EXHIBIT A

CLINICAL STUDY PROTOCOL

Title:	**Dydrogesterone primed ovarian stimulation versus fixed gonadotropin-releasing hormone antagonist protocol for accumulation of oocyte in patients with low ovarian reserve: A randomized controlled trial**
Protocol Number:	DYDR-C22-0313
National Clinical Trial (NCT) Identified Number:	NCT05847283
Sponsored by:	Tam Anh TP. Ho Chi Minh General Hospital Joint Stock Company
Funded by:	Abbott Company
Principal Investigator:	[redacted]
Version Number:	Version 3.0

August 9th 2024

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***G*LOSSARY AND ABBREVIATIONS**

ICH GCP	International Conference on Harmonisation Good Clinical Practice
CFR	Code of Federal Regulations
IND	Investigational New Drug
IDE	Investigational Device Exemption
IRB	Institutional Review Board
DOR	Diminish ovarian reserve
DPOS	Dydrogesterone-primed ovarian stimulation
PPOS	Progestin-primed ovarian stimulation
IVF	In vitro fertilization
ART	Assisted reproductive technology
FET	Frozen embryo transfer
RCT	Randomized clinical trial
ICSI	Intra Cytoplasmic Sperm Injection
DYG	Dydrogesterone
MIP	Microzined progesterone
MPA	Medroxyprogesterone acetate
AE	Adverse event
SAR	Serious adverse event
NATIONAL DI & ADR CENTRE	National centre of Drug information and adverse drug reactions monitoring
RTAC	Reproductive Technology Accreditation Certification
OHSS	Ovarian hyperstimulation syndrome

SUMMARY OF CHANGES FROM PREVIOUS VERSION:

Changed sections	Previous version: Protocol version 2.1	Update version: Protocol version 3.0	Reason of change form earlier version
Study Population	**Inclusion criteria:** ● Women's age between 18 and 37 years ● AFC ≤5 and/or AMH ≤1.2 ng/ml ● Agree to perform single frozen blastocyst embryo transfer **Exclusion criteria:** ● Oocyte recipient ● Indication of preimplantation genetic testing ● Known allergic reactions to medications in the Study (progesterone products, GnRH antagonist….) ● Basal FSH above 15mIU/mL. ● Have contraindications of ART treatment (e.g. critical or acute diseases) ● Retrieved sperm ● Repeated Implantation failure (had more than three failed embryo transfers with good quality embryos) ● Inability to comply with the study procedures	**Inclusion criteria:** ● Women's age between 18 and 37 years ● AFC ≤5 and/or AMH ≤1.2 ng/ml ● Agree to perform single frozen blastocyst embryo transfer **Exclusion criteria:** ● Oocyte recipient ● Indication of preimplantation genetic testing ● Known allergic reactions to medications in the Study (progesterone products, GnRH antagonist….) ● Basal FSH above 15mIU/mL. ● Have contraindications of ART treatment (e.g. critical or acute diseases) ● Retrieved sperm ● Repeated Implantation failure (had more than three failed embryo transfers with good quality embryos) ● Inability to comply with the study procedures ● <u>Patients with a history of thyroid cancer who are on hormone replacement therapy or those diagnosed with thyroid diseases at the time of eligibility assessment</u>	After the SAE case related to thyroid cancer, DSMB and IRB have reviewed and stated that current evidence suggests the strong possibility that there is no link between the treatment being used in the trial and thyroid cancer. To reduce any possible risk for current and prospective participants with thyroid disease in the study, the DSMB and IRB recommended that it should be excluded participants with current indications of thyroid diseases. We update the exclusion criteria according to the recommendations.
Study Duration	Estimated Study Start Date (First patient in): ~~**April, 2023**~~	Study Start Date (First patient in): **<u>June, 22th, 2023</u>**	- As of August 22, 2024, the study has pre-screened

Changed sections	Previous version: Protocol version 2.1	Update version: Protocol version 3.0	Reason of change form earlier version
	Estimated Primary Completion Date (Last patient in): ~~**April, 2025**~~ Estimated Study Completion Date: ~~**April, 2026**~~	Estimated Primary Completion Date (Last patient in): **May, 2027** Estimated Study Completion Date: **December, 2027**	2364 patients, of which 2071 patients failed pre-screening, 293 patients were screened, and 272 patients were recruited. - The pre-screening failure rate is high, so to ensure the recruitment of the approved number of 730 patients, the research team requested an extension of the study period to December 2027. - For the difficulties in recruiting patients into the study and slow recruitment progress, we have updated study timeline. - We also update Study Start Date was **June, 22th 2023**, which was actually later than the initial estimated date (April 2023) for two months.
Visit 12	**Visit 12:** (Day 163 ± 7 – Clinical pregnancy confirmation): A transvaginal ultrasound examination will be performed to determine the pregnancy defined as the presence of ~~fetal heartbeats~~ at gestation week 7 ± 1.	**Visit 12:** (Day 163 ± 7 – Clinical pregnancy confirmation): A transvaginal ultrasound examination will be performed to determine the pregnancy defined as the presence of at least one gestational sac at gestation week 7 ± 1.	Update typo error on page 16 of 46 - Visit 12

1. STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with the International Conference on Harmonisation Good Clinical Practice (ICH GCP), applicable Vietnamese Code of Federal Regulations (CFR), Terms and Conditions of Award. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Investigational New Drug (IND) or Investigational Device Exemption (IDE) sponsor, funding agency, and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this Study have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the Study. All changes to the consent form will be IRB approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

2. PROTOCOL SUMMARY

2.1. Summary

One of the barriers in patients with diminished ovarian reserve (DOR) is the significantly reduced number of oocytes resulting in fewer oocytes collected and embryos formed. Many ovarian stimulation strategies have been proposed to improve oocyte or embryo quantity which is oocyte accumulation could be a potential option with a comparable success rate and reasonable cost. Dydrogesterone-primed ovarian stimulation (DPOS) protocol effectively blocks the Luteinizing hormone (LH) surge in IVF. It favors segment ART cycles such as FET, oocyte donor, fertility preservation, and oocyte accumulation set. The protocol is more user-friendly and cheaper than the GnRH antagonist regimen regarding LH suppression during ovarian stimulation. Initial evidence suggests that oocyte quantity and quality are comparable with other ovarian stimulation regimens. However, data related to the PPOS protocol has not been well documented. There has not been an RCT with a large sample size and well-designed to provide more substantial evidence. A randomized trial to compare the effectiveness of PPOS and GnRH antagonist protocol in IVF is urgently needed.

The randomized, open-label, multi-center trial will be conducted at IVFTA HCM – Tam Anh TP. Ho Chi Minh General Hospital and IVFTA HN – Tam Anh General Hospital, Vietnam. Patients will be randomized, using a computer-generated randomization list in a 1:1 ratio, to either the DPOS group or the GnRHanta group after assessing eligibility and signing informed consent. Randomization will be centrally controlled by a data scientist without involvement in any treatment procedure. After randomization, the patients will be administered human Menopausal Gonadotrophin (HMG) 225 IU/day (IU/d) via intramuscular injection on the 2nd - 4th menstrual day. In the intervention arm, women will receive oral Dydrogesterone 10mg (Duphaston 10mg) t.i.d daily from the first day of ovarian stimulation until final oocyte maturation. In the control arm, GnRH antagonist (Ganirelix 0.25mg) subcutaneously once daily from day 5 of ovarian stimulation will be administered till the day of final oocyte maturation. Oocyte cryopreservation will be applied to collect at least 7 ± 1 oocytes. In the last ovarian

stimulation cycle, the fresh oocytes and all thawed oocytes will be fertilized by ICSI. The freeze-all strategy will be applied in both arms, and single blastocyst frozen embryo transfer will be performed in the next menstrual cycle.

The ongoing pregnancy rate of patients in IVFTA HCM was 45.0% in the Antagonist IVF stimulation group. To show or refute that hypothesis: no significant difference in ongoing pregnancy rates when using DPOS protocol (40 - 42%), we need 657 patients to produce a two-sided 95% confidence interval for the difference in population proportions with a width that is equal to 5% (margin = 5.0%, alpha = 5.0% and power = 80%). Considering a 10% loss to follow-up and protocol violation, we plan to recruit 730 participants (365 per arm). The primary endpoint is the ongoing pregnancy rate after the first completed transfer. Secondary endpoints are ovarian stimulation, embryo culture characteristics, drop-out rate, and DPOS and GnRH antagonist protocol safety.

