Study Protocol – FIBS

Study Title: Fertility treatment: Impact on symptoms of Irritable Bowel syndrome

Study Acronym: FIBS

Protocol Version and Date: 3rd version, 03/02/2026

Sponsor: VUB

Principal Investigator: Prof. Dr. Sébastien Kindt

Study Start Date: 1 December 2025

Expected Study End Date*: 31 July 2028

PROTOCOL SIGNATURE PAGE

Protocol version and date: Version 3, 03/02/2026

Protocol title: Fertility treatment: Impact on symptoms of Irritable Bowel Syndrome (FIBS)

Sponsor: VUB

Principal Investigator: Prof. Dr. Kindt

I agree:

- to assume responsibility for the proper conduct of this study
- to conduct the study in compliance with this protocol
- not to implement any deviations from or changes to the protocol without prior review and written approval from the Ethics Committee, or for administrative aspects of the study (where permitted by all applicable regulatory requirements)
- to ensure that all persons assisting me with the study are adequately informed about their study-related duties and functions as described in the protocol
- that I am aware of and will comply with the current good clinical practice (GCP) guidelines and ethical principles outlined in the Declaration of Helsinki
- to conduct the study in accordance with all applicable laws and regulations

Prof. dr. S. Kindt Signature Date

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1 Sponsor/Coordinating Investigator Information

Sponsor:	**VUB**
Principal Investigator:	**Prof. Dr. Sébastien Kindt**
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Study site(s) :	**BIVF , UZ Brussel**

2 List of Abbreviations

IBS	Irritable Bowel Syndrome
IBS-SSS	Irritable Bowel Syndrome- Symptom Severity Scale
COC	Cumulus-oocyte complex
COS	Controlled ovarian stimulation
GAD-7	Generalized Anxiety Disorder 7-item
GI	Gastrointestinal
GPA	Gravida, para and abortus
GSRS	Gastrointestinal Symptom Rating Scale
ICF	Informed Consent Form
IVF	In Vitro Fertilization
PCOS	Polycystic Ovarian Syndrome
PHQ-9	Patient Health Questionnaire 9
PPOS	Progestin-Primed Ovarian Stimulation
US	Ultrasound
VUB	Vrije Universiteit Brussel

3 Protocol Version History

Version N°	Version Date	Summary of changes
1	01/10/2025	
2	10/11/2025	Response to questions EC
3	03/02/2026	Response to questions EC

4 Trial Registration/Protocol Summary

Information	
Objectives:	The objective is to study IBS (Irritable bowel syndrome) symptoms in women who undergo controlled ovarian stimulation and oocyte retrieval, which are the cornerstones of fertility treatment. Two groups will be studied: women with PCOS (Polycystic Ovarian syndrome) and women who undergo elective oocyte cryopreservation (social freezing).
Study population:	Nulliparous women with a diagnosis of PCOS who are eligible for IVF (in vitro fertilization) treatment with freeze-only strategy (i.e. deferred embryo transfer). Nulliparous women who embark on elective oocyte

	cryopreservation. Both groups will receive PPOS (progestin-primed ovarian stimulation).
In- and exclusion criteria:	Inclusion criteria **PCOS** group : - Diagnosis of PCOS according to the 2004 Rotterdam criteria - Initiating their first cycle of IVF treatment (PPOS and freeze only protocol) - Nulliparous - ≥ 18 years - Willing to participate in the study - Understanding Dutch, French or English Exclusion criteria **PCOS** group : - Known inflammatory bowel disorder - Known major intestinal bowel disorder - Known systemic or auto-immune disorder with implication for the GI system - History of abdominal surgery (appendectomy and cholecystectomy allowed if >6 months earlier) - History of gastro-enteritis in the past 6 weeks - Previous IVF treatment cycles Inclusion criteria **social freezing** group: - Initiating their first ovarian stimulation protocol for elective oocyte cryopreservation (PPOS and freeze only protocol) - Nulliparous - ≥ 18 years - Willing to participate in the study - Understanding Dutch, French or English Exclusion criteria **social freezing** group: - Diagnosis of PCOS - Known inflammatory bowel disorder - Known major intestinal bowel disorder - Known systemic or auto-immune disorder with implication for the GI system - History of abdominal surgery (appendectomy and cholecystectomy allowed if >6 months earlier) - History of gastro-enteritis in the past 6 weeks
Factors of interest / Data to be collected:	IBS diagnosis and symptom severity Gastrointestinal symptom severity Depression levels Anxiety levels Factors to be collected **before** ovarian stimulation: • Demographics (length, weight) • The duration of infertility • Obstetrical history: GPA (Gravida, Para and abortus) • The ethnicity • For the study group: the type of PCOS Factors to be collected **after** oocyte pick-up: • The hormonal profile at start and end of ovarian stimulation • The number of follicles on the last ultrasound before pickup • The number of COC (Cumulus-oocyte complex) collected during pick-up

