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Vaginal Progesterone as Luteal Phase Support in Natural Cycle Frozen-Thawed Embryo Transfer (ProFET): a study protocol for a multi-center open-label randomized controlled trial

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9 3 **Embryo Transfer (ProFET): a study protocol for a multi-center open-label randomized**
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11 4 **controlled trial**
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43 45

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45 47 #progesterone

46 48 #natural cycle

47 49 #frozen embryo transfer

48 50 #randomized controlled trial
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59 **ABSTRACT**

61 **Introduction**

62 Vaginal progesterone supplementation is frequently given to patients receiving frozen embryo
63 transfer (FET) in the natural cycle aiming to increase the chance of pregnancy and live birth.
64 To date only a few studies have investigated if progesterone supplementation is beneficial in
65 these cycles and the level of evidence for progesterone supplementation is very low.

67 **Methods and analysis**

68 The ProFET trial is a multicenter, open-label, randomized controlled trial powered for this
69 investigation, including 1800 women with regular menstrual cycles (24-35 days), aged 18-43
70 years planned for natural cycle frozen embryo transfer (NC-FET) receiving a single blastocyst
71 for transfer. Participants are randomized (1:1:1) to either luteal phase progesterone for 3
72 weeks, luteal phase progesterone for 7 weeks or no luteal phase progesterone.

73 **Ethics and dissemination**

74 The trial was approved by the Swedish Ethical Review Authority (ID 2020-06774 and 2021-
75 02822) and the Swedish Medical Products Agency (ID nr 5.1-2020-102613). The outcome of
76 this study will be publicly disseminated.

77 **Trial registration number**

78 ClinicalTrials.gov (NCT047258649) and EudraCT (2020-005552-38).

80 **Strengths and limitations of the trial**

- 82 • The trial has a randomized design, powered to evaluate if luteal support with vaginal
83 progesterone will improve live birth rate in NC-FETs when a single blastocyst is
84 transferred.
- 85 • The trial is conducted in women planning FET in natural cycles without exogenous
86 ovulation trigger.
- 87 • If overall superiority of progesterone is demonstrated, the sample size will allow
88 evaluation if treatment duration of 7 weeks is superior to 3 weeks.
- 89 • The broad inclusion criteria of women with regular menstrual cycles empower high
90 generalizability of the results.

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3 91 • The study is open-label, neither blinded to participants, nor to treating physicians,
4 which is a limitation, however less likely to introduce bias due to the robust primary
5 92 outcome; live birth.
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12 97 INTRODUCTION

13 98
14 99 In recent years, there has been a dramatic increase in the use of frozen-thawed embryo
15 100 transfers (FET) cycles in in-vitro fertilization (IVF) all over the world. The FET rate in the
16 101 United States has doubled since 2015, accounting for 78.8 % of all embryo transfers using
17 102 non-donor Assisted Reproductive Technology (ART) in 2019(1). Similar changes are taking
18 103 place in Europe(2) and in Sweden where the FET rate now accounts for 48 % of all IVF-
19 104 cycles(3). The main reason for this increase is the improved embryo survival and high
20 105 pregnancy/live birth rates (LBRs) after transfer of vitrified/thawed blastocysts compared to
21 106 the previously used technique with transfer of thawed slow-frozen cleavage stage embryos(4,
22 107 5). Furthermore, high embryo survival rate facilitates the practice of single embryo
23 108 transfer(6), reducing multiple pregnancy rate and thereby decreasing the risk of adverse
24 109 perinatal outcomes.
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36 111 The freeze-all concept, including Gonadotropin Releasing Hormone (GnRH) agonist given to
37 112 induce oocyte maturation, has substantially changed treatment strategies in ART. Considering
38 113 efficacy aspects, five large randomized controlled trials (RCTs) have investigated the
39 114 differences in LBR following fresh embryo transfer (ET) and FET in freeze-all cycles. In
40 115 2016, a large RCT including only anovulatory patients, showed a significantly higher LBR
41 116 and a lower risk of Ovarian Hyperstimulation Syndrome (OHSS) in the freeze-all group
42 117 compared with fresh embryo transfers(7). However, in patients with regular menstrual cycles,
43 118 most trials showed no difference in ongoing pregnancy rate or LBR in the freeze-all group
44 119 compared with fresh embryo transfers (8-10), while one RCT resulted in a higher LBR with
45 120 frozen embryo transfers(11). The freeze-all concept is also now widely used when pending
46 121 risk of OHSS, and has almost the risk of eliminated OHSS, a potentially life-threatening
47 122 condition(12-14).
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58 124 The most efficient protocol for FET is still not known. A Cochrane review, including 18
59 125 RCTs, comparing different cycle regimens for FET, comprising a total of 3815 women did

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3 126 not support one treatment modality over another when investigating LBR, however, with low
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5 127 certainty of evidence(15).

