VERSION: 2
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RESEARCH PROTOCOL

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1. TITLE OF RESEARCH PROPOSAL

The effect of sexual intercourse during embryo transfer on the pregnancy rate

2. KEYWORDS

Sexual intercourse, embryo transfer, artificial reproduction technology, in-vitro fertilisation, pregnancy rate

3. BACKGROUND AND INTRODUCTION

The prevalence of infertility among reproductive-aged couples ranges between 12.6% and 17.5% worldwide.[1] ART (assisted reproductive technology) cycles have increased from approximately 140,000 in 1991 to over 3.2 million in 2018 utilising mostly from thawed frozen embryo transfers, and approximately 670,000 infants were born from ART in 2018.[2]

Seminal fluid transmits signalling agents that interact with female reproductive tissues to initiate a controlled inflammatory response causing leukocytes to infiltrate the female reproductive tract, to induce an altered transcriptional program in female tract tissues that modulates embryo developmental programming, and to initiate immune adaptations to promote receptivity to implantation and placental development through an expansion of the pool of regulatory T cells that assist implantation by suppressing inflammation, mediating tolerance to male transplantation antigens, and promoting uterine vascular adaptation and placental development.[3] Sperm itself can also play an active role in provoking the female immune response, by interacting with uterine epithelial cells to induce cytokines that assist in eliciting neutrophil recruitment and Treg cell activation.[4]

Of clinical studies, a 2019 meta-analysis of clinical trials show intravaginal or intracervical seminal plasma application around the time of oocyte pickup is associated with higher continuing pregnancy rate hence local application seminal plasma may be considered as a potential treatment to improve implantation.[5] A multicentre RCT on intercourse during the peri-transfer period of an IVF cycle involving 1343 embryos results in no significant difference between the intercourse and abstain groups in relation to the pregnancy rate (23.6 and 21.2% respectively), but the proportion of transferred embryos that were viable at 6-8 weeks was significantly higher in women exposed to semen compared to those who abstained (11.01 versus 7.69 viable embryos per 100 transferred embryos, P = 0.036, odds ratio 1.48, 95% confidence interval 1.01-2.19) indicating that exposure to semen around the time of embryo transfer increases the likelihood of successful early embryo implantation and development.[6] A 2022 randomised clinical trial shows that sexual intercourse using barrier contraception vs abstinence one night before frozen-thawed embryo transfer results in higher clinical pregnancy and implantation rates with similar spontaneous abortion rates.[7] These data taken as a whole indicate that seminal fluid, sperm and even protected vaginal intercourse may safely aid implantation during the use of ART.

However, a 2014 calendar based secondary data analysis with a positive pregnancy test as outcome shows that “Cycles in which couples had 2 or more days with intercourse during the implantation window were significantly less likely to result in a positive pregnancy test compared to cycles in which couples didn’t have intercourse in this window, after adjusting for age, race, history of regular menstrual cycles, previous pregnancy, and body mass index (Fecundability Ratio=0.62, 95% Confidence Interval: 0.42-0.91)”.[8] In contrast a more recent 2020 report finds that peri-implantation intercourse has no effect on fecundability: adjusted FR for three or more acts of peri-implantation intercourse versus none: 1.00, 95% credible interval: 0.76-1.13. Results were essentially the same with sensitivity analyses.[9]

Couples within an ART program perceived that the purpose of sexual intercourse is not only to have offspring but also to improve communication, promote intimacy, and express affection.[10] Indeed, in an ART program, “the experience of orgasm every time they had sex recently had a positive impact on reproductive health, especially pregnancy”.[11] Also, there is the “close relationship between emotional, psychological and sexual aspects, which can be integrated in the new concept of Inferto-Sex Syndrome (ISS) that can impair the ART treatment outcomes” to be considered.[12]

In most successful human pregnancies, the conceptus implants 8 to 10 days after ovulation.[13] In UMMC fertility unit, the standard invitro fertilisation regimen involved transfer of thawed blastocyst stage embryos frozen[14] at Day 5[15]. We postulate that vaginal intercourse with intravaginal ejaculation in 96 hours before thawed frozen Day 5 embryo transfer will increase the viable pregnancy rate at 12 weeks gestation as the four-day window of opportunity should create a more conducive environment for implantation. We plan to test the hypothesis by advising sexual intercourse compared to advising abstinence within the four-day window preceding Day 5 embryo transfer.