Endpoints:	The primary endpoint is to explore whether there is a difference between the IBS prevalence before and after ovarian stimulation. The score on the Rome Criteria for IBS before and after ovarian stimulation will be compared. The severity of IBS symptoms before and after the fertility treatment will be compared using the IBS-SSS (IBS- Symptom Severity Scale) questionnaire. The prevalence of gastrointestinal symptoms before and after the fertility treatment will be compared using the GSRS (Gastrointestinal symptom rating scale) questionnaire. The prevalence of anxiety levels before and after the fertility treatment will be compared using the GAD-7 (Generalized Anxiety Disorder 7-item) questionnaire. The depression levels before and after the fertility treatment will be compared using the PHQ-9 (Patient Health Questionnaire 9). The PCOS and social freezing group will each be divided in different subgroups based on the type of stimulation. The IBS prevalence (with the Rome Criteria) and the IBS symptom severity (with the IBS-SSS) will be compared in these subgroups before and after ovarian stimulation. The same will be done based on the number of treatments that were received before this treatment, the duration of -infertility, the number of previous pregnancies, the number of children, the ethnicity, the hormonal profile during COS (continuous ovarian stimulation). The PCOS group will be divided in different subgroups based on the type of PCOS. . The IBS prevalence (with the Rome Criteria) and the IBS symptom severity (with the IBS-SSS) will be compared in these subgroups before and after the fertility treatment.
Target sample size:	Since there is no study that has explored the prevalence of IBS during a fertility treatment, there is not enough data to do a power analysis before starting this study. This study will be an explorative study with a PCOS group of 60 and social freezing group of 60. After the data has been collected, a power analysis will be done and the option of including more participants will be explored. In this case, we will submit an amendment to the ethical committee. There are multiple studies that found a connection between IBS and infertility. Most of these studies are retrospective studies and are focused on male infertility. (1). A study in 2013 analyzed gastro-intestinal symptoms during IVF in infertile women. This study had 124 participants. (2) Since this study from 2013 is the most like our study, the aim for our target sample size is going to be similar, namely 120 participants.

5 Background and Rationale

5.1 Pathogenenis & diagnosis of PCOS

Polycystic Ovarian Syndrome (PCOS) affects the endocrine system resulting in excessive androgen levels and the metabolic system which can lead to, for example, hyperinsulinemia. The disease is linked to a higher risk of preeclampsia, endometrial cancer, gestational diabetes and preterm birth. (3) Obesity, anovulation, polycystic ovaries and infertility can be seen in PCOS patients. (4) Due to these risks, women with PCOS are more likely to have to receive a fertility treatment to get pregnant and to prevent losing the pregnancy prematurely. PCOS affects up to 10% of women

(prevalence changes based on the use of different criteria). (5) The Rotterdam criteria are mostly used for diagnosis. Other diagnoses should be excluded and two out of three criteria need to be met. The three criteria are irregular cycles (criteria 1), high antimüllarian hormone or polycystic ovarian morphology on transvaginal ultrasound (criteria 2) and/or biochemical or clinical hyperandrogenism (criteria 3). (6)

5.2 Types of PCOS

There are four types of PCOS. Phenotype A PCOS has ovulatory dysfunction, hyperandrogenism and polycystic ovarian morphology. Phenotype B PCOS has the same characteristics but without the polycystic ovarian morphology. These two phenotypes are considered the "classic PCOS". Phenotype C is characterized by hyperandrogenism and polycystic ovarian morphology. It is also called the ovulatory PCOS. Phenotype D PCOS is also called non hyperandrogenic PCOS, it is characterized by ovulatory dysfunction and polycystic ovarian morphology. (7)

5.3 Social freezing

The process of freezing your oocytes to preserve the ability to fertilize your own oocytes is called social (egg) freezing. The women receive ovarian stimulation (hormones) to grow as many follicles as possible before the pick-up of the oocytes. The oocytes are preserved using cryopreservation. The main reason for social freezing is the lack of an appropriate partner to start a family. (8)

5.4 Irritable bowel syndrome pathogenesis

The pathogenesis of irritable bowel syndrome has not been fully discovered. Patients experience gastro-intestinal motor problems and visceral hypersensitivity. IBS is associated with a modified gut microbiota which has an influence on immunity and the gut-brain axis. Genetic factors such as single nucleotide polymorphisms and epigenetic factors such as DNA methylation have been linked to IBS. (9) IBS patients are more likely to experience infertility. Most studies have focused on male infertility and IBS. A possible explanation for this link could be higher reactive oxygen species (ROS). However, more research is needed to explore this topic. (1) Serotonin dysregulation has been observed in patients with IBS. Patients with constipation showed a reduced level of serotonin concentrations and patients with diarrhea showed a higher level of serotonin concentrations. The serotonin concentration influences the secretion, sensation and motility of the gastrointestinal tract. More factors are possibly involved in the pathogenesis of IBS such as bacterial overgrowth, psychosocial factors, central dysregulation,… (10)

5.5 IBS and psychosocial factors

Patients with IBS have a higher risk of suffering from anxiety or depression, up to threefold higher than healthy subjects. (11) IBS symptoms exacerbate during stressful times. (12) A study investigated the stress levels during first and repeat IVF cycles and found higher stress levels in all IVF cycles. Women with lower stress levels had a higher pregnancy rate. Stress management can be beneficial for pregnancy rates and for IBS symptom severity. (13)

Studies about the relationship between depression and IBS are scarce and results are often inconsistent. Most studies find a higher rate of depression in IBS patients. The impact of depression on IBS symptom severity and prevalence hasn't been fully explored. Depression and IBS share a similar pathogenesis which makes it difficult to study these two diseases separately. Both IBS and depression show a dysfunction of the neuroendocrine systems and the immune system. (14)

5.6 IBS prevalence

The Rome Foundation Global study reported an overall prevalence of 3,8% when the Rome IV criteria are used and an overall prevalence of 10,1% when the Rome III criteria are used. (15) The Rome IV criteria question the prevalence of abdominal pain while the Rome III criteria additionally ask for abdominal discomfort (an uncomfortable sensation and not necessary pain). (16) Women have a significant higher prevalence of IBS than men, up to 3-fold higher. The risk of IBS doubles

when the person has a biological relative with IBS. The prevalence of IBS varies greatly between different countries. (17) PCOS patients are more prone to experience gastrointestinal (GI) disturbances such as irritable bowel syndrome (IBS): a recent meta-analysis showed that the risk of IBS in women with PCOS is two times higher than in a control group of women with no PCOS. (18) The prevalence of IBS during a fertility treatment has not been studied.