All analyses will be performed on an intention-to-treat basis using the R statistical package

2.2. Synopsis

Title: **Dydrogesterone primed ovarian stimulation versus fixed gonadotropin-releasing hormone antagonist protocol** for accumulation of oocyte in patients with low ovarian reserve: A randomized controlled trial

Objectives:

Primary objective:

To compare the ongoing pregnancy rate per patient in low ovarian reserve patients after accumulation oocyte cycles with either dydrogesterone primed ovarian stimulation (DPOS) or gonadotropin-releasing hormone antagonist (GnRHanta) protocol

Secondary objectives:

1. To compare the characteristics of ovarian stimulation between DPOS and GnRHanta protocol, including LH, Estradiol, and Progesterone profile (Day 1, day 5, and day 8 of ovarian stimulation, final oocyte maturation day, and 12 hours after the final oocyte maturation injection), premature LH rate, the duration of ovarian stimulation the total dose of FSH and the number of ovarian stimulation cycles.
2. To compare the outcomes of the ovarian stimulation between DPOS and GnRHanta protocol, including number of cumulus-oocyte complexes, number of MII oocytes, oocyte survival rate, fertilization rate, embryo-cleavage rate, blastocyst rate, top-quality blastocyst rate, blastocyst survival rate, implantation rate, positive pregnancy rate, clinical pregnancy rate, early miscarriage rate, multiple pregnancy rate, ectopic pregnancy rate.
3. To compare the drop-out rate of DPOS and GnRHanta protocol in accumulation oocyte cycles of patients with low ovarian reserve

4. To assess the safety of DPOS and GnRHanta protocol in accumulation oocyte cycles of patients with low ovarian reserve.
5. To compare the quality of life during ART treatment based on the WHO-BRIEF questionnaire

Endpoints:

Primary endpoint: The ongoing pregnancy rate in the first frozen embryo transfer cycle.

Secondary endpoint: LH, Estradiol, and Progesterone profile (Day 1, day 5, and day 8 of ovarian stimulation, final oocyte maturation day, and 12 hours after the final oocyte maturation injection), premature LH rate, the duration of ovarian stimulation and the total dose of FSH, the number of ovarian stimulation cycles, number of cumulus-oocyte complexes, number of MII oocytes, oocyte survival rate, fertilization rate, embryo-cleavage rate, blastocyst rate, top-quality blastocyst rate, blastocyst survival rate, implantation rate, positive pregnancy rate, clinical pregnancy rate, early miscarriage rate, multiple pregnancy rate, ectopic pregnancy rate, drop-out rate, OHSS rate, and adverse events.

Study Population:

Couples who undergo IVF treatment at ART Unit – Tam Anh General TP. Ho Chi Minh (IVFTA HCM) and ART Unit – Tam Anh General hospital (IVFTA HN) fulfill the inclusion and exclusion criteria

Inclusion criteria:

- Women's age between 18 and 37 years
- AFC $\leq$5 and/or AMH $\leq$1.2 ng/ml
- Agree to perform single frozen blastocyst embryo transfer

Exclusion criteria:

- Oocyte recipient
- Indication of preimplantation genetic testing
- Known allergic reactions to medications in the Study (progesterone products, GnRH antagonist….)
- Basal FSH above 15mIU/mL.
- Have contraindications of ART treatment (e.g. critical or acute diseases)
- Retrieved sperm
- Repeated Implantation failure (had more than three failed embryo transfers with good quality embryos)
- Inability to comply with the study procedures

- Patients with a history of thyroid cancer who are on hormone replacement therapy or those diagnosed with thyroid diseases at the time of eligibility assessment

Description of Sites/Facilities Enrolling Participants:

Site enrolling:

- IVFTA HCM (Tam Anh TP. Ho Chi Minh General hospital)
- IVFTA HN (Tam Anh General hospital)

IVFTA HCM has a volume of about 1700 - 2000 cycles per year. Of these, 67% of patients were under 37 years old, and 45% had a low ovarian reserve. Frozen embryo transfer is 97% of all cycles. The embryo and gamete cryopreservation system at IVFTA-HCM is well established for cryopreservation requirements in oocyte accumulation strategy. Quality management was applied with RTAC's code of practice.

Description of Study Intervention:

After written consent is obtained, the patient will undergo ovarian stimulation from the second-fourth day of menstruation after randomization to either arm:

1. Intervention arm: Women will receive oral Dydrogesterone 10mg (Duphaston 10mg) t.i.d daily from the first day of ovarian stimulation till the day of final oocyte maturation.
2. Control arm: Women will receive GnRH antagonist (Ganirelix 0.25mg) once subcutaneously daily from day 5 of ovarian stimulation till the day of final oocyte maturation

The hMG will be started from day 2 to day 4 in both groups. Oocyte cryopreservation will be applied to collect at least 7 ± 1 oocytes (including frozen and fresh oocytes). In the last ovarian stimulation cycle, the fresh oocytes and all thawed oocytes will be fertilized by ICSI. Freeze all strategy will be applied in both arms, and a single frozen embryo transfer will be performed in the next menstrual cycle.

Study Duration:

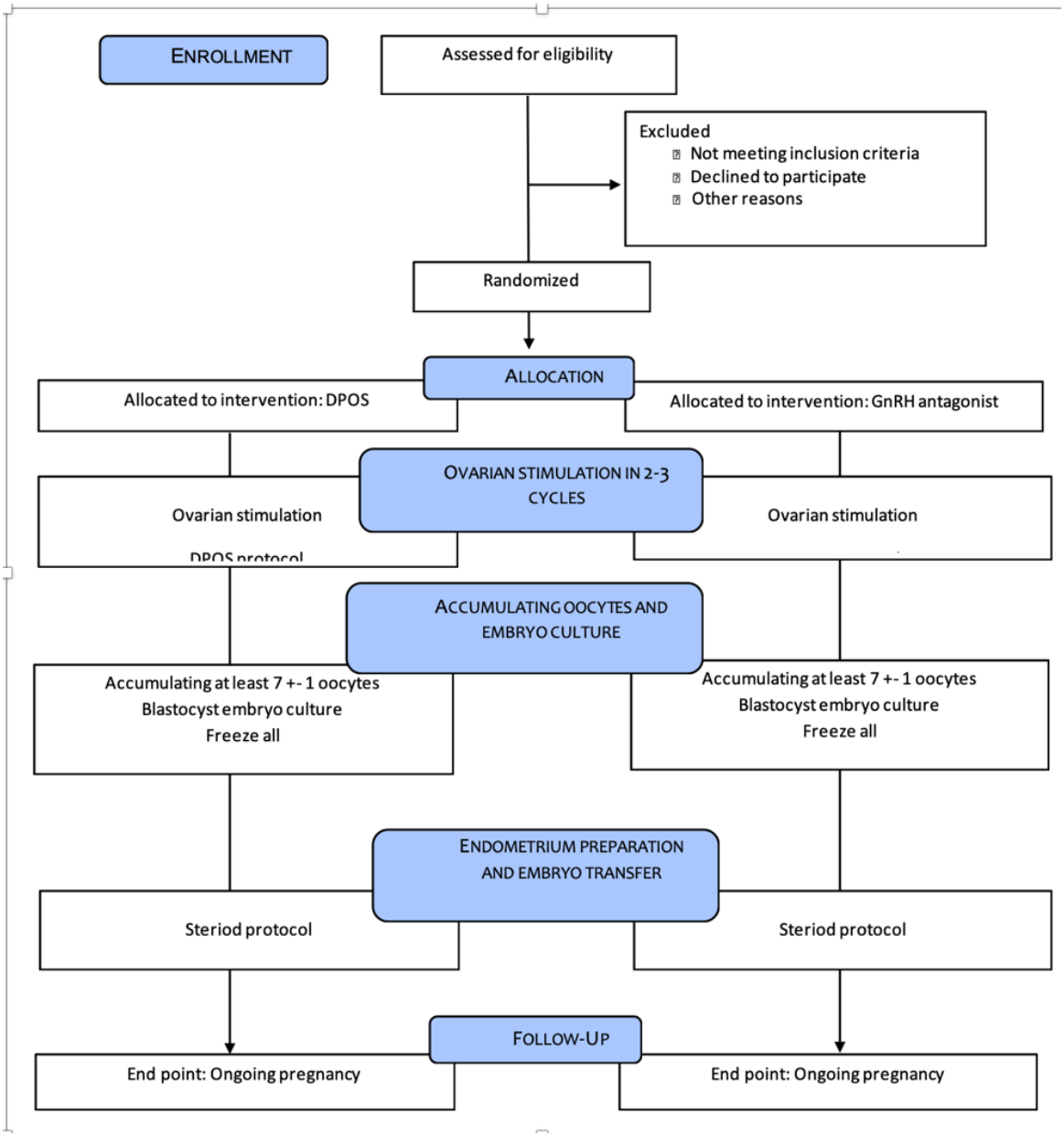
Study Start Date (First patient in): **June, 2023**