4. OBJECTIVE AND EXPECTED OUTCOMES

4.1: OBJECTIVE

To evaluate the impact of advice for sexual intercourse versus abstinence in the four days preceding thawed frozen embryo transfer in IVF

4.2: EXPECTED OUTCOMES

4.2.a: Primary outcome:

A viable pregnancy with evidence of intra-uterine gestation sac and presence of fetal heart at 10 weeks after Day 5 embryo transfer

4.2.b: Secondary outcome [16]:

- Implantation rate - defined as the number of gestational sacs observed divided by the number of embryos transferred (usually expressed as a percentage, %).
- Clinical pregnancy rate – defined as a pregnancy diagnosed by ultrasonographic visualization of one or more gestational sacs or definitive clinical signs of pregnancy. In addition to intra-uterine pregnancy, it includes a clinically documented ectopic pregnancy
- Miscarriage – defined as spontaneous loss of a clinical pregnancy before 22 completed weeks of gestational age, in which the embryo(s) or fetus(es) is/are nonviable and is/are not spontaneously absorbed or expelled from the uterus
- Multiple pregnancy – defined as pregnancy with more than one embryo or fetus.
- Ectopic pregnancy – defined as a pregnancy outside the uterine cavity, diagnosed by ultrasound, surgical visualization or histopathology.
- Sexual intercourse activity in the 4 days preceding to the Day 5 embryo transfer

5. METHODOLOGY

5.1 STUDY DESIGN

This study a parallel group randomized controlled trial involving convenience sampling. Intervention group will be advised to **engage in sexual intercourse 96 hours prior to the Day 5 embryo transfer** while a control group will be couples advised to abstain from sexual intercourse throughout the cycle.

5.2 PLACE OF STUDY

This trial will be conducted in Fertility clinic, Obstetrics and Gynaecology department, University Malaya Medical Centre (UMMC).

5.3 POPULATION OF STUDY

Women of reproductive age undergoing IVF treatment at UMMC

5.4 CRITERIA

Inclusion criteria

1. Women undergoing frozen embryo transfer (FET)
2. Day 5 embryo transfer
3. Medicated frozen embryo transfer cycle
4. Endometrial thickness (ET) of more than 8mm on starting the luteal phase support

Exclusion criteria

1. Women undergoing fresh embryo transfer
2. ET of less than 8mm on starting the luteal phase support
3. Triple embryo transfers

5.5 METHODS

i) Recruitment and randomization:

All patients for IVF treatment who have reached the Oocyte Pick UP (OPU) and decided for Frozen Embryo Transfer (FET) will be identified and given the leaflet information sheet. At the start of FET cycle, patient is approached and explained regarding the study. Recruitment is done at this point with informed consent obtained. Randomization will be done once the endometrial thickness is ≥ 8mm with trilaminar appearance on sonography.

Randomization sequence will be generated online using https://www.sealedenvelope.com/simple-randomiser/v1, in blocks of 4 or 8, following 1 to 1 ratio by a clinic assistant who will not be involved in the recruitment process. Allocation will be sealed in a numbered opaque envelope. The couple will receive random envelopes which will categorize the couple into two groups; intervention group, will be advised for sexual intercourse 96 hours prior to the Day 5 embryo transfer, while the control group, will be advised for abstinence from sexual intercourse throughout the embryo transfer cycle. Patients are planned for a Day 5 frozen embryo transfer as per inclusion criteria.

Patients will be prescribed with luteal phase support medications for 5 days. Until the day of embryo transfer, couple will be advised to follow respective instructions based on the envelope received. Sexual activity diary will be given to the patient to chart the of sexual intercourse activity. Patient diagram information will also be given for time reference to avoid confusion.

Embryo transfer will be proceeded as scheduled. The clinician performing embryo transfer will not be involved in the study.

ii) Protocol:

To prepare the endometrium, exogenous administration of estrogen and progesterone was used for the FET cycle. The patient will be scanned at Day 2-3 of menses to assess the endometrial lining (less than 6mm), ensure no presence of polyps, or other abnormalities and to look for presence of ovarian follicles or cyst. If patient's condition is optimum, patient will be started with estradiol valerate 8mg daily. Scan will be repeated again at Day 7-8 from the day of initial scan to look for endometrial thickness and appearance; if thickness ≥ 8mm, with trilaminar appearance, luteal phase support will be started and patient is planned for FET. If the endometrial thickness is inadequate, dose of estradiol valerate will be increased or to co-administer estradiol gel 0.06% and to repeat scan in another 2-3 days until optimum endometrial thickness is achieved. Throughout the FET cycle assessment, the cycle will be abandoned in the presence of endometrial polyps, hydrometra, or inadequate thickness despite adjustments of dose/route.