5.7 IBS subtypes

IBS has three subtypes. IBS-C has predominantly constipation, IBS-D has predominantly diarrhea and IBS-M is a mix of subtypes C and D. (19)

5.8 IBS symptoms during fertility treatments

Women can experience cramping, bloating and abdominal pain during the IVF cycles. After oocyte retrieval, diarrhea and constipation can occur. Most research about these symptoms have been focused on ovarian hyperstimulation syndrome (OHSS) and only few have tried to uncover how many women suffer from these symptoms outside of the OHSS context. (20)

This study will focus on the IBS symptoms experienced during fertility treatment. Some examples of drugs in fertility treatment include aromatase inhibitors, gonadotropin-releasing hormone (GnRH) and clomiphene. Aromatase inhibitors, for example letrozole inhibit the aromatization of androgen and thus reduces estrogen levels. This leads to increased GnRH release which results in more FSH production. (21) Aromatase inhibitors can cause headache, diarrhea and nausea. (22) GnRH-antagonists such as Cetrotide are used to prevent a premature rise of LH.(23) GnRH-agonists such as Decapeptyl are used for controlled ovarian hyperstimulation.(24) In a small study from 2015, severe dysmotility after taking GnRH analogs was linked to a high prevalence of endometriosis. Further research is needed, but it does hint at the possibility that patients with endometriosis have a higher chance of suffering from severe gastrointestinal symptoms. (25) Clomiphene citrate inhibits the negative feedback of estrogen to the pituitary and hypothalamus by binding estrogen receptors competitively. It stimulates the release of FSH which enhances follicle maturation. (26) Most common side effect of clomiphene are abdominal pain, diarrhea, anorexia and nausea. (27)

5.9 Rationale for this study

Different fertility drugs can cause nausea, diarrhea, constipation, abdominal pain,... It is unknown how many women suffer from these symptoms and how large the impact of these symptoms is during fertility treatment. The prevalence of IBS varies between 7,9 % to 49,3% in different studies, while the Rome Foundation Global study found a prevalence of 3,8%. (15, 28) The prevalence of IBS varies greatly between different subgroups. This study aims to give an indication of the prevalence and severity of gastrointestinal symptoms during fertility treatment. This knowledge can lead to better understanding and support during fertility treatment. Moreover, women undergoing fertility treatment will have an idea of what to expect when it comes to IBS-symptoms.

5.10 Rationale for study design

The role and significance of IBS symptoms in women who undergo fertility treatment has not yet been studied. Therefore, we designed this explorative study to get an overview of IBS symptoms during fertility treatment. There are many different subgroups of women undergoing a fertility treatment. This study chooses PCOS and social freezing. Women with PCOS are more at risk of developing gastrointestinal disturbances. (18) Elective oocyte cryopreservation is becoming more popular and this subgroup is a significant proportion of the women who come to the fertility clinic in UZ Brussels. Since it is predicted to become even more popular in the future, it would be helpful to explore the IBS symptoms during this treatment.

6 Study Objectives and Endpoints

6.1 Primary Objective

The primary objective of this study is to investigate whether fertility treatment is associated with changes in the **prevalence** and **severity** of Irritable Bowel Syndrome (IBS) symptoms. Specifically, the study aims to:

1. Assess whether the **proportion of participants meeting IBS diagnostic criteria** (based on ROME III and IV) differs **before and after fertility treatment**.
2. Evaluate whether there is a **statistically significant change in IBS symptom severity** as measured by the **IBS Symptom Severity Score (IBS-SSS)** before and after treatment.

6.1.1 Primary research hypotheses

H1.
The prevalence of participants meeting diagnostic criteria for Irritable Bowel Syndrome (IBS), as defined by the ROME III and ROME IV criteria, differs significantly before and after fertility treatment.

H2.
IBS symptom severity, as measured by the IBS Symptom Severity Score (IBS-SSS), changes significantly following fertility treatment compared with baseline.

6.2 Secondary Objectives

The secondary objective is to identify which **psychological and clinical factors** are associated with the presence or change in IBS symptoms in women undergoing fertility treatments, including those with **Polycystic Ovary Syndrome (PCOS)** and those undergoing **social oocyte freezing.**

The secondary objectives include:
- Changes in depression levels
- Changes in anxiety levels
- Differences in IBS symptoms based on the type of stimulation
- Differences in IBS symptoms based on the number of treatments received before this treatment
- Differences in IBS symptoms based on the duration of infertility
- Differences in IBS symptoms based on ethnicity
- Differences in IBS symptoms based on the type of PCOS
- Differences in IBS symptoms based on the hormonal profile during COS
- Differences in IBS symptoms based on the US reaction

6.2.1 Secondary research hypotheses

H3.
Changes in depressive symptom levels are significantly associated with changes in IBS symptom severity in women undergoing fertility treatment.

H4.
Changes in anxiety levels are significantly associated with changes in IBS symptom severity in women undergoing fertility treatment.

H5.
Changes in IBS symptoms differ significantly according to the type of ovarian stimulation used during fertility treatment.

H6.
Changes in IBS symptoms differ significantly based on the number of fertility treatments received prior to the current treatment.

H7.
Changes in IBS symptoms differ significantly according to the duration of infertility.

H8.
The prevalence and/or change in IBS symptoms differ significantly across ethnic groups.

H9.
Among women with Polycystic Ovary Syndrome (PCOS), changes in IBS symptoms differ significantly according to PCOS phenotype.

H10.
Changes in IBS symptoms are significantly associated with the hormonal profile during controlled ovarian stimulation (COS).

H11.
Changes in IBS symptoms differ significantly according to the ovarian response assessed by ultrasound during COS.

