


BMJ Open Effectiveness of progesterone supplementation in women presenting low progesterone levels on the day of frozen embryo transfer: a randomised controlled trial

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ABSTRACT

Introduction Progesterone is an essential hormone involved in the process of implantation and pregnancy maintenance. Evidence from recent studies has supported the importance of serum progesterone level around the time of embryo transfer in hormonal replacement therapy frozen embryo transfer cycles and recommended the need for individualised luteal support. Low progesterone around the time of embryo transfer is found to be associated with decreased rate of pregnancy after frozen embryo transfer. This single-centre, longitudinal, randomised, interventional controlled study aims to compare the rate of ongoing pregnancy between two groups of women with progesterone level below 10 ng/mL on the day of frozen embryo transfer: the study group using 800 mg vaginal micronised progesterone supplemented with 50 mg intramuscular progesterone per day and the control group using only 800 mg vaginal micronised progesterone.

Methods and analysis We enrol patients who are undergoing frozen embryo transfers with blastocyst-stage or cleavage-stage embryos and who satisfy the inclusion and exclusion criteria. After signing the informed consent, participants are randomised into two groups: the study group using vaginal micronised progesterone supplemented with progesterone intramuscular 50 mg per day and the control group using only vaginal micronised progesterone. Randomisation will be performed using R software at a 1:1 allocation ratio. Sequentially numbered, opaque sealed envelopes are used for allocation. The primary outcome is the rate of ongoing pregnancy. To demonstrate a difference of 10% with regard to rate of ongoing pregnancy, at least 370 participants per arm are required (type I error $\alpha=0.05$, power=0.8). Assuming a dropout rate of 10%, a total of 824 patients (412 per group) will be invited.

Ethics and dissemination This study was approved by the Ethics Committee of Tu Du Hospital on 17 May 2021 (reference number: 1251/QĐ-BVTD). All participants provide informed consent before being enrolled in the study. The results of our study will be submitted to reproductive medicine conferences and journals.

Trial registration number NCT04897269.

Strengths and limitations of this study

- This is a single-centre, longitudinal, randomised, interventional controlled pharmacological study.
- The intended sample size is large with 824 participants.
- Our study is not designed to detect a small difference despite the large sample size.

INTRODUCTION

Progesterone is an essential hormone in the process of implantation and pregnancy maintenance. Its crucial roles include luteal phase support, modulation of maternal immune response, suppression of inflammatory response, reduction of uterine contractility and improvement of uteroplacental circulation.¹ Progesterone is a steroid produced primarily by the corpus luteum and the placenta. An investigation conducted by Csapo *et al*² showed that luteectomy in early pregnancies induced abortion. Progesterone is necessary to obtain a secretory phase transformation of the endometrium. During the luteal phase, it prepares the endometrium for pregnancy by stimulating proliferation in response to human chorionic gonadotropin (hCG).³ During conditions of insufficient progesterone, which is defined as a luteal phase defect, progesterone may not maintain normal secretory endometrium and may prevent normal embryo implantation and growth.⁴ A mid-luteal serum progesterone <10 ng/mL was established to predict a luteal phase defect.⁵

Nowadays, frozen-thawed embryo transfer (FET) is being performed worldwide due to several factors, including implementation of

vitrification with an extremely high survival rate, prevention of ovarian hyperstimulation syndrome and avoiding the negative effect of late follicular elevating progesterone on embryo implantation.⁶ With regard to endometrium preparation in the FET cycles, no protocol is definitely better than the others.^{7,8} Hormonal replacement therapy (HRT) is a commonly used protocol⁸ due to its flexibility and convenience⁹ in programming the embryo transfer day and excellent results.¹⁰ In this method, oestrogen is administered from the beginning of the menstrual cycle to inhibit follicle stimulating hormone (FSH) intercycle rise and follicular growth, resulting in the corpus luteum not being formed. Exogenous progesterone administration may begin 2, 3 or 5 days before the scheduled day of transfer depending on the stage of the frozen embryos.