6 128 Safety aspects in ART are of great importance in treatment decision. Recently, interest has
7
8 129 risen concerning the role of the corpus luteum (CL) in frozen cycles and studies evaluating
9
10 130 the risks of altered vascular adaptation associated with pregnancies following FET according
11
12 131 to the presence or absence of CL have been published(16). Corpus luteum is known to
13
14 132 produce estrogen and progesterone, but also relaxin, a hormone that regulates the maternal
15
16 133 cardiovascular and renal systems and hence mediates the hemodynamic changes occurring
17
18 134 during pregnancy. In a prospective cohort study including almost 700 women, programmed
19
20 135 cycles (artificial cycles using estrogen and progesterone for endometrial preparation) in FET
21
22 136 with no CL present were associated with an almost three-fold increased risk of preeclampsia
23
24 137 compared with modified natural cycles (natural cycles triggered by human chorionic
25
26 138 gonadotrophin (hCG), for ovulation) with one CL present(16). Furthermore, in a recent
27
28 139 Swedish large registry study, including almost 10 000 pregnancies/deliveries after FET,
29
30 140 doubled rates of both hypertensive disorders of pregnancy and postpartum hemorrhage were
31
32 141 found in programmed cycles compared to natural cycles(17). These studies thus support FET
33
34 142 in natural cycles.

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36 143
37 144 The role of progesterone as luteal phase support (LPS) in natural cycles FET (NC-FET) has
38
39 145 been briefly studied. A systematic review and meta-analysis from 2020, including one RCT
40
41 146 and three retrospective studies, found no evidence of an improved clinical pregnancy rate
42
43 147 after progesterone support in NC-FET(18). A more recent systematic and meta-analysis,
44
45 148 showed a benefit of progesterone as luteal phase support in NC-FET for LBR(19). However,
46
47 149 the two meta-analyses included a mix of RCTs and observational studies and had a wide
48
49 150 heterogeneity regarding progesterone treatment regimens. The authors concluded that further
50
51 151 large, randomized studies are needed to improve the certainty of evidence.

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53 152
54 153 In view of the limited knowledge concerning a possible advantage of progesterone as luteal
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56 154 phase support in NC-FET , the aim of this large RCT is to investigate if progesterone as luteal
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58 155 phase support increases LBR compared with no progesterone. In addition, assessment of
59
60 156 perinatal and obstetric outcomes will be performed. Furthermore, the trial will investigate if
157
158 157 the duration of progesterone support matters and assess the association between serum
159
160 158 progesterone levels in early luteal phase and IVF outcome(20-22).

160 **OBJECTIVES**

161

162 **Primary objective**

163 To investigate if LPS by vaginal progesterone increases the chance of a live birth after FET in
164 a natural cycle compared with no LPS. If progesterone support is superior to no treatment, we
165 will further investigate if 7 weeks of treatment is more effective than 3 weeks.

166

167 **Secondary objectives**

168

- 169 1. To compare study groups regarding secondary outcomes including biochemical,
170 clinical and ongoing pregnancy, as well as miscarriage.
- 171 2. To compare perinatal and obstetrical outcomes.
- 172 3. To compare self-reported side effects in women receiving and not receiving LPS with
173 vaginal progesterone.
- 174 4. Investigate the association between serum progesterone levels before FET and LBR .

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176

177 **METHODS AND ANALYSIS**

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179 **Study design**

180 This multicenter, open-label, randomized, controlled Phase IV trial includes the participation
181 of eight fertility clinics in Sweden and one in Iceland. All clinics perform standardized
182 treatment according to the public healthcare system guidelines in Sweden and Iceland. Patient
183 enrollment began in April 2021 and is planned to continue until September 2023.

184

185 A total of 1800 women undergoing NC-FET after conventional IVF or intracytoplasmic
186 sperm injection (ICSI) treatment at one of the nine participating clinics will be recruited. As a
187 clinical routine, patients scheduled for NC-FET contact their fertility clinic on the first day of
188 the menstrual bleeding to schedule the treatment. Subsequently, a study nurse or doctor will
189 identify and contact patients who fulfill the inclusion criteria to ask for interest in
190 participating. Study information is sent to the patient by regular mail or through a secured
191 website. Signed written or digitally informed consent is returned to the clinic either by regular
192 mail or by contact through the website.

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3 **194 Eligibility criteria**

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5 **195** Inclusion and exclusion criteria are specified in Table 1.
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8 **Table 1. ProFET trial. Inclusion and exclusion criteria.**

Inclusion criteria	Exclusion criteria
Natural cycle FET with blastocyst	Oocyte donor cycles
Regular menstrual cycle (24-35 days)	Uterine malformations: cervical anomalies, submucosal uterine fibroid or endometrial polyps requiring surgery.
Age 18-43 years	Hypersensitivity to vaginal progesterone
BMI 18.5-35 (kg/m ²)	Medical contraindication to progesterone treatment
Understand written and spoken Swedish, English or Arabic and have signed a written informed consent.	Serious concomitant disease contraindicating ART and pregnancy
	Preimplantation genetic testing (PGT)
	Previously included in the ProFET study
	Participation in another study with an investigational product within the last 30 days