6.3 Endpoints

The IBS prevalence before and after the fertility treatment will be compared using the Rome Criteria for IBS. The severity of IBS symptoms before and after the fertility treatment will be compared using the IBS-SSS questionnaire. The difference in gastrointestinal symptoms before and after the fertility treatment will be compared using the GSRS questionnaire. The prevalence of anxiety levels before and after the fertility treatment will be compared using the GAD-7 questionnaire. The depression levels before and after the fertility treatment will be compared using the PHQ-9.

The PCOS and social freezing group will each be divided in different subgroups based on the type of stimulation. The IBS prevalence (with the Rome Criteria) and the IBS symptom severity (with the IBS-SSS) will be compared in these subgroups before and after the fertility treatment. The same will be done based the duration of infertility, the ethnicity, the hormonal profile during COS and the US reaction. The PCOS group will be divided in different subgroups based on the type of PCOS. . The IBS prevalence (with the Rome Criteria) and the IBS symptom severity (with the IBS-SSS) will be compared in these subgroups before and after the fertility treatment.

7 Study Design

7.1 Study Design

This study is an explorative non-interventional prospective study. Questionnaires will be used as form of intervention. The intervention will take place before and after hormonal stimulation (on the day of pick-up). The aim of this study is to get a view on the prevalence of IBS symptoms in the PCOS and social freezing group.

7.2 Date Range for collected study data

The first inclusions will be done in December 2025 and the last inclusions will be in July 2026. This means that study data will be collected till February 2027.

8 Study Population

8.1 Population of interest

Women with PCOS who are nulliparous and initiate IVF treatment.
Woman who are nulliparous and initiate ovarian stimulation for social freezing.
Both groups will receive PPOS (progestin-primed ovarian stimulation) and will get the freeze-all procedure after pick-up of oocytes.
Patients will be recruited at the fertility clinic of the UZ Brussels.

8.2 Inclusion & exclusion criteria

Inclusion criteria **PCOS** group :
- Diagnosis of PCOS according to the 2004 Rotterdam criteria
- Initiating their first cycle of IVF treatment (PPOS and freeze only protocol)
- Nulliparous
- ≥ 18 years
- Willing to participate in the study
- Understanding Dutch, French or English

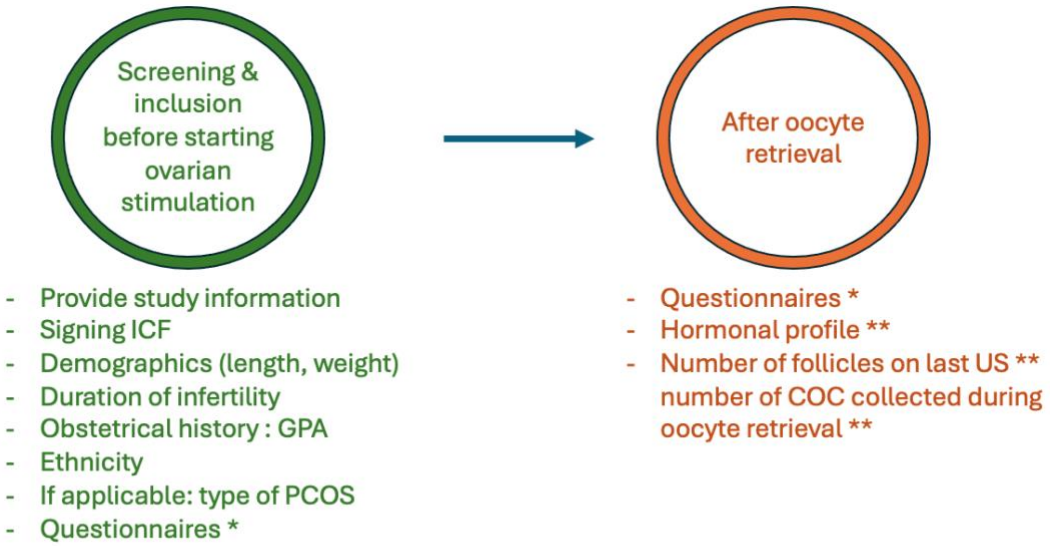
Exclusion criteria **PCOS** group :
- Known inflammatory bowel disorder
- Known major intestinal bowel disorder
- Known systemic or auto-immune disorder with implication for the GI system
- History of abdominal surgery (appendectomy and cholecystectomy allowed if >6 months earlier)
- History of gastro-enteritis in the past 6 weeks

Inclusion criteria **social freezing** group:
- Initiating their first ovarian stimulation protocol for elective oocyte cryopreservation (PPOS and freeze only protocol)
- Nulliparous
- ≥ 18 years
- Willing to participate in the study
- Understanding Dutch, French or English

Exclusion criteria **social freezing** group:
- Diagnosis of PCOS
- Known inflammatory bowel disorder
- Known major intestinal bowel disorder
- Known systemic or auto-immune disorder with implication for the GI system
- History of abdominal surgery (appendectomy and cholecystectomy allowed if >6 months earlier)
- History of gastro-enteritis in the past 6 weeks

9 Study Assessments and Procedures

9.1 Schedule of Activities



* IBS-SSS, Rome IV criteria, GSRS, PHQ-9, GAD-7
** part of standard care

9.2 Detailed Study Assessments

9.2.1 Screening

When a patient visits the fertility clinic and is considered a possible participant for the study, they will be informed about the study. Inclusion and exclusion criteria will be checked. The doctor will go over the informed consent form (ICF) and the patient will get enough time to ask questions. If the patient is eligible and wants to participate in the study, they will sign the ICF.

9.2.2 Visit before fertility treatment

Informed consent will be obtained. The IBS-SSS (IBS Symptom Severity Scale) (appendix 2) and Rome IV Criteria (appendix 1) are used to determine resp. IBS symptom severity and presence. The GSRS (appendix 3) is used to get a more general insight in gastrointestinal symptoms. The Patient Health Questionnaire-9 (PHQ-9) (appendix 5) will be used to determine the depression level of the patient. the Generalized anxiety Disorder 7-item (GAD-7) (appendix 4) examines the anxiety levels. These questionnaires will be available in three languages: Dutch, French and English. The questionnaires will be filled out on paper. In total, it will take about 15 minutes to fill out all the forms.