Accumulating evidence has supported the importance of the level of serum progesterone at the time of embryo transfer in HRT FET cycles and recommended the need for individualised luteal support. Labarta *et al*¹¹ showed that the rate of ongoing pregnancy was significantly lowered in the group of women with serum progesterone level <9.2 ng/mL on the day of embryo transfer compared with women with progesterone level >9.2 ng/mL. In a retrospective cohort study of Gaggiotti-Marre *et al*,¹² low serum progesterone level (≤ 10.64 ng/mL) 1 day before FET was associated with a lower rate of live births following FET of euploid embryos. Similarly, Cédric-Durnerin *et al* showed that serum progesterone level <10 ng/mL was observed in 37% of FET cycles and was associated with significantly lower rates of pregnancy (34% vs 48%, $p=0.04$) and live births (17% vs 31%, $p=0.01$).¹⁰ Thereafter, some studies have assessed the benefit of progesterone supplementation in FET cycles when the progesterone level was below the cut-off. In two recent studies of Yarali *et al* and Álvarez *et al*,^{13,14} 25 mg subcutaneous progesterone was added if progesterone level 1 day before embryo transfer was below the cut-off. The rates of ongoing pregnancy were comparable between the progesterone supplementation group and the control group whose progesterone level was above the cut-off. Until now, there have been no randomised control trials assessing the effect of adding progesterone on the day of embryo transfer.

Progesterone is most commonly administered as vaginal suppositories. Vaginal progesterone, with the first uterine pass effect,¹⁵ is absorbed through the vagina and transmitted primarily to the uterus and therefore achieves higher tissue levels than the oral, subcutaneous or intramuscular route.¹⁶ Moreover, it is painless, self-administered and rarely induce allergic reactions.¹⁶ It is recommended that a low level of serum progesterone after vaginal administration may be rescued by an addition of another route of progesterone.¹⁰ Most of the recent authors used intramuscular progesterone in their studies.^{13,14}

This randomised controlled trial is designed to evaluate the effect of progesterone supplementation in patients with low serum progesterone level on the day of embryo transfer. The primary outcome is the rate of ongoing

pregnancy between two groups of women with progesterone level below 10 ng/mL on the day of frozen embryo transfer: the study group using 800 mg vaginal micronised progesterone supplemented with 50 mg progesterone intramuscular per day and the control group using only 800 mg vaginal micronised progesterone.

MATERIALS AND ANALYSIS

Design, setting and participants

This is a single-centre, longitudinal, randomised, interventional controlled pharmacological study enrolling women attending in vitro fertilization (IVF) Tu Du at Tu Du Obstetrics and Gynecology Hospital in Vietnam.

Patients undergoing frozen embryo transfers with blastocyst-stage or cleavage-stage embryos and who satisfy the inclusion and exclusion criteria provide blood samples for progesterone measurement on the day of embryo transfer. Patients with progesterone level below 10 ng/mL on the day of embryo transfer are randomised into two groups: the study group (50 mg intramuscular progesterone supplementation and 800 mg vaginal micronised progesterone) and the control group (800 mg vaginal micronised progesterone per day).

The following are the detailed inclusion criteria for participants:

- ▶ Patients undergoing Assisted Reproductive Technology (ART) with frozen embryo transfer from 18 to 45 years of age.
- ▶ Body mass index (BMI) from ≥ 18.5 kg/m² to 22.9 kg/m².¹⁷
- ▶ Endometrial preparation with hormone replacement in frozen embryo transfer cycles with day 3 or day 5 embryos.

