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Biomedical Research Protocol

Comparison of blood flow in the arteriae uterinae in ovarian stimulation cycles for IVF / ICSI, in hormonal replacement cycles and Natural cycles for Frozen Embryo Transfer

1901-ABU-002-BL

IVI Foundation

Project Title

Comparison of blood flow in the arteriae uterinae in ovarian stimulation cycles for IVF / ICSI, in hormonal replacement cycles and Natural cycles for Frozen Embryo Transfer

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Responsibilities and Signatures

By signing this protocol from the project entitled:
Those undersigning state that:

- This study respects the ethical and legal rules and follows good clinical practice in its implementation
- It has the material and human resources needed to carry out the study, without interfering in other studies or clinical tasks usually entrusted to them
- They are committed that each subject is treated and controlled according the approval granted by the Ethics Committee for Clinical Research, Institutional Review Board, remaining committees and the involved authorities
- Collaborators included in this study are adequately trained for its implementation, they will have an active participation, and they consent thereto.

Center and/or Laboratory Director

Prof. Dr. Human Fatemi
IVI RMA Abu Dhabi Director

Date

Principal Investigator

Dr. Barbara Lawrenz
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Date

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Abstract

To evaluate the influence of ovarian stimulation for IVF / ICSI on the blood flow in the arteriae uterinae as well as whether there is an influence of the type of endometrial preparation for frozen embryo transfer (FET) with either hormonal replacement therapy or natural cycle on the blood flow of the arteria uterina left / right. Blood flow in the arteriae uterinae will be measured by Doppler ultrasound and calculating the pulsatility index (PI) and resistance index (RI).

Until now, it is not clear, whether hormonal stimulation for IVF / ICSI and administration of estradiol tablets in the context of preparing the endometrium for embryo transfer will influence the blood flow in the aa. uterinae at all. Until now, the optimal means of endometrial preparation for "Frozen Embryo Transfer" cycle is not clear and study results are contradictory. It is known, that there are remarkable differences in the endocrinological milieu between natural cycles and hormonal replacement cycles.

This study will measure the blood flow in the aa. uterinae in women, undergoing firstly ovarian stimulation for IVF / ICSI and later either HRT or NC for FET.

List of acronyms

ICSI	Intracytoplasmic sperm injection
IVF	In vitro fertilization
A.	Arteria
aa.	arteriae
PI	Pulsatility Index
RI	Resistance Index
FET	Frozen Embryo Transfer
NC	Natural Cycle
HRT	Hormonal Replacement Cycle
GnRH	Gonadotropin-Releasing-Hormone

AIM: main objective/research question

The primary aim of this observational prospective study is to evaluate, whether there is a difference in the blood flow, calculated as PI and RI in the arteriae uterinae right / left between women, undergoing HRT- or NC-cycle for FET. Measurements on the day of cycle start and the day of progesterone rise in NC-cycles / start of progesterone administration in HRT-cycles (+/- 1 - 2 days) will be performed.

Secondary Objectives/research questions

- To evaluate differences (delta) between the mean PI (between right and left A. uterine) and mean RI (between right and left A. uterine) on the day of cycle start and the day of ovulation induction - 1 - 2 days
- To compare thickness of the lining between HRT or NC.
- To compare number of days of estradiol exposure before the embryo transfer between HRT or NC.

Introduction

A. Background:

A wide spectrum of different factors, including uterine receptivity and endometrial thickness (Abdalla et al, 1994; Noyes et al, 1995) and endometrial texture (Bakos et al, 1993), have been implicated as crucial factors in implanation. It has also been suggested that uterine receptivity is related to the uterine blood flow. Due to the availability of ultrasound Doppler measurements, the uterine blood flow can be assessed non-invasively by transvaginal Doppler ultrasonography.

It is also known, that there are remarkable differences in the endocrinological milieu between natural cycles, ovarian stimulation cycles for IVF / ICSI and hormonal replacement cycles. Those differences may lead to different flow pattern in the vessels, providing blood supply to the uterus.

Until now, there are no data whether there is a difference in the blood flow of the aa. Uterinae .