9.2.3 Visit after fertility treatment

After the fertility treatment (ovarian stimulation) the patient will come to the fertility clinic for pick-up of the oocytes. During this visit the following questionnaires will be filled-out: IBS-SSS (appendix 2) , Rome IV criteria (appendix 1) , GSRS (appendix 3) , PHQ-9 (appendix 5) and the GAD-7 (appendix 4) .

9.3 Assessment types

9.3.1 IBS symptoms

The IBS-SSS (IBS Symptom Severity Scale) (appendix 2) and Rome IV Criteria (appendix 1) are used to determine IBS symptoms. The result of the IBS-SSS is a score of 0 to 500. Each of the five topics is rated from 0 to 100. The topics are number of days with abdominal pain, satisfaction with bowel habits, abdominal pain, bloating/distention and IBS-related quality of life. A 15% change in the score

is considered clinically significant. (29) A score of 75-175 is considered mild, a score of 175 to 300 is considered moderate and a score of >300 is considered severe. (30) The Rome IV Criteria for IBS describe recurrent abdominal pain on average at least 1day per week in the last 3 months, associated with two or more of the following criteria: associated with a change in the frequency of stool, associated with a change in the form of stool and/or related to defecation. The criteria should be present for the last 3 months and symptoms should be present at least 6 months before the diagnosis is made. (16)

IBS prevalence differs greatly based on if the Rome IV or Rome III criteria are used. The Rome Foundation Global study reported an overall prevalence of 3,8% when the Rome IV criteria are used and an overall prevalence of 10,1% when the Rome III criteria are used. (15) The Rome IV criteria ask for abdominal pain while the Rome III criteria ask for abdominal discomfort (an uncomfortable sensation and not necessary pain). (16) Because of this big difference in prevalence, this study will first ask for abdominal pain (Rome IV criteria) and afterwards ask for abdominal discomfort (Rome III Criteria). This gives us a more complete picture of the IBS symptoms.

9.3.2 Gastrointestinal symptoms

The Gastrointestinal Symptom Rating Scale (appendix 3) contains 15 questions divided over five domains: abdominal pain, reflux syndrome, indigestion syndrome, constipation syndrome and diarrhea syndrome. Each question is scored from 1-7 depending on the inconvenience that the person experienced in the previous week. A higher score correlates with more inconvenient symptoms. (31) This questionnaire is used to get a more general overview of gastrointestinal symptoms.

9.3.3 Depression levels

The Patient Health Questionnaire-9 (PHQ-9) (appendix 5) will be used to determine the depression level of the patient. The questionnaire contains the nine DSM-V criteria for depression. Each question can be scored from “0” (not at all) to “3” (nearly every day). A score of “0-4” is considered minimal depression, “5-9” is mild depression, “10-14” is moderate depression, “15-19” is moderately severe and “20-27” is severe. (32)

9.3.4 Anxiety levels

Thirdly, the Generalized anxiety Disorder 7-item (GAD-7) (appendix 4) examines the anxiety levels. The questionnaire consists of seven questions with each time four response options (not at all = 0, several days = 1, more than half the days = 2 and nearly every day = 3). A total scores determine the severity of anxiety: 0-4 = minimal anxiety, 5-9= mild anxiety, 10-14 = moderate anxiety and 15 or more = severe anxiety. (33)

9.3.5 Results on the GAD7 and/or PHQ9 questionnaires

The GAD and PHQ-9 both are **screening tools** meant for preliminary assessment of anxiety and depression in patients. No specific training is required, and the questionnaires are meant for **self-administration** to the patient. Scoring is only **indicative** of possible underlying disorders, requiring a more comprehensive clinical assessment for confirmation. Therefore, for higher scores, the decision to refer for further specialised assessments relies on the clinical evaluation by the attending physician of the fertility clinic. The scoring will be performed by the student. Scores > 10 for the GAD and PHQ-9 will be communicated to the attending physician.

Both questionnaires will be scored according to the scoring sheet:

Scoring sheet:
GAD-7:
- 5-9: mild symptom severity
- 10-14: moderate
- > 15: severe

PHQ-9:
- 0-4: none
- 5-9: mild depression
- 10-14: moderate depression
- 15-19: moderate to severe depression
- 20-27: severe depression

10 Data Collection and Management

10.1 Monitoring

The investigator must make all trial documentation and related records available in case a monitoring visit or audit by the Sponsor is requested. Also in case of regulatory inspections all trial documentation should be made available to the inspector(s). All participant data must be handled and treated confidentially.
The Sponsor's monitoring frequency will be determined prior to the start of the trial. A monitoring plan will be generated detailing the frequency and scope of monitoring for the trial. Throughout the course of the trial the monitoring plan can be adjusted as necessary.

10.2 Data Collection

All questionnaires will initially be collected on paper. Each paper questionnaire will be assigned a unique study code and will not contain any identifiable participant information. The linkage between study codes and participant identities will be securely stored and accessible only to the study investigators. Completed paper questionnaires will subsequently be entered into the REDCap system in a timely manner.

An Electronic Data Capture system "***REDCap***" will be used for data collection. The system is validated and access to all levels will be granted/revoked by the Sponsor representative. Trial data should be entered within reasonable time after the subject attended the visit. Corrections/modifications will be automatically tracked by an audit trail detailing date and time of the correction and the name of person performing the correction.