Estimated Primary Completion Date (Last patient in): **May, 2027**

Estimated Study Completion Date: **Dec, 2027**

Participant Duration:

2.3. Schema


ENROLLMENT
Assessed for eligibility
Excluded
Not meeting inclusion criteria
Declined to participate
Other reasons
Randomized
ALLOCATION
Allocated to intervention: DPOS
Allocated to intervention: GnRH antagonist
OVARIAN STIMULATION IN 2-3 CYCLES
Ovarian stimulation
DPOS protocol
Ovarian stimulation
ACCUMULATING OOCYTES AND EMBRYO CULTURE
Accumulating at least 7 +- 1 oocytes
Blastocyst embryo culture
Freeze all
Accumulating at least 7 +- 1 oocytes
Blastocyst embryo culture
Freeze all
ENDOMETRIUM PREPARATION AND EMBRYO TRANSFER
Steriod protocol
Steriod protocol
FOLLOW-UP
End point: Ongoing pregnancy
End point: Ongoing pregnancy

2.4. Schedule of activities (SOA)

Stage	Procedure	Before Ovarian stimulation (OS)	OS cycle (repeated in 2 - 3 OS cycles)						Embryo culture	Endometrium preparation	Embryo transfer day	Pregnancy testing	Pregnancy follow-up	
			1st Day of OS	5th Day of OS	8th Day of OS	Trigger day	12h after the trigger shot	OPU day					7th week of pregnancy	12th week of pregnancy
	Visiting	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**	**Visit 9**	**Visit 10**	**Visit 11**	**Visit 12**	**Visit 13**
Pre-screening	Information providing	X												
	Demographics	X												
	Medical history	X												
	Pre IVF testing	X												
Screening	Ultrasound		X											
	AMH		X											
	Pre-anesthesia testing and examination		X											
	Informed consent		X											
Enrollment	Randomization		X											
Ovarian stimulation	FSH		X											
	LH		X	X	X	X	X							
	E2		X	X	X	X	X							
	P4		X	X	X	X	X							
	Ultrasound for OS		X	X	X	X								
	OPU							X						
	Oocyte cryopreservation							X						

Stage	Procedure	Before Ovarian stimulation (OS)	OS cycle (repeated in 2 - 3 OS cycles)						Embryo culture	Endometrium preparation	Embryo transfer day	Pregnancy testing	Pregnancy follow-up	
			1st Day of OS	5th Day of OS	8th Day of OS	Trigger day	12h after the trigger shot	OPU day					7th week of pregnancy	12th week of pregnancy
	Visiting	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**	**Visit 9**	**Visit 10**	**Visit 11**	**Visit 12**	**Visit 13**
Embryo culture	Oocyte thawing (in the last OS cycle)							X						
	ICSI (in the last OS cycle)							X						
	Embryo assessment								X					
	Embryo cryopreservation								X					
Embryo transfer	Ultrasound for ET									X				
	Embryo thawing										X			
	Embryo transfer										X			
Follow-up	bhCG testing											X		
	Ultrasound for Pregnancy Monitoring												X	X
QOL	WHO-BRIEF	X				X								
Report	Adverse event review and evaluation	X	X	X	X	X	X	X	X	X	X	X	X	X
	Complete the case report form	X	X	X	X	X	X	X	X	X	X	X	X	X

3. INTRODUCTION AND RATIONALE

Even when assisted reproductive techniques are offered, ovarian stimulation still is a significant challenge in reproductive medicine. Diminished ovarian reserve (DOR) is often associated with poor ovarian stimulation response, a high cancellation rate, and a significant decline in pregnancy rate during in vitro fertilization (IVF) cycle. Among infertile women, diminished ovarian reserve (DOR) incidence ranges from 6% to 64% at different ages. Based on data of 181,536 fresh, autologous ART cycles reported to SART by US clinics in 2004 and 2011 (1), the prevalence of LOR patients increased from 19 to 26% from 2004 to 2011, and it relates to poor IVF outcomes and low pregnancy rate for these patients (2,3). Unfortunately, the prevalence of DOR has also increased by 42% among patients younger than 40 years old (1). DOR is associated with a decrease in oocyte number and quality and contributes to a decrease in the clinical pregnancy rate and live birth rate and an increase in the miscarriage rate (4). Various studies showed the live birth rate per cycle of 11.1%, 11.4%, and 6.7%, according to ART-register data in the United Kingdom, Canada, and Egypt (5–7). Chang et al. found that young patients with DOR had significantly decreased rates of biochemical pregnancy, clinical pregnancy, and embryo implantation but increased rates of miscarriage compared to young patients without DOR (8).

Several cohort and meta-analysis studies showed that the number of oocytes retrieved for in vitro fertilization (IVF) positively predicts ART treatment (9–11). The appropriate number of oocytes was reported to be between 6 and 15 for optimizing the live birth rate (9,12). If fewer oocytes are retrieved, the live birth delivery rate decreases (9,13). Unfortunately, one of the barriers in patients with DOR is the significantly reduced number of oocytes resulting in fewer oocytes collected and embryos formed. S.J. Morin et al. (2018) reported that the number of oocytes collected in the DOR group (6 oocytes [2-10]) decreased significantly compared to the non-DOR group (14 oocytes [8-19]) ($P < 0.001$). The number of usable blastocysts was also significantly lower in the DOR group than in the non-DOR group (2 embryos [0-3] vs. 4 embryos [1-8]), while the cycles with no usable blastocyst increased significantly (17.0% vs. 5.3%; $P< 0.001$) (14).

Many ovarian stimulation strategies have been proposed to improve oocyte or embryo quantity to increase the chances of embryo transfer and pregnancy outcomes (15,16). Cobo et al. (2012) reported an oocyte storage strategy in LOR patients showing the cumulative live-birth rate was higher in the accumulation group 36.4% (95% CI, 30,3 – 42%) than in the fresh group 23.7% (19.9 – 27.4%, $P < 0.05$) with significant lower embryo-transfer cancellation rate (9.1% of accumulation group vs. 34% of the fresh group) (17). The accumulation of oocytes from several ovarian stimulation cycles is currently possible with the aid of novel vitrification technologies. Therefore, the strategy could potentially increase the number of embryos with comparable success rates and reasonable costs (17–19).

The Progesterone priming ovarian stimulation (PPOS) protocol could be a potential treatment in an oocyte accumulation setting to reduce the financial burden and stress. Progesterone with high concentrations has long been shown to block the LH surge. Its inhibiting role on follicular growth has been the foundation of progestin-only contraceptives, which, after prolonged use, suppress follicular growth and thus block ovulation (20,21). The generation of the LH surge is suggested by two periods: one oestradiol-dependent (early follicular phase) and one oestradiol-independent (late follicular phase) which the signal is transmitted to the GnRH neurosecretory system following the release of the LH surge. If the pituitary gland is exposed to Progesterone for an extended period, Progesterone downregulates its receptors in the pituitary gland (22), inhibiting the pituitary release of LH. Progesterone is essential in inhibiting the estrogen-dependent LH surge pathway (23). Progesterone also involves

slow-down pulsatility of luteinizing hormone in the pituitary gland and hypothalamus (24). Based on the mechanism, the effect was gradually applied to ovarian stimulation. Various studies show that Progestin-primed ovarian stimulation (PPOS) effectively blocks the LH surge in IVF (25–27). Compared with the GnRH antagonist protocol, Chen et al. (2020) showed that the incidence of premature LH surge in the PPOS group was lower than that in the antagonist group (0 vs. 5.88%, $P < 0.05$) (20). PPOS favors segment ART cycles such as FET, oocyte donor, fertility preservation, and oocyte accumulation set. In DOR patients, ovarian could be simulated in multiple processes to obtain more oocytes for the increasing number of embryos (17). However, numerous stimulation cycles can stress LOR patients as they undergo several procedures, injections, and financial burdens. Instead, the PPOS might be more user-friendly and cheaper than the GnRH antagonist regimen regarding LH suppression during ovarian stimulation (27,28).