Luteal phase support consists of oral dydrogesterone 10mg TDS and vaginal micronized progesterone 200mg TDS for 5 days before the frozen embryo transfer date. For the purpose of this study, with the assumption that sexual intercourse takes place at night, micronized progesterone will be advised to be inserted per rectal for the night dose.

iii) Embryo preparation

The embryo was frozen using vitrification method (Irvine Scientific, USA) at cleavage (Day 2 – Day 3) or blastocyst stage (Day 5). The embryo is placed in the vitrification solution for 8-10 minutes according to the embryo developmental stage. On the day of frozen embryo transfer, the embryo is devitrified using the vitrification media thaw kit at least for 13 minutes and survival of the cell is assessed. The thawed embryo will be prepared for transfer.

iv) Follow up

Patient will follow a routine follow up course in UMMC: BHCG tracking after 2 weeks of embryo transfer and an ultrasound scan at 10 weeks after embryo transfer. The serum beta sub-unit of human chorionic gonadotrophin (BHCG) levels were examined using chemiluminescence analysis (Atellica IM 1300 Analyzer, SIEMENS). A biochemical pregnancy was defined as a serum quantitative B-HCG of ≥ 50IU/L.

Patient will be followed up via phone calls throughout the pregnancy until the delivery of baby.

6. SAMPLE SIZE

The sample size calculation was done based on the overall clinical pregnancy rate at Fertility clinic UMMC which is 40%. According to the ratio 1:1, risk ratio of 1.5, with a 10% drop out rate, a minimum sample size of 110 are expected per group.

7. DATA ANALYSIS

The primary data analysis will be based on an intention to treat, even if couples do not comply to their study allocation. Two sample t tests (two tailed) will be used to test for equality of the means of continuous variables. Chi square test and confidence interval (CI) calculations are used to test the equality of categorical variables. Statistical analysis will be entered into SPSS (Version 27, IBM, SPSS Statistics).

8. RESEARCH DATA

Where will the data be kept?
Data collection will include demographic information, fertility treatment details, exposure data (occurrence of sexual activity), and outcome data (pregnancy test results, implantation rates, viable pregnancy).
Digital entry of data collection will be done using a password protected Excel sheet and data management software (SPSS software). Each participant will be anonymised with a unique ID in the main dataset with no identifiable details. It will be a restricted access storage whereby only authorized person (ie primary researcher) can view or edit the data.
Physical data such as consent forms, and sexual intercourse diary will be personally kept in a locked cabinet by the primary researcher.

Who will have access to the research data?
The primary researchers

How long will the data be kept?
At least for 7 years after thesis submission. After the retention period, physical documents will be destroyed by shredding and digital files will be deleted securely using file wiping software.

9. GANTT CHART

Year	2024	2025	2025	2026		
Activity/Months	Sep - Oct	April	June	June - Aug	Aug - Nov	
Research proposal and preparation						
Presentation to ethics committee and approval/ISRCTN						
Patient recruitment and data collection						
Data entry						
Data analysis						

Thesis writing					[redacted]	
Thesis submission					[redacted]	

10. POTENTIAL IMPACT

The diagnosis of being infertile is a powerful stress factor which affects couples' relationship and sexuality. The effort of producing an offspring via ART program can be emotionally burdening for the couple from invasive procedures and taxing processes. This study will hope to improve communication, promote intimacy, and express affection between couples. Apart from getting the female reproductive tract primed for implantation from early exposure of semen, sexual intercourse may reduce the heightened anxiety and distress thus producing a positive experience during embryo transfer. This study will also contribute to the current understanding of the role of seminal plasma in the female reproductive tract, and appropriate timing of exposure in priming it for implantation.