10.3 Data collection from patient file

The patient file will be utilized to collect specific data for the purpose of this study. The following information will be gathered: age, the type of medication administered for ovarian stimulation, the hormonal profile, the number of follicles observed on the final ultrasound, and the number of oocytes retrieved during oocyte pick-up (OPU). This data will be obtained post-OPU.

10.4 Database Management and Quality Control

The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the eCRF and in all required reports.
The investigator must keep source documents for each subject in the study. All information on the eCRF must be traceable to these source documents, which are generally stored in the subject's medical file. The source documents should contain all demographic and medical information, including laboratory data, ultrasound data, etc., and the original signed informed consent forms. Data reported on the eCRF that derive from source documents should be consistent with the source documents or the discrepancies should be explained.

The investigator and the sponsor should maintain the study documents as specified in the "Essential Documents for the Conduct of a Clinical Trial" chapter 8 of ICH-GCP and as required by the applicable regulatory requirement(s).
These are documents which individually and collectively permit evaluation of a study and the quality of the data produced and include groups of documents, generated before the study

commences, during the clinical study, and after termination of the study and include but are not limited to: study protocol, amendments, submission and approval of EC, raw data of subjects including lab tests, insurance contracts, signed informed consent forms, confidential subjects identification code, eCRF, curricula vitae of the investigator and other participants in the study, study staff lists and responsibilities, monitoring reports and final study report.
The investigator and the sponsor should take measures to prevent accidental or premature destruction of these documents.
The investigator and the sponsor must retain study documents as long as needed to comply with ICH-GCP, national and international regulations. By signing the protocol, the investigator and the sponsor agree to adhere to these requirements.

10.5 Statistical Considerations and Data Analysis

The primary objective of this study is to investigate whether fertility treatment is associated with changes in the **prevalence** and **severity** of Irritable Bowel Syndrome (IBS) symptoms. Specifically, the study aims to:

3. Assess whether the **proportion of participants meeting IBS diagnostic criteria** (based on ROME III and IV) differs **before and after fertility treatment**.
4. Evaluate whether there is a **statistically significant change in IBS symptom severity** as measured by the **IBS Symptom Severity Score (IBS-SSS)** before and after treatment.
5. Identify which **psychological and clinical factors** are associated with the presence or change in IBS symptoms in women undergoing fertility treatments, including those with **Polycystic Ovary Syndrome (PCOS)** and those undergoing **social oocyte freezing**.

Participants will either meet the criteria for IBS or not meet the criteria for IBS (based on the ROME III and IV criteria). This will correlate to a binary outcome: yes (1) or no (0) answer. This answer will be defined before and after the fertility treatment. A logistic regression model will be used to determine the chance of P(1) which is the chance of meeting the IBS criteria (yes answer). This regression model will be used for the PCOS group and the social freezing group. This model estimates the probability of an event occurring (meeting the IBS criteria) based on a given set of independent variables . The independent variables include GAD-7 score, PHQ-9 score, ethnicity, duration of the desire to have children, the number of follicles found on the US,…

These independent variables will be evaluated with the AIC (Akaike information criterion). AIC is used to compare different possible regression models and define which model is the best fit for the given data. If the AIC is lower, the data is a better fit. The regression model will be made with different independent variables and each time the AIC will be calculated. The model with the lowest AIC will be the final model.

Participants will fill in the IBS-SSS and obtain a score between 0 and 500 . The difference in score before and after the fertility treatment will be calculated. This score difference, which is a continuous outcome, will be used in a multiple linear regression model. This model estimates how several independent variables influence a single outcome. The regression analysis will show which independent variables (such as ethnicity, anxiety scores, duration of the desire to have children,…) have an influence on the score difference of IBS-SSS questionnaire. AIC will be used to evaluate the different possible models which will include different independent variables. The model with the lowest AIC will be used.

All analyses will be performed with SPSS.

11 Ethical Considerations

11.1 Ethical Conduct of the Study

11.1.1 Declaration of Helsinki

The trial will be performed in accordance with the Declaration of Helsinki, the conditions and principles of Good Clinical Practice, the protocol and applicable local regulatory requirements and laws.

11.1.2 Ethics Committee

Before the start of the trial or implementation of any amendment, approval of the trial protocol and amendments, informed consent forms and other relevant documents will be obtained from the applicable ethical committee(s).

11.2 Recruitment and Informed Consent

Each participant shall provide a signed and dated Informed Consent Form before performance of any study-related activities. The IC form that is used will be approved by reviewing EC and will be in a language that the participant can read and understand. The ICF will be in accordance with current ICH and GCP guidelines and with applicable local regulations.

11.3 Study Data Protection

The collection and processing of personal data from participants enrolled in the study will be limited to those data that are necessary to fulfill the objectives of this study.
These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data protection laws and regulations.

11.4 Subject Identification

The participant identification will be treated as confidential and will be filed by the investigator in an identification log. This log is kept at the participating site and shall not be copied. In all reports and communication between the site and the Sponsor the participant shall be identified with a participant study number.

12 Insurance

VUB is, as Sponsor of the trial, responsible for ensuring appropriate general/product liability insurance and as required in accordance with applicable laws and regulations, country-specific liability insurance coverage for claims made by a trial subjects for injury arising from the subject's participation in the trial.

13 Reporting and Dissemination

The data and information collected during this trial will be reported in a publication in a scientific/medical journal. Reporting of trial results will be performed according to local regulations.

For the correct authorship rules we refer to the International Committee of Medical Journal Editors: https://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html

14 Finance and Conflict of Interest Statement

Investigators and study team members will provide the Sponsor with sufficient, accurate financial information in accordance with local regulations to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities/ethics committee. Any update of information on financial interests should be disclosed during the course of the study.