Many PPOS protocols have been proposed in which the three most agents include Dydrogesterone (DYG), Microzined Progesterone (MIP), and Medroxyprogesterone acetate (MPA) (20). There were no important differences between the three PPOS protocols in terms of ovarian stimulation response as well as pregnancy outcomes(20,21,29). MIP seems to be of unusual choice because of its vaginal administration. DYG 20 – 30 mg/day has shown to be effective in preventing early LH surge (30,31), although MPA 10 mg/day exerts a more substantial suppression on endogenous LH than DYG (31). However, excessive inhibition of LH levels in the poor response group relates to poor outcomes, and LH supplementation even is indicated in women (32,33). Notably, the extensive worldwide use of DYG for the treatment of threatened and recurrent miscarriage, as well as for the luteal phase support in infertile patients, also suggests its long-term safety (21). Ovarian Stimulation (DPOS) may be an effective and safe protocol in the DOR population.

Nevertheless, data related to the PPOS protocol has not been well documented, including DPOS. More and more centers in China are using the protocol because it is simpler and cheaper (26,34–36). Initial evidence suggests that oocyte quantity and quality are comparable with other ovarian stimulation regimens (20,21,27). Based on retrospective studies, the rate of euploid embryos and the live birth rate were similar in the PPOS group compared with the control group (37,38). However, there has not been an RCT with a large sample size and well-designed to provide more robust evidence. A randomized trial to compare the effectiveness of DPOS and GnRH antagonist protocol in IVF is urgently needed.

4. OBJECTIVES

4.1. Primary objectives:

- To compare the ongoing pregnancy rate per patient in low ovarian reserve patients after accumulation oocyte cycles with either dydrogesterone primed ovarian stimulation (DPOS) or fixed gonadotropin-releasing hormone antagonist (GnRHanta) protocol

4.2. Secondary objectives:

- To compare the characteristics of ovarian stimulation between DPOS and GnRHanta protocol, including LH, Estradiol, and Progesterone profile (Day 1, day 5, and day 8 of ovarian stimulation, final oocyte maturation day, and 12 hours after the final oocyte maturation injection), premature LH rate, the duration of ovarian stimulation and the total dose of FSH, the number of ovarian stimulation cycles.

- To compare the outcomes of the ovarian stimulation between DPOS and GnRHanta protocol, including number of cumulus-oocyte complexes, number of MII oocytes, oocyte survival rate, fertilization rate, embryo-cleavage rate, blastocyst rate, top-quality blastocyst rate, blastocyst survival rate, implantation rate, positive pregnancy rate, clinical pregnancy rate, early miscarriage rate, multiple pregnancy rate, ectopic pregnancy rate.
- To compare the drop-out rate of DPOS and GnRHanta protocol in accumulation oocyte cycles of patients with low ovarian reserve
- To assess the safety of DPOS and GnRHanta protocol in accumulation oocyte cycles of patients with low ovarian reserve.
- To compare the quality of life during ART treatment based on WHO-BRIEF questionnaire

5. STUDY DESIGN

This open-label, randomized, controlled, multi-center clinical trial compares the efficacy and safety of the oral Dydrogesterone primed ovarian stimulation versus fixed gonadotropin-releasing hormone antagonist protocol for accumulating oocytes in patients with low ovarian reserve.

- Study Type: Interventional (Clinical Trial)
- Estimated Enrollment: 730 participants (365 per arm)
- Allocation: Randomized
- Intervention Model: Parallel Assignment
- Masking: Open-label
- Primary Purpose: Treatment
- Official Title: Dydrogesterone primed ovarian stimulation versus fixed gonadotropin-releasing hormone antagonist protocol for accumulation of oocyte in patients with low ovarian reserve: A randomized controlled trial

The Study will be conducted according to the following scheme:

Pre-screening

- **Visit 1** (Day – 180 to 1): The patients who have IVF indication will undergo a physical examination - including vitals sign, a gynecological exam; a review of their medical history and contaminant medication, a transvaginal ultrasound and baseline routine tests (blood tests – including AMH, cervical cancer screening – Pap's smear and HPV, breast ultrasound…). Potentially eligible couples will be given the information sheet about the Study during their first consultation

Screening and Enrollment

- **Visit 2** (Day 1: the first day of OS cycle - Ovarian stimulation (OS) starts on the 2nd – 4th of mense):
    - The patients undergo a transvaginal ultrasound to access Antral Follicle Count, ovaries and uterus.
    - Laboratory tests (blood tests, urine test and ECG) will be taken for pre-anesthesia examination; in addition, the hormone values include FSH (follicle-stimulating hormone) – only for the first OS cycle, E_2 (Estradiol), LH (luteinizing hormone), P4 (Progesterone) and AMH if the result is not

available within 6 months. A pre-anesthesia examination will ensure the patient is eligible for the oocyte retrieval procedure.

- Screening for eligibility will be performed by treating physicians.
- Eligible participants will be invited to a full discussion with investigators about the Study and given the informed consent form. Patients will be given a period (3-4 hours) during anesthesia testing to understand the information and decide whether they agree to participate in the Study. The investigators will obtain written informed consent from all couples before enrolment. To maximize retention in the trial, a consultation will be available to couples to ensure they understand the procedures well and to address any questions or complaints that arise during the Study.

Treatment period: The patient will be treated with an oocyte accumulation strategy, and estimated ovarian stimulation cycles range from two to four cycles to collect at least 7 ± 1 oocytes.

For the first ovarian stimulation cycle:

- **Visit 2** (Day 1: the first day of OS cycle - Ovarian stimulation (OS) starts on the 2nd – 4th of mense): After informed consent is obtained, the patient will be indicated randomly with one of the following regimens:

  Group I: oral DYG (Duphaston) 10mg t.i.d from the first day of OS to the day of final oocyte maturation

  Group II: GnRH antagonist (Ganirelix 0.25 mg) will be initiated on the fifth day of OS to the day of final oocyte maturation

- **Visit 3** (1st cycle **-** Day 5 of OS): Transvaginal ultrasound and blood sampling (for hormone check-up) will be performed.
- **Visit 4 (**1st cycle **-** Day 8 of OS)**:** Transvaginal ultrasound and blood sampling (for hormone check-up) will be performed.
- **Visit 5** (1st cycle **-** Day 10 ± 2 of OS)**:** Transvaginal ultrasound and blood sampling (for hormone check-up) will be performed. A final oocyte maturation injection will be performed.
- **Visit 6** (1st cycle **-** Day 11 ± 2 of OS)**:** blood sampling (for hormone check-up) will be performed after 12 hours of final oocyte maturation injection.
- **Visit 7** (1st cycle **-** Day 12 ± 2 of OS): Oocyte retrieval and oocyte cryopreservation will be performed. AE/SAE will be recorded routinely.

For the following ovarian stimulation cycle (s): the patient will be stimulated with the previous OS protocol. The visits of the patient will be repeated as the first OS cycle.

For the last ovarian stimulation cycle: Based on the aim to collect at least 7 ± 1 oocytes, the clinician will determine the last ovarian stimulation cycle on the day of final oocyte maturation. It is defined as a cycle in that the total number of frozen and expected fresh oocytes are at least 7 ± 1 oocytes. The patient will be stimulated with the

previous OS protocol. The visits of the patient will be repeated as the first OS cycle. However, the frozen oocytes of the previous OS cycle will be thawed. All fresh and thawed oocytes will be fertilized by ICSI at **Visit 7**.

- **Visit 8:** (Day 77 ± 7 – Embryo culture report): The patient will receive a Blastocyst embryo culture report and decide whether embryo cryopreservation or not.
- **Visit 9: (**Day 90 ± 7 – Endometrial preparation): Endometrial preparation will start on the 2nd – 4th of mense.
- **Visit 10:** (Day 109 ± 7 – Embryo transfer): Progesterone will be started when endometrial thickness reaches 8 mm or more for 6 - 7 days. Embryo transfer will be performed under ultrasound.
- **Visit 11:** (Day 121 ± 7): On day 11, after embryo transfer, subjects will have a routine pregnancy test (serum β-hCG) to confirm the subject's biochemical pregnancy
- **Visit 12:** (Day 163 ± 7 – Clinical pregnancy confirmation): A transvaginal ultrasound examination will be performed to determine the pregnancy defined as the presence of at least one gestational sac at gestation week 7 ± 1 .
- **Visit 13** (Day 198 ± 7 - End of treatment): A transvaginal ultrasound examination will be performed to determine the Ongoing pregnancy, defined as fetal heartbeats at gestation week 12 ± 1 .