B. Justification:

Until now, the changes in the blood flow of the aa. uterinae during hormonal stimulation for IVF / ICSI as well as during the preparation for FET are unclear. Additionally, the “best” approach to prepare the endometrium and further on to perform a frozen embryo transfer is still not clear and the data are contradictive. This study will evaluate whether there is any change in blood flow during hormonal stimulation for IVF/ICSI and during different types of endometrial preparation for FET. In case this study would show that the blood flow of the arteria uterina right and left is influenced negatively by one of the approaches, used for endometrial preparation of the FET, then this should be taken into account in future when deciding for one or the other approach.

Methodology

A. Study Design

- Prospective cohort study, observational and unicentric (in IVIRMA Middle East Fertility Clinic, Abu Dhabi, UAE).

B. Study period and context

- Patients who are treated for primary / secondary infertility in IVIRMA Middle East Fertility Clinic, Abu Dhabi, UAE
- Estimated study duration: 6 - 8 month
- Study would start after approval of the study protocol by EC of IVIRMA Middle East Fertility Clinic Abu Dhabi.
- Study would end after inclusion of the number of patients, estimated due to the sample size calculation

C. Reference Population

Pat. with primary / secondary infertility, who undergo an ovarian stimulation treatment for IVF/ICSI and subsequently are planned for FET with vitrified embryos, either as HRT-FET or as NC-FET

D. Subject Inclusion/exclusion criteria

Inclusion criteria:

- Patients who undergo ovarian stimulation in a GnRH-antagonist protocol for IVF / ICSI
- Patients who have vitrified embryo(s)
- Preparation for FET either in HRT or NC cycle

Exclusion criteria:

- Poor responder according to Bologna criteria (Ferraretti et al.) as follows:
- At least two of the following three features must be present:
    - -(i) Advanced maternal age (≥40 years) or any other risk factor for POR;
    - -(ii) A previous POR (≤3 oocytes with a conventional stimulation protocol);
    - -(iii) An abnormal ovarian reserve test (i.e. AFC, 5–7 follicles or AMH, 0.5 –1.1 ng/ml).
- Uterine surgery for removal of fibroids (hysteroscopic, laparoscopic) or removal of uterine septum
- Endometriosis
- Asherman-Syndrome
- Previous cytotoxic treatment
- Previous radiation of the uterus / adnexal region
- Known hypertension
- Intake of Aspirin or similar medication which might influence the blood flow
- Status after tubal ligation
- Status after surgery in the adnexal region on 1 side

E. Intervention and Follow-up

- Patient will present themselves on day 2 / 3 of their period.

- Patients will undergo ovarian stimulation for IVF / ICSI in a GnRH-antagonist protocol: Start of stimulation medication (HMG, recFSH) on either day 2 or 3 of the cycle, dosage according to the ovarian response. From day 5 of stimulation a GnRH-antagonist will be added to prevent premature ovulation. Medication for ovulation induction (hCG, GnRH-agonist or dual trigger) will be given when at least 3 follicles are ≥ 17 mm.
- Patients will be planned for FET in either HRT or NC, according to the physician´s decision.
- Factors to recommend HRT FET: irregular cycle, PCO, unavailability for treatment monitoring
- Factors to recommend NC FET: regular cycle 28 -33 days, availability for treatment monitoring

Endometrial preparation approaches:

Artificial (HRT) Cycles:

- Commence E2 4mg from day 2/3 of period for 3 days
- Increase E2 to 6mg on day 4 of E2 treatment. E2 dose may be increased according to clinician discretion based on endometrial thickness.
- The patient will be scanned transvaginally throughout the HRT cycle to not only monitor endometrial development but to also exclude the presence of a dominant follicle on the ovaries.
- In conjunction with ultrasound monitoring the patient will undergo serial measurements of serum LH, estradiol and progesterone levels.
- Commence the initial progesterone dose of 100mg at 22hrs (vaginal suppository) after ≥ 10 days and ≤ 16 days of estradiol administration when the minimal endometrial thickness achieved is 6mm with a trilaminar appearance.
- Subsequently increase progesterone administration to 100mg vaginally three times daily. Continue estradiol administration 6mg (3 tablets daily). Embryo transfer is scheduled 5 days following the initial initiation of progesterone

Spontaneous natural cycles:

- Commencing on the second day of menses and intermittently throughout the patients' natural cycle ultrasound scans will be performed to monitor follicular growth.
- In conjunction with ultrasound monitoring the patient will u/7ndergo serial measurements of serum LH, estradiol and progesterone levels to accurately determine the timing of ovulation. These serum hormonal levels will be measured with an automated Elecsys® immunoanalyzer (Roche Diagnostics, Mannheim, Germany).
- The LH surge will be considered to have begun when the concentration rises by 180% above the most recent serum value and continues to rise thereafter (Irani et al. 2017, Fatemi et al., 2010).
- Day 1 after the LH rise, a decrease in estradiol concentration is identified. Twenty four hours later progesterone concentrations rise with a level of greater than or equal to 1.5nmol /L confirming ovulation (day 0) (Irani et al., 2017; Speroff et al.). This is considered as day 0 with initiation of vaginal progesterone 100mg (vaginal suppository) at 22pm that night. The following day (day 1) the patient increases progesterone administration to 100mg vaginally three times daily (8 hourly) and continues this regime until 7 weeks gestation as per clinic protocol. Embryo transfer is scheduled 5 days (day 5) following confirmation of ovulation (day 0).

For all study arms, transvaginal scan will be performed after the patient had emptied the bladder and is positioned in lithotomic position.

Measurement of the Doppler in the aa. uterinae:

A sagittal section of the uterus will be obtained and the cervical canal and internal cervical os will be identified. The transducer will be gently tilted from side to side and color flow mapping will be used to identify each uterine artery along the side of the cervix and uterus at the level of the internal os. Pulsed wave Doppler will be used with the sampling gate set at 2 mm to cover the whole vessel and care will be taken to ensure that the angle of insonation is less than 30º. When three similar consecutive waveforms are obtained, the PI and peak systolic velocity (PSV) will be measured and the mean PI of the left and right arteries calculated.

Images of the blood flow measurements will be taken and stored in an access-restricted folder. After completion of the study recruitment and data analysis, the images will be destroyed.

Timing for measurement of the Doppler in the aa. Uterinae will be performed:

- Ovarian stimulation cycle: day 2 or 3 of the cycle; day of ovulation induction or 1 to 2 days before in case patient will not be seen directly on the day of ovulation induction
- FET cycle:
- HRT: day 2 or 3 of the cycle and day of start of progesterone administration or 1 to 2 days before in case patient will not be seen directly on the day when she starts progesterone
- NC: day 2 or 3 of the cycle and day of start of progesterone rise or 1 to 2 days before in case patient will not be seen directly on the day when progesterone starts to rise

F. Collection of biological samples and information

No biological samples will be collected for this study

Statistical methodology

A. Data base

The database will be rigorously defined with the variables destined to be analyzed according to the objectives set. The necessary information will be exported from the clinical information manager, SIVIS, to a table in Excel format through a database-based query system. The data which is not recorded in SIVIS will be collected in a password-coded EXCEL file to guarantee the data protection.

The exported data will be duly codified in order to protect the clinical and personal information of the patients according to the applicable law in the place where the research project is carried out.

Finally, and prior to the statistical study, an exploratory data analysis will be carried out to review the quality of the information extracted.

B. Study Variables

EXPOSURE VARIABLES (INDEPENDENT)

- Type of endometrial stimulation. Categorical dichotomous variable (HRT or natural cycle)

MAIN-OUTCOME MEASURE (DEPENDENT)

- Difference in the blood flow, calculated as PI and RI, between the HRT- and the NC-FET cycles, on the day of progesterone administration / progesterone rise. Quantitative continuous variable

SECONDARY OUTCOME MEASURES (DEPENDENT)

- δ_{S11} : Continuous quantitative variable measured as the differences between average PI value on the day of cycle start and the day of ovulation induction - 1 - 2 days.
- δ_{S12}: Continuous quantitative variable measured as the differences between average RI value on the day of cycle start and the day of ovulation induction - 1 - 2 days.
- Thickness of the lining. Continuous quantitative variable measured in millimeters (mm).
- Number of days of estradiol exposure before the embryo transfer. Discrete quantitative variable.

CONTROL VARIABLES

- Cycles days before start of progesterone administration.
- In HRT cycles: amount (mg) of administered Estradiol.
- Age of the patient
- BMI of the patient
- Number of previous pregnancies
- Number of previous C-sections
- Number of previous miscarriages
- Smoker status: Smoking yes/no

C. Sample Size

So far, no study has been published evaluating the PI and RI of the arteria uterina between HRT- and NC-FETs.