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16 Appendices

APPENDIX 1: ROME IV IBS QUESTIONNAIRE (English version) (Rome Foundation)

Question	*Answer*
40. In the last 3 months, how often did you have pain anywhere in your abdomen?	⓪ Never ① Less than one day a month ② One day a month ③ Two to three days a month ④ Once a week ⑤ Two to three days a week ⑥ Most days ⑦ Every day ⑧ Multiple times per day or all the time
40b. In the last 3 months, how often did you have discomfort anywhere in your abdomen ?	⓪ Never → If you answered "Never" on question 40 and 40b: ***Skip question 41*** ① Less than one day a month ② One day a month ③ Two to three days a month ④ Once a week ⑤ Two to three days a week ⑥ Most days ⑦ Every day ⑧ Multiple times per day or all the time
41. How often did this pain in your abdomen happen close in time to a bowel movement -- just before, during, or soon after? (Percent of times with pain)	⓪ 0% Never ① 10% ② 20% ③ 30% ④ 40% ⑤ 50% ⑥ 60% ⑦ 70% ⑧ 80% ⑨ 90% ⑩ 100% Always

42. How often did your stools become either softer than usual or harder than usual when you had this pain? (Percent of times with pain)

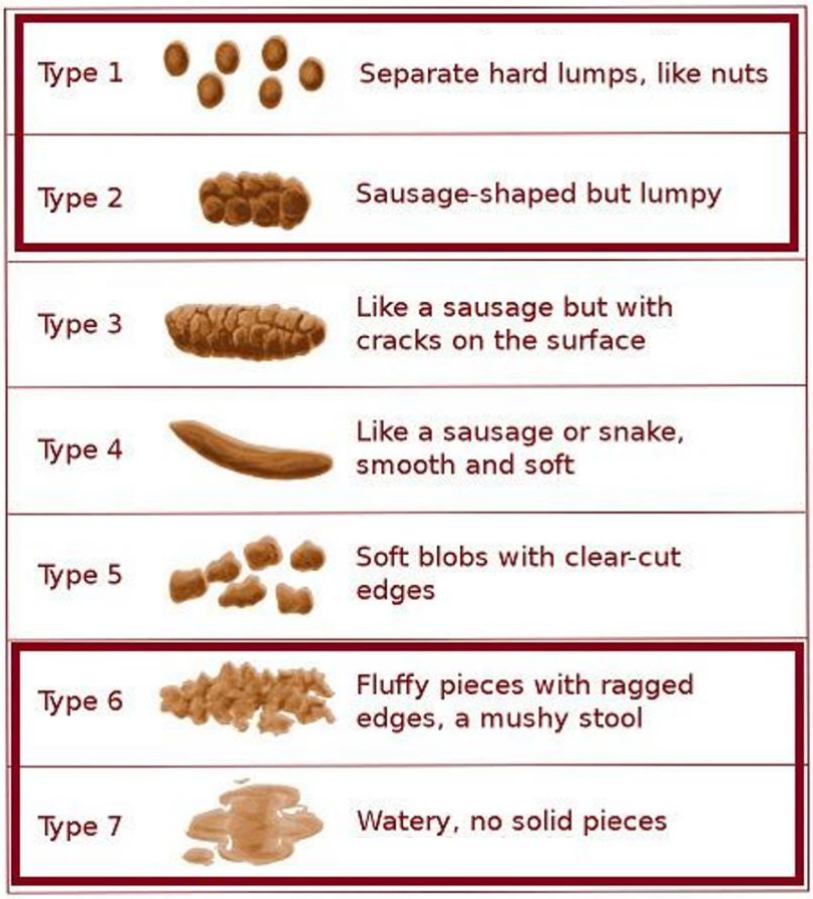
⓪ 0% Never
① 10%
② 20%
③ 30%
④ 40%
⑤ 50%
⑥ 60%
⑦ 70%
⑧ 80%
⑨ 90%
⑩ 100% Always

43. How often did your stools become either more frequent than usual or less frequent than usual when you had this pain? (Percent of times with pain)

⓪ 0% Never
① 10%
② 20%
③ 30%
④ 40%
⑤ 50%
⑥ 60%
⑦ 70%
⑧ 80%
⑨ 90%
⑩ 100% Always

48. Has it been 6 months or longer since you started having this pain?

⓪ No
① Yes



Bowel movements of Type 1 or 2 and also of Type 6 or 7 in the picture above can be considered to be abnormal. Type 1 or 2 means you are constipated, and Type 6 or 7 means you have diarrhea.

64. In the last 3 months, when you had abnormal stools, what were they usually like?

① Usually constipation (like Type 1 or 2 in the picture)
② Usually diarrhea (like Type 6 or 7)
③ Both diarrhea and constipation - that is, more than 1/4 of all the abnormal bowel movements were constipation and more than ¼ were diarrhea
④ Not applicable, because I never or rarely had abnormal bowel movements

APPENDIX 2: IBS Symptom Severity Scale (IBS-SSS) (English version) (Rome Foundation)

1a. **Do you currently (in the past 10 days) suffer from abdominal (stomach) pain?**
No ***Skip to question 3a***
Yes

1b. **How severe was your abdominal (stomach) pain in the past 10 days? (Please indicate a number from 0 to 100, with 0 meaning "no pain" and 100 meaning "very severe pain")**
0 -- No pain
10
20
30
40
50
60
70
80
90
100 -- Very severe pain

2**. Please enter the number of days you had the abdominal pain in the past 10 days. (For example, if you enter 4 it means that you had pain 4 out of 10 days. If you have pain every day, enter 10.)**
0 days
1
2
3
4
5
6
7
8
9
10 days

3a. **Do you currently (in the past 10 days) suffer from abdominal distention (bloating, swollen or tight stomach)?**
Women: Please ignore distention related to your period when answering this question.
No ***Skip to question 4***
Yes

3b. **How severe was your abdominal distention/tightness in the past 10 days? (Please indicate a number from 0 to 100, with 0 meaning "no distention" and 100 meaning "very severe distention")**
0 -- No distention
10
20
30
40
50
60
70
80
90
100 -- Very severe distention