6. STUDY POPULATION

Patients come for examination and treatment at ART Unit – Tam Anh TP. Ho Chi Minh General hospital (IVFTA HCM) and Tam Anh General hospital (IVFTA HN)

6.1. Inclusion criteria

- Woman age ≤ between 18 and 37 years
- AFC ≤ 5 and/or AMH ≤ 1.2 ng/ml
- Agree to perform freeze-all strategy and single frozen blastocyst embryo transfer

6.2. Exclusion criteria

- Oocyte recipient
- Indication of preimplantation genetic testing
- Known allergic reactions to medications in the Study (progesterone products, GnRH antagonist….)
- Basal FSH above 15mIU/mL.
- Have contraindications of ART treatment (e.g. critical or acute diseases)
- Retrieved sperm
- Repeated Implantation failure (≥ 3 failed embryo transfers with good-quality embryos)
- Inability to comply with the study procedures.
- Patients with a history of thyroid cancer who are on hormone replacement therapy or those diagnosed with thyroid diseases at the time of eligibility assessment

7. STUDY INTERVENTION

7.1. Pre-screening

At **Visit 1**, the couples with IVF indication will undergo a physical examination - including vitals signs, a gynecological exam; a review of their medical history and contaminant medication, a transvaginal ultrasound, and baseline routine tests. The blood tests include total blood count, blood group, AMH (within 6 months), TSH, FT4, Anti-TPO, HIV, HCV, HBsAg, Syphilis, Tuberculosis, Rubella, Chlamydia PCR, Pap's smear and HPV, breast ultrasound. Potentially eligible couples will be given the information sheet about the Study during their first consultation (**Visit 1**).

7.2. Screening and Enrollment

The patients will be checked for inclusion criteria on the second - fourth menstrual day (Visit 2). Laboratory tests (blood tests, urine test and ECG) will be taken for pre-anesthesia examination; in addition, the hormone values include FSH (follicle-stimulating hormone) – only for the first OS cycle, E_2 (Estradiol), LH (Luteinizing hormone), P4 (Progesterone) and AMH if the result is not available within 6 months. A pre-anesthesia examination will be performed to ensure that the patient is eligible for the oocyte retrieval procedure.

On the day (Visit 2), sscreening for eligibility will be performed by treating physicians. If the patients are eligible, we will invite them to a full discussion about the Study and give the informed consent form. We will inform verbal and written consent and explain the relevant information such as study context, objectives, and data collection procedure. Patients will be given a period (3-4 hours) during anesthesia testing to understand the information and decide whether they agree to participate in the Study. The investigators will obtain written informed consent from all couples before enrolment. To maximize retention in the trial, a consultation will be available to couples to ensure they understand the procedures well and to address any questions or complaints that arise during the Study.

When there is an eligible participant to be enrolled in the Study, clinicians from the specific site will enter the study data management center to get the allocation of patients according to a computer-generated randomization list in a 1:1 ratio, with a variable block size of 2, 4, 6 or 8. After randomization, if a participant wishes to change her assigned protocol in the first cycle, she will be considered a crossover but analysed in the group she was assigned (intention-to-treat). However, if she wishes to change the assigned protocol from the second ovarian stimulation cycle or transfer more than one embryo after randomization, it will be considered a protocol violation and excluded from the analysis. Due to the type of interventions, this Study will be label-opened to clinicians who perform ovarian stimulation, oocyte retrieval, and embryo transfer.

7.3. Study intervention administration

7.3.1. *Ovarian stimulation*

At Visit 2, eligible patients will be randomized to the DPOS group or GnRH antagonist group:

For DPOS arm (Group I): Patients will be co-administered with Human Menopausal Gonadotrophin (HMG) 225 IU/day (IU/d) via intramuscular injection and oral DYG (Duphaston) 30mg/d from menstrual cycle day 2 - 4 (CD2 – CD4) **(Visit 2)** to the day of final oocyte maturation (Dual trigger with Ovitrelle 250 mcg and Diphereline 0,2mg).

For GnRH antagonist arm (Group II): In the fixed GnRH antagonist protocol, daily s.c. administration of GnRH antagonist (Ganirelix 0.25 mg) will be initiated on the 5th day of stimulation. HMG 225 IU will be administered daily from menstrual cycle day 2 - 4 (CD2 – CD4) **(Visit 2),** and follicular monitoring will be performed after 4 days of

injections. Treatment with HMG and GnRH antagonist will continue daily until the day when final oocyte maturation is triggered (Dual trigger with Ovitrelle 250 mcg and Diphereline 0,2mg)

Follicular development will be monitored by ultrasound scanning and measurement of LH, E2 and P4 on Day 5 **(Visit 3)** and Day 8 **(Visit 4)**. Scanning and hormonal measurement will be repeated depending on the size of the follicles. A dual trigger with Ovitrelle 250 mcg and Diphereline 0,2mg will be used when at least one leading follicles of 17 mm **(Visit 5)**. After 12 hours of final maturation injection, hormonal measurement also will be performed **(Visit 6)**.

7.3.2. Oocyte retrieval and cryopreservation

After 36 hours of final maturation injection **(Visit 7)**, all follicles greater than 12mm in diameter will be aspirated. In the low ovarian reserve group, oocyte cryopreservation will be applied to collect at least 7 ± 1 oocytes. The patient will be stimulated with the previous OS protocol for the following ovarian stimulation cycle. The visits of the patient will be repeated as the first OS cycle.

Oocytes will be received after 36 hours post-hCG. Then, the cumulus-oocyte complexes (COCs) will be cultured for 2 hours in a CXCM medium (Irvine, USA) (37oC, CO2 6%, O2 5%). After 2 hours, the oocytes will be denuded, and the presence of the first polar body will assess oocyte maturity.

Matured oocytes will be frozen by vitrification (CRYOTEC® Method) (39). Firstly, the oocytes will be balanced in an equilibration solution (ES). When the shape of the oocyte fully recovers, it will be transferred to a Vitrification Solution (VS) with a minimum equilibrium (the limit time is 15 min). The oocyte will be exposed to a vitrification solution by maintaining the same mixture of cryoprotectants but double-concentrated for 50 – 60 seconds. Loading takes place within the next 10 seconds by placing oocytes on the device contained in the minimum volume— immediately plunging into liquid nitrogen-induced vitrification. Three oocytes maximum will be loaded per device container. Oocytes were stored in liquid nitrogen tanks for a variable storage time.

7.3.3. Oocyte thawing and ICSI

For the last ovarian stimulation cycle, based on the aim to collect at least 7 ± 1 oocytes, the clinician will determine the last ovarian stimulation cycle on the day of final oocyte maturation. It is defined as a cycle in that the total number of frozen and expected fresh oocytes is more than seven. The patient will be stimulated with the previous OS protocol. The visits of the patient will be repeated as the first OS cycle. However, the frozen oocytes of the previous OS cycle will be thawing; all fresh and frozen oocytes will be fertilized by ICSI at **Visit 7**.

For the fresh oocyte, the oocytes will be received after 36 hours post-hCG. Then, the cumulus-oocyte complexes (COCs) will be cultured for 3 hours in a CXCM medium (Irvine Scientific., USA) (37oC, CO2 6%, O2 5%). After 3 hours, the oocytes will be denuded, and oocyte maturity will be assessed by the presence of the first polar body and continued to culture for another hour before ICSI.

The thawing process will follow the CRYOTEC® Method (39). After removing the liquid nitrogen, the oocytes will be transferred immediately into the 37oC thawing solution (TS) within 1 minute. Then the oocyte will be transferred to a diluent solution (DS) for 3 minutes at room temperature. The warming procedure will be completed using two washes (one lasting 5 minutes, the other 1 minute) in the buffer solution at room temperature. After warming, oocytes were placed under standard culture conditioners at 6%CO2 and atmospheric at 37°C for 2 hours before the intracytoplasmic sperm ịnjection. After warming, the survival oocyte is evaluated. Warmed oocytes are considered

'morphologically surviving' if there is no dark/degenerated or contracted ooplasm and no cracked zona pellucida. Oocytes with an abnormal oolemma or ooplasm at the time of ICSI should not be excluded (40)

Semen samples will be obtained by masturbation after 3–5 days of ejaculatory abstinence. After the samples are liquefied at room temperature, a semen analysis will follow the WHO (2021) guidelines (41). The density gradient procedure will use the Isolate (Irvine Scientific., USA) discontinuous density gradient. Briefly, one aliquot of liquefied semen will be loaded onto 40%, and 80% gradients (each 1.0 ml) with the 80% fraction at the bottom of a 15 ml tube and then will be centrifuged at 300 g for 10 min at room temperature. After centrifugation, the sperm pellet will be washed twice in 3 ml of pre-warmed MHMC [Irvine Scientific., USA] and centrifuged for 5 min at 300 g. The supernatant will be discarded, and the final pellet will be resuspended in a pre-warmed MHMC medium.