The following are the exclusion criteria:

- ▶ Oocyte donor cycles.
- ▶ Surrogacy.
- ▶ In vitro maturation of the oocytes.
- ▶ Preimplantation genetic testing.
- ▶ Abnormal uterus (fibroid types 0–3 according to the International Federation of Gynaecology and Obstetrics (FIGO) classification, endometrial polyp, adenomyosis, congenital malformation).
- ▶ Uterine fibroid types 4 and 5 (FIGO classification) >4 cm.
- ▶ History of recurrent implantation failure (>2 times of embryo transfer failure).
- ▶ Caesarean scar defect (isthmocoele).
- ▶ Endometrial thickness <7 mm on the day of embryo transfer.

Recruitment and randomisation

After completing the IVF profile, patients undergo ovarian stimulation, oocyte retrieval and embryo transfer. The remaining embryos are cryopreserved for future use in frozen embryo transfer (FET) cycle. In the FET cycle, patients come to the clinic on days 1–4 of their period to start endometrial preparation. Embryo transfer occurs

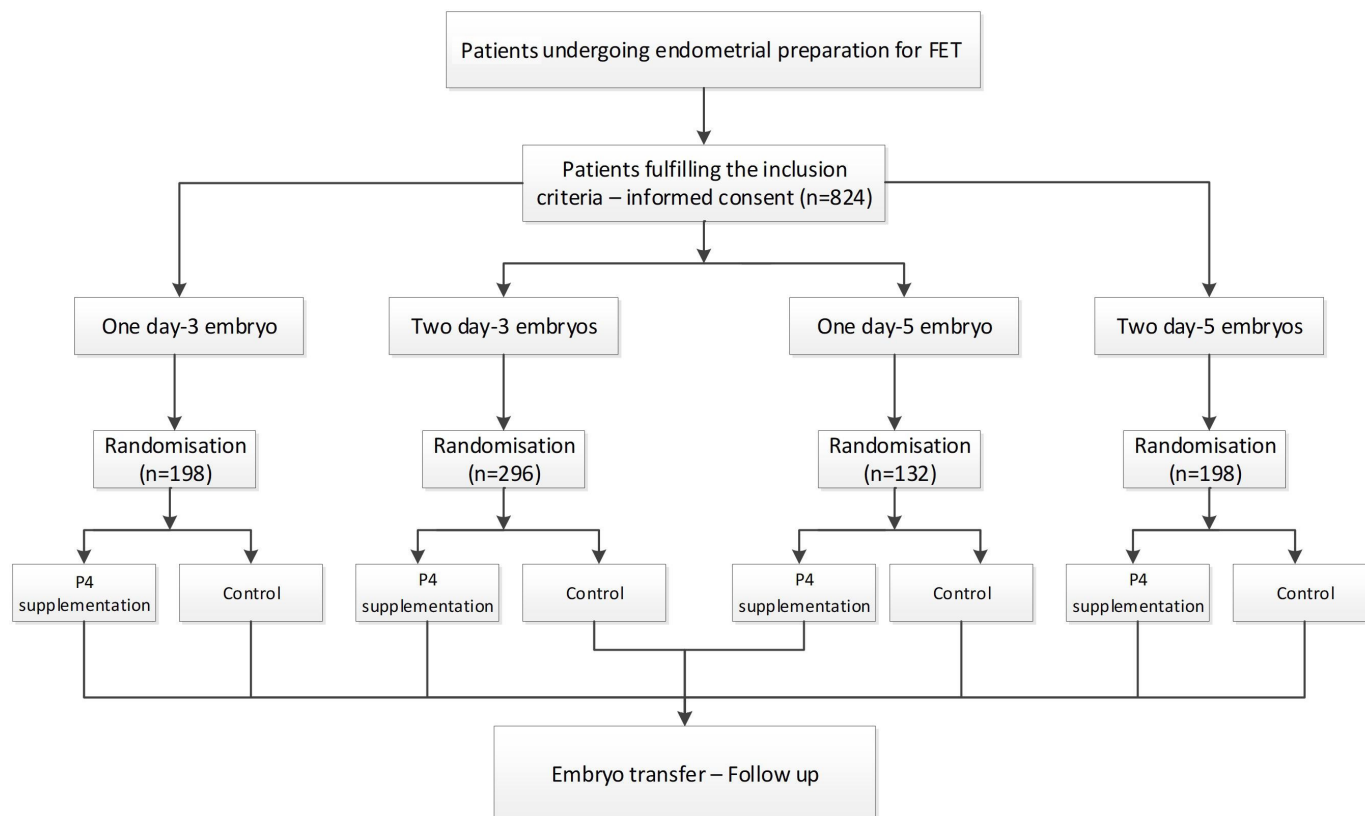


Figure 1 Flow chart of recruitment. FET, frozen-thawed embryo transfer. (P4 - progesterone)

on the third day of progesterone administration for day 3 embryos and on the sixth day for day 5 embryos. On the day of starting progesterone, patients fulfilling the eligibility criteria are invited to participate in the study; the study was explained to all participants. Patients who agree to join sign the informed consent. On the day of embryo transfer, blood is drawn for progesterone measurement. If progesterone level is equal or below 10 ng/mL, patients are randomised to the control group or to the study group.

We employ a stratified randomisation scheme together with permuted block randomisation. Patients are stratified by the number of embryos transferred (one vs two embryos) and the stage of embryos transferred (day 3 vs day 5) (figure 1). Randomisation is then performed using R V.4.0.3 software with package blockrand for each stratum at a 1:1 allocation ratio and with block size determined as 2, 4, 6 and 8. The randomisation list was prepared by an independent statistician not participating in the recruitment.

Sequentially numbered, opaque sealed envelopes are used for allocation. Envelopes receive numbers in advance and are opened sequentially, only after the participant's name has been written on the appropriate envelope. One member of the research team who labelled the containers is aware of the allocation. Participants and other researchers are unaware of treatment allocation until the envelope is opened. Treatment is not masked to the participants, care providers and investigators, but masked to the outcome assessor.