11. APPENDICES

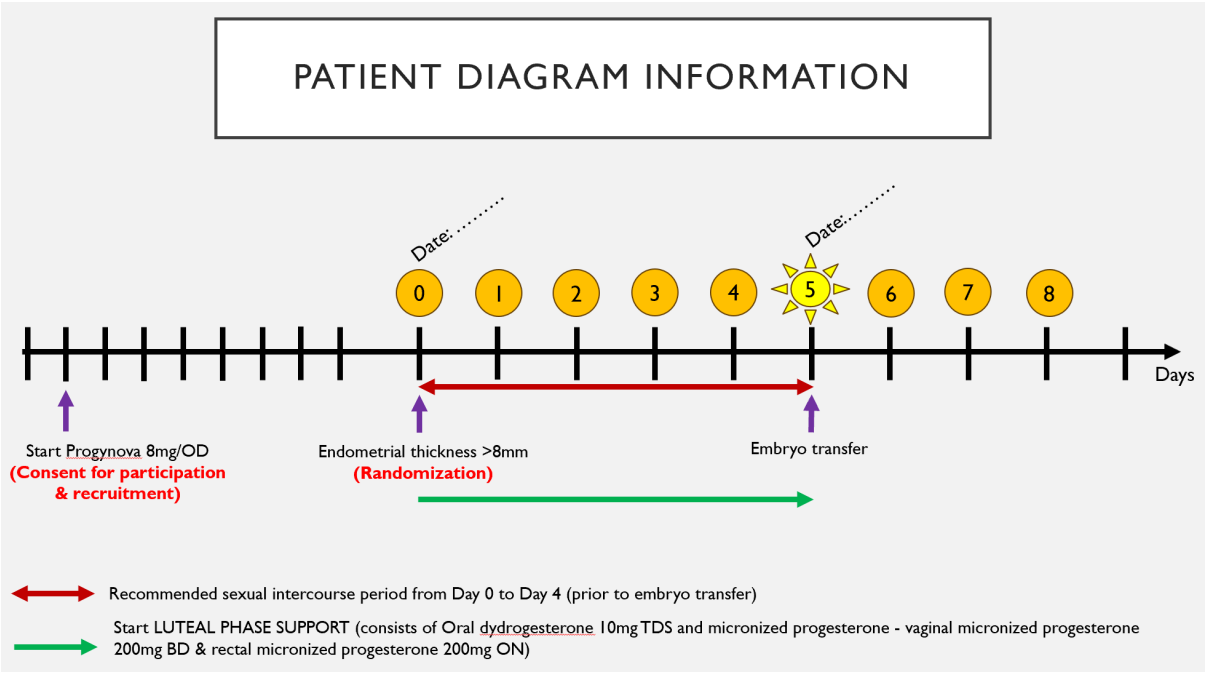
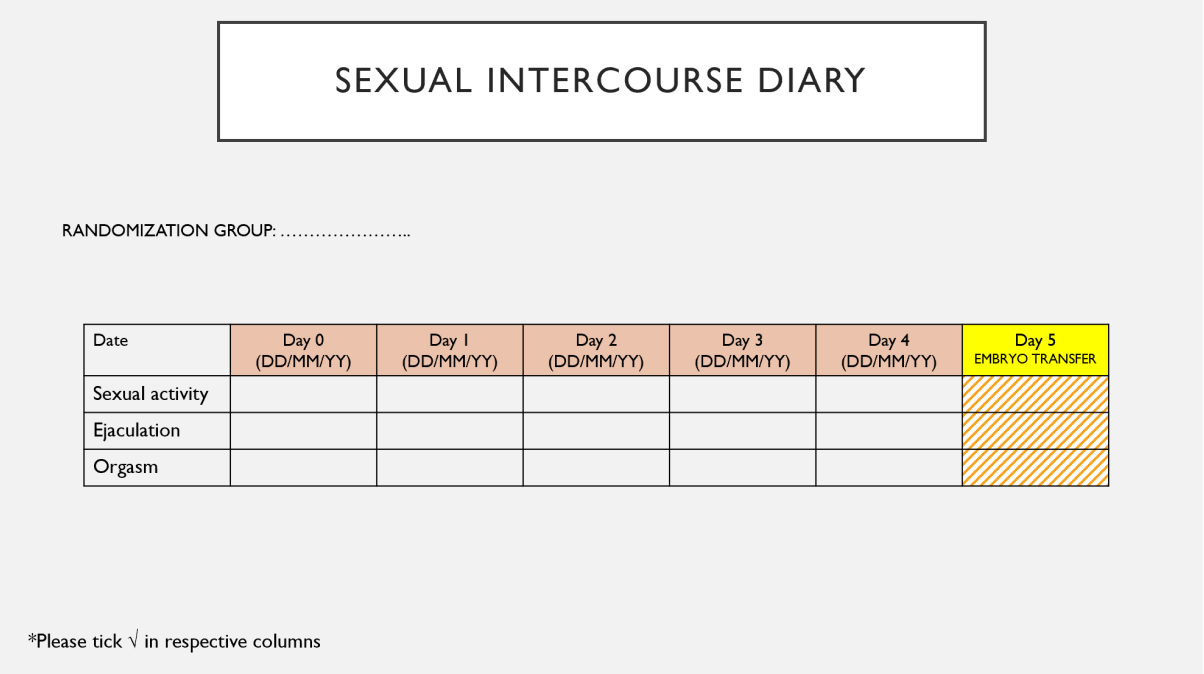