Intracytoplasmic sperm injection (ICSI) is performed at 40 hours post-hCG for both the fresh and frozen oocytes and continues to culture in the CXCM medium (Irvine Scientific., USA) (37oC, CO2 6%, O2 5%). Once injected, oocytes were placed in the individual wells. Wells will be filled with 20ul of CXCM medium (Irvine, USA) (37oC, CO2 6%, O2 5%). Fertilization check at 17±1 hours post-ICSI and only normally fertilized zygotes show two pronuclei (2PN) are continued to culture to day 3. The morphology of culture embryos will be assessed at 68±1 hours, 116±2 hours, and 140 ± 2 hours after injection. The evaluated parameters include cell number, symmetry, and percentage of fragmentation for cleavage day 3. Cleavage day 3 embryos with greater than 6 blastomeres will be considered day 3 embryos. All embryos are continued to culture until day 5.

On day 5, the quality of blastocysts will depend on the expansion of the blastocoele cavity and the integrity of both the inner cell mass and trophectoderm cells. Embryos are categorized into four grades (Grade 1: AA, AB, BA, Grade 2: BB, Grade 3: BC, CB, and Grade 4: others). The highest-graded blastocysts with a blastocoel expansion greater than 3 (42) are vitrified (CRYOTEC® Method). The slow-growing embryos (the blastocoel expansion lesser than 3, cleaved embryos) continue to culture to day 6.

On day 6, the blastocysts are assessed and vitrified similarly to day 5 embryos. Freeze all in both arms, then the frozen embryo will be transferred in the next cycle.

7.3.4. Embryo cryopreservation

At **Visit 8**, after 5-6 days of culture, all embryos are cryopreserved by vitrification (one embryo/container) using CRYOTEC® Method. We will be frozen blastocysts that have the expansion of grade 3 or more, and the quality of the inner cell mass and trophectoderm cells have grade B or C (not used for CC). The procedures for oocytes will be similar to those described above, such as the vitrification step, loading, and storage. Firstly, the embryos will be balanced in an equilibration solution (ES). When the shape of the embryos is fully recovered, they will be transferred to a Vitrification Solution (VS) with a minimum equilibrium (the limit time is 15 min). The embryos will be washed in the VS2 well and spread onto the Cryotec (one blastocyst/Cryotec). After freezing, the containers will be stored in liquid nitrogen.

7.3.5. Endometrium preparation and embryo transfer

At **Visit 9,** all patients undergoing FET will receive oral estradiol valerate (Valiera®; Laboratories Recalcine) 6 mg/day from the 2nd to 4th day of menses for 6 days. The endometrial thickness will be monitored from day six onwards. From day 8-9 of menses, the estradiol dose could be adjusted to 12mg/day according to the development of the

endometrium. Progesterone will be started when endometrial thickness reaches 8 mm or more. Patients will receive micronized Progesterone (Cyclogest® 400mg; Actavis) at the dose of 400mg twice daily (morning and evening) plus dydrogesterone (Duphaston 10mg) at the dose of 10mg twice daily (morning and evening). Estradiol valerate will be decreased to 4 - 8mg/day when starting Progesterone. A single embryo will be thawed on the day of embryo transfer after six or seven days of Progesterone, depending on day-5 or day-6 embryo. After thawing, surviving embryos will be transferred into the uterus under ultrasound guidance. Estradiol and Progesterone will be continued until the day of the pregnancy test. If the pregnancy test is positive, the patients will continue to use 800 mg micronized Progesterone or 800 mg micronized Progesterone plus 20 mg dydrogesterone until 7 weeks of gestation. The blastocyst will be thawed on embryo transferring day **(Visit 10)** according to the CRYOTEC® Method. After warming, the survival blastocyst is evaluated. Blastocyst cryo-survival be defined as at least 75% of cells perceived to be intact after thawing (40). After thawing, the embryo will be cultured in a CXCM medium (37oC, CO2 6%, O2 5%). The embryo will be transferred to the uterus two hours later under ultrasound.

7.3.6. Quality of life assessment

These participants completed questionnaires, which included a standardized quality of life measure (World Health Organisation Quality of Life -- Brief Assessment (WHOQoL-BREF)). The WHOQoL-BREF assesses four domains of quality of life: 1) physical 2) psychosocial 3) social relationships, and 4) environment.

The total score will be converted to a 100-point scale (according to WHO guidelines). The questionnaire will be assessed at two points:

- On the Day 2 of menstruation in the first cycle **(Visit 1)**
- On the day of the final maturation injection of last OS cycle **(Visit 6 of the last cycle)**

7.4. Study intervention follow-up

7.4.1. Ovarian hyperstimulation syndrome assessment.

In low ovarian reserve patients, the risk of OHSS is rare. However, following oocyte retrieval, if the number of collected oocytes is more than 20 with an hCG trigger, they will be managed according to the OHSS management policy of IVFTA HCM. In short, the high-risk patient will be documented and educated regarding symptoms and when to seek further help. Clinical examination on the Day 3 and Day 5 of embryo culture in person is mandatory for all high-risk OHSS cases **(Visit 8)**.

OHSS diagnosis accords with RCOG guideline 2016. The category of OHSS severity is listed in Table 1. Outpatient management is applied for women with mild and moderate OHSS. Hospital admission will be considered when having severe or critical OHSS symptoms requiring paracentesis. Severe OHSS is an SAE and will be reported.

Table 1. Category of Ovarian hyperstimulation syndrome severity

Category	Features
Mild	Abdominal bloating Mild abdominal pain Ovarian size usually < 8 cm3
Moderate	Moderate abdominal pain Nausea ± vomiting Ultrasound evidence of ascites

	Ovarian size is usually 8 - 12 cm3
Severe	Clinical ascites (± hydrothorax). Oliguria (< 300 ml/day or < 30 ml/hour) Hematocrit> 45% Hyponatraemia (Na^+/ máu < 135 mmol/l) Hypo-osmolality (osmolality < 282 mOsm/kg) Hyperkalaemia (K^+ > 5 mmol/l) Serum Albumin (< 35 g/l) Ovarian size usually> 12 cm
Critical	Tense ascites/ large hydrothrorax Hct > 55% White cell count > 25 000/ml Oliguria/ Anuria Thromboembolism ARDS (Acute Respiratory Distress Syndrome)
Note: Hct = haematocrit; WBC = white blood cell; CrCl = creatinine clearance; Cr = creatinine; Na+ = sodium; K+ =potassium Ovarian size may not correlate with the severity of OHSS in cases of assisted reproduction because of the effect of follicular aspiration	

7.4.2. Pregnancy outcome follow-up

Serum hCG will be measured 11 days after embryo transfer **(Visit 11)**, and if positive, an ultrasound scan of the uterus will be performed at gestational weeks 7 **(Visit 12)** and 12 **(Visit 13)**. At 11–12 weeks of gestation, participants will be referred to the IVFTA HCM - Tam Anh TP. Ho Chi Minh General hospital and IVFTA – Tam Anh General hospital for prenatal care. For those who cannot participate in the prenatal care program at Tam Anh TP. Ho Chi Minh General hospital or Tam Anh General hospital, for any reason, will contact the participants via telephone/email to collect data. We also ask these participants to scan the results of each visit in every contact.

8. OUTCOME MEASUREMENTS

The primary endpoint is ongoing pregnancy after the first embryo transfer. Ongoing pregnancy is defined as pregnancy with a detectable heart rate at 12 weeks' gestation or beyond after the completion of the first transfer. Cycles in which no blastocyst is available for transfer will be considered failures. Several fertility outcomes, characteristics, and outcomes of the ovarian stimulation, patient safety, drop-out rate, and quality of life during oocyte accumulation will be assessed as secondary endpoints. Full details and definitions are provided in Table 2.