Intervention and comparator

Progesterone supplementation group

In this group, patients will be prescribed 50 mg intramuscular progesterone supplementation and 800 mg vaginal micronised progesterone (Cyclogest) per day for 14 days until the day of beta-hCG testing.

Control group

Patients continue their regular medication (800 mg vaginal micronised progesterone per day) (Cyclogest) until the day of beta-hCG testing.

If the beta-hCG test is positive, patients will be continued on their prescriptions depending on the study or control group as mentioned above until 7 weeks of pregnancy when the fetal heart is confirmed. This means that in the control group patients are continued on 800 mg vaginal micronised progesterone per day and in the study group patients keep on using 50 mg intramuscular progesterone and 800 mg vaginal micronised progesterone until 7 weeks of pregnancy.

Outcomes

Primary outcome

- ▶ Ongoing pregnancy: ongoing pregnancy is defined as a pregnancy of one or more fetuses with heart beat confirmed by ultrasound at 12 weeks gestation.

Secondary outcome

- ▶ Biochemical pregnancy: a pregnancy diagnosed by detection of beta-hCG in the serum.¹⁸

- ▶ Implantation rate: the number of gestational sacs observed divided by the number of embryos transferred (expressed as percentage).¹⁸
- ▶ Miscarriage: spontaneous loss of a clinical pregnancy before 12 completed weeks of gestational age, in which the embryo or the fetus is non-viable and is not spontaneously absorbed or expelled from the uterus.¹⁸
- ▶ Clinical pregnancy: a pregnancy diagnosed by ultrasonographic documentation of at least one fetus with a discernible heart beat.¹⁸

Endometrial preparation protocol

After completing the IVF profile, patients undergo ovarian stimulation, oocyte retrieval and embryo transfer. The remaining embryos are cryopreserved for future use in frozen embryo transfer cycle. In the FET cycle, patients come to the clinic on days 1–4 of their period to start endometrial preparation with estradiol valerate. Estradiol valerate is administered at a dosage of 4–8 mg per day. Ultrasound scans are performed every 3–7 days to monitor the development of the endometrium. The dosage of estradiol may be increased up to 16 mg per day to maximise the growth of endometrium. When the endometrium reaches 8 mm or higher and the duration of endometrial preparation is ≥ 12 days, vaginal progesterone is started. Embryo transfer occurs on the fourth day of progesterone administration for day 3 embryos and on the sixth day for day 5 embryos.