Figure 1: Recommended sexual intercourse 96 hours preceding embryo transfer



SEXUAL INTERCOURSE DIARY

RANDOMIZATION GROUP: ……..

Date	Day 0 (DD/MM/YY)	Day 1 (DD/MM/YY)	Day 2 (DD/MM/YY)	Day 3 (DD/MM/YY)	Day 4 (DD/MM/YY)	Day 5 EMBRYO TRANSFER
Sexual activity						
Ejaculation						
Orgasm						

*Please tick √ in respective columns

Figure 2: Sexual intercourse diary

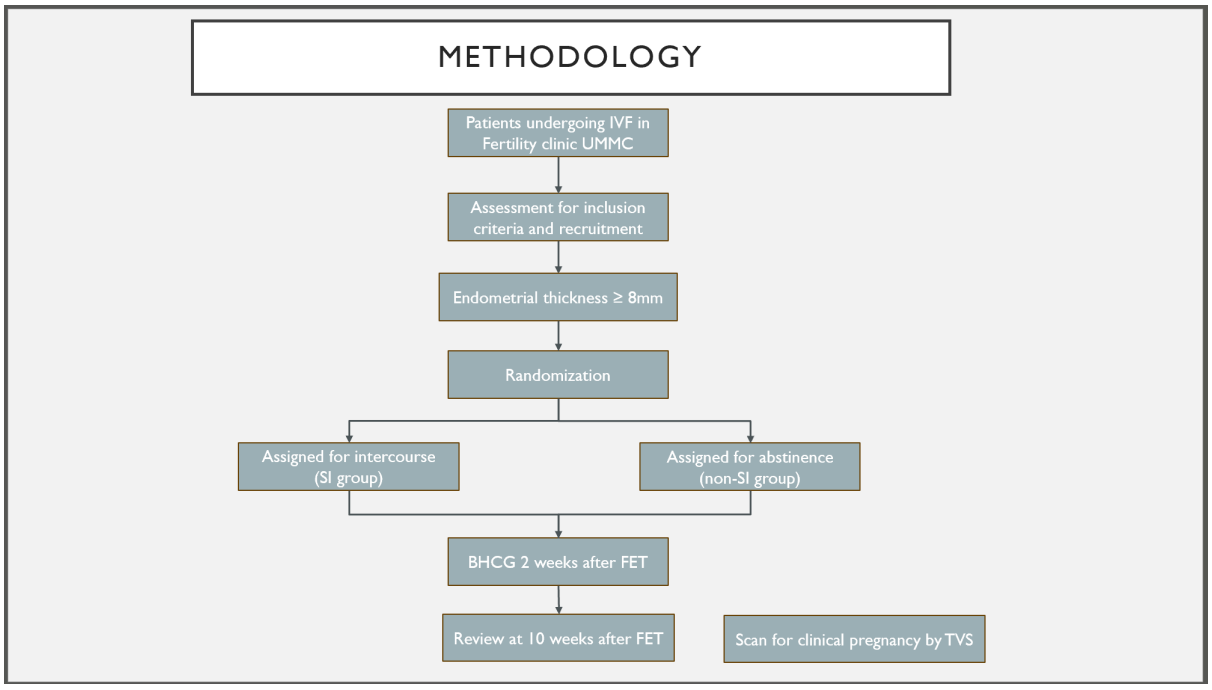


Figure 3: Study design

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Please state whether you have submitted this research proposal for funding, now or before

✓ Yes: If Yes, which grant? Department of O&G grant

This proposal will be kept strictly private and confidential. It will not be shared with anyone without your prior approval.

Name of Researcher (CAPITAL): ATIKAH BINTI MOHAMED HALIM

Signature of Researcher: 

Date: 5TH JUNE 2025