Table 2. Secondary endpoints and their definition

Secondary endpoints	Definition
LH, Estradiol and progesterone profile	LH, Estradiol, and progesterone level are measured on day 1, day 5, and day 8 of ovarian stimulation, final oocyte maturation day, and 12 hours after the final oocyte maturation injection
Rate of premature LH surge	Premature LH surge (PLS) is increased serum LH more than twice the baseline or more than 15 mIU/ml

	The rate of Premature LH surge is defined as number of PLS appearances per number of ovarian stimulation cycles
Duration of ovarian stimulation	Number of ovarian stimulation days (from the first day of FSH injection to final oocyte maturation injection day)
The total dose of FSH	A number of international units of HMG are administrated during ovarian stimulation
Number of Cumulus-oocyte complex (COCs)	Number of Cumulus-oocyte complexes after oocyte retrieval
Number of MII oocyte	Number of MII oocytes after denuding
Number of survival oocyte	Number of survival oocytes after thawing
Fertilization rate per oocyte inseminated/injected	Fertilisation is defined as the appearance of two PN at 17±1 hour per inseminated/injected
Embryo-cleavage rates	Number of embryos on Day 3 after ICSI day
Blastocyst rate	Numbers of embryos on Day 5 and Day 6 after ICSI
Number of survival blastocyst	Number of survival embryos on Day 5 and Day 6 after thawing
Top-quality blastocyst rate	Numbers of embryos on Day 5 and Day 6 with good quality after ICSI
Positive pregnancy test	A positive pregnancy test is defined as a serum hCG level greater than 25 mIU/mL 11 days after the first transfer
Implantation rate	Implantation rate is defined as the number of gestational sacs per number of embryos transferred 3 weeks after the first transfer
Biochemical pregnancy	Biochemical pregnancy is defined as a pregnancy diagnosed only by the detection of beta hCG in serum or urine
Miscarriage	Complete loss of clinical pregnancy at 12 weeks of gestation
Multiple pregnancy rate	Multiple pregnancy rate is explained as two or more gestational sacs or positive heartbeats by transvaginal sonography 5 weeks after embryo placement
Ectopic pregnancy rate	Ectopic nidation of pregnancy confirmed by sonography or laparoscopy at 12 weeks of gestation
OHSS	Ovarian hyperstimulation is diagnosed according to RCOG guideline 2016
Known Adverse events regarding medications	Adverse events regarding medications according to local information products - including: • Duphaston: Abdominal pain, bloating, severe headache, dizziness, depression, hot flushes, abnormal vaginal discharge, joint pain, urinary problems or nausea, fatigue or extreme tiredness, somnolence or sleepiness, vaginal hemorrhage, pruritus (severe itching of the skin). • Orgalutran: redness, pain or swelling at injection site, headache, nausea, tiredness.

	• Diphereline: nausea, tiredness, mood changes, depression, redness, swelling, pain and /or bruising at the injection site, swelling of the hands and feet (edema) muscle and bone pain, pain in arms and legs, dizziness, headache, impotence, loss of libido. • Ovitrelle: injection site, headache, vomiting, nausea (feeling sick), abdominal (belly) pain, abdominal distension (feeling of bloating) • Valiera: Headache, nausea, breast pain, abdominal cramp, bloating, vaginal spotting. • Cyclogest: Abdominal distension (swelling in the abdomen), abdominal pain, constipation, sleepiness, tiredness, hot flush, breast pain. • IVF-M: Headache, injection site pain, injection site allergic reaction, abdominal pain, abdominal cramps or nausea.
Drop-out	Drop-out is defined as any patient discontinuing the Study or the investigator withdrawing them from the Study for any reason.
Quality of life score	Quality of life is assessed by Vietnamese WHO-BRIFE questionnaire

9. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT

9.1. Discontinuation of study intervention

Discontinuation from DPOS study does not mean discontinuation from the treatment, and remaining study procedures should be completed as indicated by the study protocol. Suppose a clinically significant finding is identified (including, but not limited to changes from baseline) after enrollment. In that case, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

9.2. Participant discontinuation/withdrawal from the Study

Participants are free to withdraw from the Study at any time upon request.

An investigator may discontinue or withdraw a participant from the Study for the following reasons:

- Pregnancy that is not from an embryo transfer cycle
- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the Study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

The reason for participant discontinuation or withdrawal from the Study will be recorded on the Case Report Form (CRF). Subjects who sign the informed consent form and are randomized but do not receive the study intervention may be replaced. Subjects who sign the informed consent form, and are randomized and receive the study intervention, and subsequently withdraw, or are withdrawn or discontinued from the Study, will not be replaced.

9.3. Lost to follow-up

A participant will be considered lost to follow-up if she fails to return for scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant, reschedule the missed visit, counsel the participant on the importance of maintaining the assigned visit schedule, and ascertain if the participant wishes to and/or should continue in the Study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, she will be considered to have withdrawn from the Study with a primary reason of loss to follow-up.

10. STUDY ASSESSMENT AND PROCEDURES

10.1. Feasibility of Study

IVFTA-HCM is a recognised center in Vietnam with high volume and modern equipment. IVFTA-HCM has a volume about 1700 - 2000 cycles per year. Of these, 67% of patients under 37 years old and 45% had a low ovarian reserve. Frozen embryo transfer is 97% of all cycles. The embryo and gamete cryopreservation system at IVFTA-HCM is well-established for freezing requirements in the oocyte collection strategy. RTAC code of practice was applied in quality management.

10.2. Safety and other assessments

The investigator will inform subjects and the reviewing accredited medical research ethics committee if anything occurs, based on which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The Study will be suspended pending further review by the accredited medical research ethics committee, unless suspension would jeopardize the subjects' health. The investigator will take care that all subjects are kept informed

11. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

11.1. Adverse Events and adverse drug reactions

Adverse events (AE) are defined as any undesirable experience occurring to a subject during the trial, whether or not considered related to the intervention.

An adverse drug reaction (ADR) is defined as an appreciably harmful or unpleasant reaction resulting from an intervention related to the use of a medicinal product; adverse effects usually predict hazard from future administration and warrant prevention, or specific treatment, or alteration of the dosage regimen, or withdrawal of the product

11.2. Reporting of adverse events

11.2.1. AE Recording

All adverse events reported spontaneously by the subject or observed by the investigator or their staff will be recorded. Any AE should be recorded on the Adverse Events form in the CRF and source documents. In every visiting, AE/SAE will be recored by subject or observed by medical staff. The AE/SAE related medication also will be asked routinely in the oocyte pick-up day **(Visit 7)** of every ovarian stimulation cycle.

AEs will be collected from the time the subject signed the study specific informed consent until the last visit **(Visit 13)** according to the pregnancy outcomes (the primary endpoint is the ongoing pregnancy).

11.2.2. AE assessment

Each AE is to be evaluated by the investigator for duration, severity, seriousness and causal relationship to the investigational drug. The action taken with study drug, the concomitant treatment/therapy introduced and the outcome, and as well as whether the event led to study termination will also be recorded.

Severity

The severity of the AE should be characterized as ""“mild, moderate or severe"” according to the following definitions:

- Mild events are usually transient and do not interfere with the subject’s daily activities.
- Moderate events introduce a low level of inconvenience or concern to the subject and may interfere with daily activities.
- Severe events interrupt the subject's usual daily activity.

Drug-Event Relationship

The causal relationship between the study drug and the AE should be characterized according to the following:

- Unrelated – there is not a reasonable possibility that the study drug caused the AE.
- Unlikely – suggests that only a remote connection exists between the study drug and the event. Other conditions, including concurrent illness, progression or expression of the disease state or reaction to concomitant medication, appear to explain the AE.
- Possible – suggests that the association of the AE with the study drug is unknown, however, the event is not reasonably supported by other conditions.
- Probable – suggests that a reasonable temporal sequence of the AE with drug administration exists and, in the Investigator's clinical judgment, it is likely that a causal relationship exists between the drug administration and the AE, and other conditions (concurrent illness, progression or expression of the disease state, or concomitant medication reactions) do not appear to explain the AE.

Outcome

The outcome of the adverse event should be classified according to the following definitions:

- Recovered / resolved: the event has resolved (no further symptoms are present and no treatment is being received by the subject).
- Recovered / resolved with sequelae: the event has resolved but there may be lingering effects present (e.g., a scar following a cut or abrasion).
- Fatal: the subject died as a result of the event. This code should only be used for the event that caused the death, not any event that was present at the time of the subject's death. Fatal events require immediately reporting to the Sponsor (or an authorized representative).
- Unknown: may only be used in the event that the subject is lost to follow-up and no reliable data can be obtained.

11.2.3. AE reporting

All of AE will be reported to the Instituional Review Board of Tam Anh TP. Ho Chi Minh General hospital and Ethical Committee for Biomedical Research on Human Subjects of Vietnamese Ministry of Health in periodic reports.