4. **How dissatisfied are you with your bowel functioning in the past 10 days? (Please indicate a number from 0 to 100, with 0 meaning "Not dissatisfied" and 100 meaning "very dissatisfied")**
0 -- Not dissatisfied
10
20
30
40
50
60
70
80
90
100 -- Very dissatisfied

5. **How much did abdominal pain or discomfort or altered bowel functioning affect or interfere with your life in general in the past 10 days? (Please indicate a number from 0 to 100, with 0 meaning "Not at all" and 100 meaning "completely")**
0 -- Not at all
10
20
30
40
50
60
70
80
90
100 -- Completely

Appendix 3: Gastrointestinal Symptom Rating Scale (GSRS) (Astrazeneca, 1995) (English version)

THE GASTROINTESTINAL SYMPTOM RATING SCALE
(GSRS)

Please read this first:
This survey contains questions about how you have been feeling and whatit has been like DURING THE PAST WEEK. Mark the choice that best applies to you and your situation with an "X" in the box.

1. Have you been bothered by PAIN OR DISCOMFORT IN YOUR UPPER ABDOMEN OR THE PIT OF YOUR STOMACH during the past week?

- ♦ No discomfort at all
- ♦ Minor discomfort
- ♦ Mild discomfort
- ♦ Moderate discomfort
- ♦ Moderately severe discomfort
- ♦ Severe discomfort
- ♦ Very severe discomfort

2. Have you been bothered by HEARTBURN during the past week? (By heartburn we mean an unpleasant stinging or burning sensation in the chest.)

- ♦ No discomfort at all
- ♦ Minor discomfort
- ♦ Mild discomfort
- ♦ Moderate discomfort
- ♦ Moderately severe discomfort
- ♦ Severe discomfort
- ♦ Very severe discomfort

3. Have you been bothered by ACID REFLUX during the past week? (By acid reflux we mean the sensation of regurgitating small quantities of acid or flow of sour or bitter fluid from the stomach up to the throat.)

- ♦ No discomfort at all
- ♦ Minor discomfort
- ♦ Mild discomfort
- ♦ Moderate discomfort
- ♦ Moderately severe discomfort
- ♦ Severe discomfort
- ♦ Very severe discomfort

4. Have you been bothered by HUNGER PAINS in the stomach during the past week? (This hollow feeling in the stomach is associated with the need to eat between meals.)

- ♦ No discomfort at all
- ♦ Minor discomfort
- ♦ Mild discomfort
- ♦ Moderate discomfort
- ♦ Moderately severe discomfort
- ♦ Severe discomfort
- ♦ Very severe discomfort

5. Have you been bothered by NAUSEA during the past week? (By nausea we mean a

feeling of sickness that may lead to retching and vomiting.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

6. Have you been bothered by RUMBLING in your stomach during the past week? (Rumbling refers to vibrations or noise in the stomach.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

7. Has your stomach felt BLOATED during the past week? (Feeling bloated refers to swelling often associated with a sensation of gas or air in the stomach.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

8. Have you been bothered by BELCHING during the past week? (Belching refers to the release of wind from the stomach via the mouth, often associated with easing a bloated feeling.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

9. Have you been bothered by BREAKING WIND during the past week? (Breaking wind refers to the need to release air or gas from the bowel, often associated with easing a bloated feeling.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

10. Have you been bothered by CONSTIPATION during the past week? (Constipation refers to a reduced ability to empty the bowels.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

11. Have you been bothered by DIARRHOEA during the past week? (Diarrhoea refers to a too frequent emptying of the bowels.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

12. Have you been bothered by LOOSE STOOLS during the past week? (If your stools (motions) have been alternately hard and loose, this question only refers to the extent you have been bothered by the stools being loose.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

13. Have you been bothered by HARD STOOLS during the past week? (If your stools (motions) have been alternately hard and loose, this question only refers to the extent you have been bothered by the stools being hard.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

14. Have you been bothered by an URGENT NEED TO HAVE A BOWEL MOVEMENT during the past week? (This urgent need to go to the toilet is often associated with a feeling that you are not in full control.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

15. When going to the toilet during the past week, have you had the SENSATION OF NOT COMPLETELY EMPTYING THE BOWELS? (This feeling of incomplete emptying means that you still feel a need to pass your motions despite having

exerted yourself to do so.)

- No discomfort at all
- Minor discomfort
- Mild discomfort
- Moderate discomfort
- Moderately severe discomfort
- Severe discomfort
- Very severe discomfort

PLEASE CHECK THAT ALL QUESTIONS HAVE BEEN ANSWERED!
THANK YOU FOR YOUR CO-OPERATION.

Appendix 4: General Anxiety Disorder 7 (GAD-7) (Spitzer 2006) (English version)

Over the last 2 weeks, how often have you been bothered by the following problems?	Not at all	Several days	Over half the days	Nearly every day
1. Feeling nervous, anxious, or on edge	0	1	2	3
2. Not being able to stop or control worrying	0	1	2	3
3. Worrying too much about different things	0	1	2	3
4. Trouble relaxing	0	1	2	3
5. Being so restless that it's hard to sit still	0	1	2	3
6. Becoming easily annoyed or irritable	0	1	2	3
7. Feeling afraid as if something awful might happen	0	1	2	3

Appendix 5: Patient Health Questionnaire-9 (PHQ-9) (Kroenke 2001) (English version)

Over the last 2 weeks, how often have you been bothered by any of the following problems?	Not at all	Several days	More than half the days	Nearly every day
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself - or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite - being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or hurting yourself in some way	0	1	2	3

If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all ☐
Somewhat difficult ☐
Very difficult ☐
Extremely difficult ☐