Sample size estimation

In a previous study, the rate of ongoing pregnancy of the group with low progesterone level is reported to be 32.7%.¹¹ To demonstrate a difference of 10% with regard to the rate of ongoing pregnancy, at least 370 participants per arm are required (type I error $\alpha=0.05$, power=0.8). Assuming a dropout rate of 10%, a total of 824 patients (412 per group) will be invited. We employed a stratified randomisation scheme together with permuted block randomisation. Based on the current distribution of patients' characteristics in the department, participants are stratified by the number of embryos transferred (40% for one vs 60% for two embryos) and the stage of embryo transferred (60% for day 3 vs 40% for day 5) into four equal groups:

1. 198 participants with one embryo transferred on day 3;
2. 296 participants with two embryos transferred on day 3;
3. 132 participants with one embryo transferred on day 5; and
4. 198 participants with two embryos transferred on day 5.

Statistical methods

We will analyse data according to an intention-to-treat principle. To demonstrate baseline variables, continuous data will be presented with mean values and SD or median with IQR according to the normality of data. Categorical data will be described by frequencies and

percentages. Comparison of rates of ongoing pregnancy (primary outcome), implantation rates (secondary outcome) and other binary outcomes between the two arms will be performed using χ^2 test accompanied by risk ratio and 95% CI. Quantitative variables will be analysed using Student's t-test or Mann-Whitney test. Multivariable logistic regression will be employed to adjust for potential confounders if the randomisation fails to balance other risk factors between the two arms. All statistical analyses will be performed using R software (Vienna, Austria).

Progesterone measurement

Serum progesterone level samples are analysed by automated progesterone immunoassays (Cobas 8000). Tu Du Lab is certified by ISO 15189.

Study duration

The study started in May 2021, with a recruitment duration of 18–24 months.

Patient and public involvement

There was no patient and public involvement in the development of the research question, study design or recruitment, and conduct and reporting of the study.

Data monitoring and interim analysis

Data are monitored, updated and secured by two clinicians and one statistician. The interim analysis will be performed when the first 400 participants have been recruited.

Adverse event monitoring

Adverse events are closely monitored during the study. On the day of embryo transfer, the participants in the study group are given an instruction leaflet which includes information about side effects, adverse events related to the drug, how to prevent and manage these irritations, and the phone number to call if they feel discomfort. Also, the staffs phone to the patients 2 to 3 times during the luteal support to ask for their compliance to intramuscular progesterone and adverse events.

Common adverse events or adverse reactions related to intramuscular progesterone are pain, swelling at the injection site and breast tenderness. Rare adverse events include severe allergic reactions. All serious adverse events, serious adverse reactions or suspected unexpected serious adverse reactions are recorded and reported to the adverse events system of Tu Du Hospital. When the patient reports an adverse event/adverse reaction (AR) and it is not serious, it is managed as instructions and consultants of the specialists in our clinic. The patient can continue their participation in the study. If the adverse event/adverse reaction is serious, the patient is emergently treated at the Department of Emergency of Tu Du Hospital and is taken out of the study.

Patients can stop participating in the study at any period of time they want. In that case, they are excluded from the study.

ETHICS AND DISSEMINATION

Our study was approved by the Ethics Committee of Tu Du Hospital on 17 May 2021 (reference number: 1251/QĐ-BVTD). All participants provide informed consent before being enrolled in the study. For confidentiality, personal patient information is not shared. The results of our study will be submitted to reproductive medicine conferences and journals.

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