11.2.4. AE follow-up

All AEs occurring during the Studystudy are to be followed up in accordance with good medical practice until resolved or judged no longer clinically significant, or if a chronic condition, until fully characterized. All follow-up results are to be reported to the Instituional Review Board of Tam Anh TP. Ho Chi Minh General hospital.

11.3. Serious Adverse Events

11.3.1. Definition

A serious adverse event (SAE) is any untoward medical occurrence or effect, at any dose, that:

- results in death;
- is life threatening (at the time of the event);
- requires hospitalization or prolongation of existing inpatients' hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- is considered an important medical event (an event that may not be immediately life- threatening or result in death or hospitalization, but based on medical judgment may jeopardize the subject and may require medical or surgical intervention to prevent any of the outcomes listed above (i.e., death of subject, life-threatening, hospitalization, prolongation of hospitalization, congenital anomaly, or persistent or significant disability/incapacity).

11.3.2. Reporting SAE

Any SAE, whether or not related to the study drug, must be reported immediately (within 24 hours of the investigator's awareness of the event) by telephone or email to Instituional Review Board of Tam Anh TP. Ho Chi Minh General hospital . Pregnancy in a study subject is not considered an adverse event. Serious complication of pregnancy are considered as a SAE.

Fatal or life-threatening SAEs must be reported urgently within 07 workdays of investigator's awareness of the event. Other SAEs must be reported within 15 workdays. The reports will be sent to Agency of Science, Technology and Training, Ethical Committee for Biomedical Research on Human Subjects of Vietnamese Ministry of Health and National centre of Drug information and adverse drug reactions monitoring (National DI and ADR center).

Investigator shall report adverse events that occur during the course of the Study directly to the competent regulatory authorities as required by and in accordance with the Applicable Law

12. STATISTICAL CONSIDERATIONS

12.1. Sample size determination

The ongoing pregnancy rate of patients in ART center – Tam Anh HCM hospital was 45.0% in the Antagonist IVF stimulation group. To show or refute that hypothesis: no significant difference in ongoing pregnancy rates when using DPOS protocol (40 - 42%) (43–45), we need 657 patients to produce a two-sided 95% confidence interval for the difference in population proportions with a width that is equal to 5% (margin = 5.0%, alpha = 5.0% and power = 80%). Considering a 10% loss to follow-up and protocol violation we plan to recruit 730 participants (365 per arm).

12.2. Randomization

After signed informed consent, patients will be randomly assigned in a 1:1 ratio to receive one protocol: Antagonist or DPOS. Randomization will be centrally controlled by a data scientist, who is not involved in any treatment procedure. When there is an eligible participant to be enrolled in the Study, clinicians from the specific site will enter the study data management center to get the allocation of patients according to a computer-generated randomization list in a 1:1 ratio, with a variable block size of 2, 4, 6 or 8. Apart from randomization, patients will be followed up and treated according to the protocol at each center.

12.3. Statistical analyses

Statistical analysis will be conducted according to the ITT principle, in which all randomized women will be conducted for the comparison of primary outcomes between treatment groups. The per-protocol analysis may be conducted, but these results would be considered reference only. All tests will be two-tailed, and differences with p–value < 0.05 will be considered statistically significant. The statistical software used was R, version 4.0.5 (R Foundation for Statistical Computing). We will develop a detailed statistical analysis plan that will be completed before data–lock.

12.3.1. Characteristics of patients

Patient's characteristics will be shown through descriptive analysis, and the balance between the two arms will be assessed. For continuous variables, the normality test will be estimated using frequency histograms and the

Shapiro's test. If the parameters are normally distributed, they will be presented as mean with standard deviation (SD) and compared using Student's t-tests or ANOVA tests. If the parameters are non-normally distributed, their medians and inter-quantile ranges (IRQs) will be reported, and non-parametric tests will be utilized to test the distribution of these variables. For categorical variables, data will be shown by the proportions of the two arms, and they will be compared using Pearson's chi-square test or Fisher's exact test where appropriate. In addition, we will also report the numbers of recruitment, participants lost to follow-up, protocols violation, and other relevant descriptive data.

12.3.2. Primary outcome

The primary outcome, ongoing pregnancy rate will be compared using Pearson's chi-square test or Fisher's exact test for unadjusted analysis. We will also compute the unadjusted risk ratio (RR) and its 95% confidence interval (95% CI). In the event of a prominent imbalance of potential confounders between arms, we will perform multivariable Log-Binomial or Poisson Regression with a robust variance estimate to compute adjusted RR and its 95% CI.

12.3.3. Secondary outcome

For continuous variables, results will be shown as mean (SD) and between-group differences will be considered using Student's t-test. For dichotomous outcomes, relative risk (RR) and 95% confidence interval (CI) values will be calculated.

12.3.4. Missing data and sensitivity analysis

Missing values regarding baseline characteristics, we will first perform an analysis by excluding missing values; we will then perform multiple imputations to impute missing values and conduct subsequent analysis to estimate the robustness of the findings. For the loss of follow-up and protocol violation, we will attempt sensitive analyses to explore the effect of these factors on the trial findings.

12.3.5. Data and safety monitoring board

An independent Data and Safety Monitoring Board (DSMB) will be established to review and interpret data generated from the Study and to review revisions of the protocol before their implementation. Its primary objectives are to ensure the safety of study subjects and the integrity of research data. The DSMB advises on research design issues, data quality and analysis, and research participant protections for the Study.

The DSMB will hold regular conference calls to review the protocol concerning ethical and safety standards, monitor the safety of the trials, monitor the integrity of the data concerning the original study design, and provide advice on study conduct. The DSMB will review the progress of the trial, adjudicate adverse events, and decide on any premature closure of the Study. The DSMB consists of three members. Voting members consist of individuals who are impartial, independent of the investigator(s), and who have no financial, scientific, or other conflicts of interest with the Study.

12.3.6. Planed interim analyses

The interim analysis will be performed by an independent statistician who will not be directly involved in the Study, after the completion of data collection of the first 366 randomized patients. In interim analyses, data will be assessed for safety, efficacy, and futility. Safety will be assessed in terms of serious adverse events. The interim analysis will be conducted using a two-sided significant test with the Haybittle–Peto spending function and a type I error rate of 5% with stopping criteria of p <0.001. According this report, the DSMB will guide whether to stop or continue the Study.

13. ETHICAL CONSIDERATION

13.1. Regulation statement

The Study will be conducted according to the principles of the Declaration of Helsinki (World Medical Association Declaration Of Helsinki Ethical Principles for Medical Research Involving Human Subjects Version Edinburgh, Scotland, October 2000, with Note of Clarification on Paragraph 29 added by the WMA General Assembly, Washington 2002 end Note of Clarification on Paragraph 30 added by the WMA General Assembly, Tokyo 2004) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts.

The National Review Board approved protocol V 2.1 on February 16th, 2023, with No: 28/CN-HĐĐĐ

The National Review Board approved the amended protocol V3.0 on October 22nd, 2024, with No: 237/CN- HĐĐĐ

13.2. Recruiment and consent

The investigator must explain to each subject the nature of this Study, its purpose, procedures, expected duration and the potential risks and benefits involved in study participation along with any discomfort it may entail. Each subject must be informed that participation in the Study is voluntary and that withdrawal of consent will not affect her right to the most appropriate medical treatment or affect the doctor's relationship.

Informed consent will be given by means of a standard written statement. It will be written so as to be easily understood by the subject. The subject will be given the time to read and understand the statement herself before signing her consent and dating the document. The subject will be provided with a copy of the written statement once signed.

Patients randomized to the DOPS group will be paid fees associated with medication.

13.3. Privacy

Participating subjects will be assigned a 5-digit number. This personal code will be on all forms retrieved from participants

14. ADMINISTRATIVE ASPECTS AND PUBLICATION

14.1. Handling and storage of data and documents

Data will be collected using a questionnaire and the Microsoft excel software. Data monitoring will be done by the principal investigator, based at Tam Anh TP. Ho Chi Minh General hospital. Data handling will be done anonymously, with only the patient code available to the local investigator. Patients will be asked for informed consent.

14.2. End of study report

At the end of the study, a final report of Study results will be sent to the National and Local Institutional Review Board.

14.3. Public disclosure and publication policy

No specific arrangements will be made between any sponsors and the investigator concerning the public disclosure and publication of the research data. The principal investigator will publish the results of the Study as soon as appropriate.

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