo IVF according to the hospital's protocol.

V. RESPONSIBILITIES OF RESEARCH SUBJECTS WHEN PARTICIPATING IN RESEARCH

You need to follow the instructions of your doctor and midwife and complete all steps in the treatment and research process as described above because of the study's possible risks and side effects. When performing IVF, you will have risks and side effects of IVF treatment, such as:

1) Complications of the oocyte retrieval process, such as vaginal bleeding, internal bleeding due to ovarian cyst rupture, bladder bleeding, and infection at the retrieval site: rarely occur; you will be evaluated by a doctor. , monitor and treat if there is a risk or this condition occurs as in step 5 of the research process

2) Ovarian hyperstimulation is when the ovaries respond greatly to ovarian stimulation drugs. Ovarian hyperstimulation can cause bloating, abdominal pain, and polymembranous effusion. Your doctor will evaluate your condition, monitor and treat you if there is a risk or this condition occurs, as in step 5 of the research process.

3) Cycle cancellation: a condition in which no ovarian follicles are larger than 12 mm after ten days of ovarian stimulation. Your condition will be evaluated with canceled cycles, and your ovaries will be stimulated again in the next cycle.

4) Embryo transfer but negative pregnancy test results: due to the nature of the study, only data is recorded on the first embryo transfer, so if the pregnancy test result is negative, the study will end, but you will still be able to continue fertilization treatment. Test tube sperm according to hospital protocol.

VII. BENEFITS OF PARTICIPATING IN RESEARCH

You will be thoroughly explained and monitored throughout the in vitro fertilization treatment process.

VIII. INFORMATION SECURITY

Your information will be kept confidential in your medical record. When collecting data, all personal information will be encrypted, stored separately, and used only for research purposes.

IX. DO NOT PARTICIPATE IN RESEARCH

The choice to participate in the study is yours and voluntary. If you have been accepted into the study but change your mind, you can withdraw from the study at any time. She will still be cared for and treated like a routine in vitro fertilization case.

X. COSTS OF TREATMENT AND COSTS OF RESEARCH

You will not be paid for participating in the research. You will still pay the cost of COS injections and the cost of performing in vitro fertilization as in normal treatment cases, according to hospital regulations.

XI. CONTACT

If you have any questions about the study, please contact Dr. Than Trong Thach at +84908 400 040 or by email at thachthan007@gmail.com.

APPENDIX 3

CONSENT TO PARTICIPATE IN RESEARCH

Research name

"COMPARISON OF FERTILISATION RESULTS BETWEEN TWO OVARIAN STIMULATION PROTOCOLS WITH PROGESTIN AND ANTAGONIST IN VIETNAM POPULATION. "

Researcher and contact information

Main researcher: Dr. Than Trong Thach

Contact Phone: +84908.40.00.40

Email: thachthan007@gmail.com

I voluntarily participated in this study. I have read and clearly understood the purpose and procedure of the research. The study was explained clearly; I had time to ask questions about the study, and I was satisfied with all the answers.

_____

Name of study participant Signature Date

Confirmation of the person conducting the research

I, the undersigned, commit to fully explaining research information to research participants and ensuring that research participants clearly understand the purpose, procedures, risks, and benefits of participating in the study.

_____

Name of person conducting research Signature Date

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