The study of *Tekay et al.* compared the PI of the arteria uterina in a total of 57 patients in fresh ET´s after ovarian stimulation and FET with HRT-preparation of the endometrium. However, this article does not study all the variables of our interest. Besides, it has low statistical power and our populations are different, so we consider that it is more convenient to carry out a pilot study to study descriptively the variables of interest in our population.

As this will be a pilot study, we plan to include 30 patients in each arm (HRT / NC), therefore we would estimate that we will have to include approximately 80 patients in total, as at the time of ovarian stimulation it will not be clear which type of FET patient will undergo later on. This approach was chosen in order not to interrupt patient recruitment. As this is expected to be a 2 : 1 recruitment (HRT : NC), an intra-group homogeneity analysis will be performed and if there are differences, patients from this group will be randomly selected for statistical analysis. Besides, in anticipation of potential participants wishing to withdraw from the study, an additional 5% of patients to the total sample will be added to cover possible losses, recruiting a total of 84 participants.

D. Statistical data analysis

EXPLORATORY DATA ANALYSIS

A statistical summary of the data collected in the study describing patient's population, cycle's most relevant parameters together with clinical results will be provided.

- Categorical variables will be presented in frequency tables, providing absolute frequencies and proportion of each level of each factor in the sample.
- Continuous variables will be summarized according the mean, standard deviation and 95%-confidence interval, all of them will be represented by diagrams or densities quartiles.

Exploratory data analysis will allow evaluating data quality and detecting anomalies.

HOMOGENEITY ANALYSIS

Although the inclusion criteria and study design tend to preserve the homogeneity of study, it is important to ensure comparability between groups for each control variable. Since each patient is assigned to a group according to clinical criteria and not by random assignment between groups, it will be important to perform a homogeneity analysis to ensure that the effect studied may be due to the type of endometrial preparation and not to the effect that may be caused by a control variable behaving differently between the two study groups.

Consequently, categorical variables will be compared using the χ^2 test, and for quantitative variables, a t-Student test will be applied (if we can assume normality, if it is not possible, we will use the Kruskal-Wallis test).

OBJECTIVE ASSESSMENT

To assess the primary objective, and considering the descriptive nature of any pilot study, confidence intervals of 90%, 95% and 99% will be provided for the blood flow level in each group, and for the difference

between the comparison groups. A basic comparison test will also be provided on an indicative basis. In order to do this, and considering the size of the sample, we will first apply tests of the normality of the data. If these tests are favourable, a t-Student test will be applied. In case of not being able to assume normality, we will apply a non-parametric test.

We will proceed in the same way for the rest of the secondary objectives.

Work plan

Phase I: drafting of protocol (Barbara Lawrenz, Laura Melado, Desislava Markova) and review / approval through UAGI and EC approval

Phase II: Patient recruitment and follow-up (Barbara Lawrenz, Laura Melado)

Phase III: Data analysis (Barbara Lawrenz, designated statistician from UAGI)

Phase IV: Drafting and reviewing of manuscript, submission and publication (Barbara Lawrenz, Laura Melado, Desislava Markova, Carol Coughlan, Human Fatemi)

	2019											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Phase I												
Phase II												
Phase III												
Phase IV												
Phase V												

	2020											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Phase I												
Phase II												
Phase III												
Phase IV												
Phase V												

Ethical issues

This Research Project respects the fundamental principles of the Declaration of Helsinki, the Council of Europe Convention on Human Rights and Biomedicine, the UNESCO Universal Declaration on the Human Genome and Human Rights, as well as the requirements of Health Authority Abu Dhabi, UAE, for protection of personal data and bioethics.

For the inclusion of all patients in the study, it is needed signed informed consent, which is approved by the Ethics Committee from the research centres

Funding

No funding needed

Needs funding	No		
Externally Funded project	No	Funding Institution	Haga clic aquí para escribir texto.
External Funding plan (if no funding is available yet)	No funding needed		
IVIRMA Funding requested	Choose an item.	Internally Funded project	
Funding time range			
Budget			

Insurance

IVIRMA Abu Dhabi has an Insurance Policy in force that conforms to current legislation and with coverage to compensate and indemnify cases of ill health or injury, which may arise in connection with their participation in the study, within routine clinical practice.

However for this observational study, no insurance is needed

Publication and diffusion

- Ultrasound in Obstetrics and Gynecology, IF 5.6
- Fertility and Sterility, IF 4.8
- ESHRE
- ASRM

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