36
37 **196** FET; Frozen embryo transfer, BMI; Body mass index.
38 **197**

39
40 **198 Treatment and intervention**

41 **199** At the endogenous surge of LH (luteinizing hormone ; a hormone that naturally rises to
42 **200** trigger ovulation), study participants are randomized 1:1:1 to one of three groups:
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45 **201**

46 **202** A. No vaginal progesterone

47 **203** B. Vaginal progesterone for 3 weeks

48 **204** C. Vaginal progesterone for 7 weeks
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52 **205**

53 **206** Patients randomized to luteal phase progesterone are instructed to administrate 100 mg
54 **207** vaginal progesterone (Lutinus[®]; Ferring Pharmaceuticals, Saint-Prex, Switzerland) three
55 **208** times daily starting 3 days after the LH-surge. Participants are asked to leave a blood sample
56 **209** for analysis of serum progesterone regardless of group-allocation. A blood sample will be
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3 210 drawn in the morning 3 days after the LH-surge, before any start of progesterone. The result
4
5 211 will not be available to the patient. On day 5-6 after LH-surge, a blastocyst is transferred
6
7 212 according to standard embryo transfer procedure. Patients randomized to vaginal progesterone
8
9 213 will continue administration of progesterone until a pregnancy test. In the case of a positive
10
11 214 pregnancy test patients will continue with vaginal progesterone for a total of 3 or 7 weeks
12
13 215 respectively. In the case of a negative pregnancy test or miscarriage later on the patient will
14
15 216 stop progesterone treatment. See figure 1.

15 217

17 218 **Randomization**

18
19 219 Study data are recorded in an electronic case report file (e-CRF) designed by Medicase
20
21 220 (Sahlgrenska Science Park, Gothenburg, Sweden) which also includes a randomization
22
23 221 program. Randomization is stratified for:

24 222

- 25 223 - Previous ET not resulting in positive pregnancy test, number (0-2, ≥ 3)
- 26 224 - Parity 0/ ≥ 1
- 27 225 - Age (<35/ ≥ 35 years)
- 28 226 - Treatment site

32 227

34 228 **Blinding procedure**

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36 229 The trial is not blinded, neither to patients nor to treating physicians. Analyses are done by an
37
38 230 independent statistician.

39 231

41 232 **Data collection**

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43 233 Patient-related data are collected and variables are registered in the e-CRF program at the
44
45 234 following time points:

46 235

- 48 236 1) screening before LH-surge
- 49 237 2) randomization at LH-surge
- 50 238 3) FET at LH + 5 or 6 days
- 51 239 4) result of pregnancy-test (urine sample)
- 52 240 5) early pregnancy scan (7 weeks + 5 days to 9 weeks + 0 days) in case of positive
53 241 pregnancy test
- 54 242 6) through a follow up (by telephone) after gestational week 23 + 0 days
- 55 243 7) from the patient's and the newborn's medical records after delivery

244 **Sample collection**

245 Blood samples will be collected at LH + 3 days, whenever possible. A blood sample of 5 ml is
246 analyzed for serum progesterone level. The blood samples will be sent to and analyzed at the
247 Swedish certified laboratory Unilabs and are then discarded.

248

249 **Transvaginal ultrasound scans**

250 If the patient conceives, an early transvaginal pregnancy scan will be made at gestational age
251 7 weeks + 5 days to 9 weeks + 0 days, for estimation of number of gestational sacs, number of
252 fetuses, crown-rump length and viability.

253

254 **Questionnaires**

255 The participants will be asked to fill out a questionnaire regarding registration of possible
256 study medication side effects. The form is filled out regardless of group allocation and
257 submitted in connection with vaginal ultrasound at gestational age 7 weeks + 5 days to 9
258 weeks + 0 days – or earlier in the case of a negative pregnancy test or miscarriage. Specified
259 reported symptoms will be recorded as adverse events (AEs) in the e-CRF. Serious adverse
260 events (SAEs) will be followed until two weeks after delivery.

261

262 **Data management**

263 Data is transferred to an online e-CRF; Medicase. The Medicase database is based on coded
264 subject ID numbers used in the trial. Data are stored on a server located at Sahlgrenska
265 University Hospital, Gothenburg, Sweden, with a daily backup. Only research staff at the
266 Sahlgrenska University Hospital will have access to the final dataset. Ownership of data is
267 determined by co-operation agreements as well as data processing agreements between
268 Sahlgrenska University Hospital and the participating clinics.

269

270 **Data sharing plan**

271 The data underlying this article will be shared on reasonable request to the main responsible
272 author.

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276 STATISTICS

277

278 Outcome measurements

279 Primary outcome is live birth. Secondary outcomes include biochemical and clinical
280 pregnancy rates, miscarriage rates and obstetric and neonatal outcomes in the study groups.
281 Self-reported side effects will be reported as mild, moderate or severe. Progesterone levels 3
282 days after LH-surge will be measured in units of nmol/L.

283

284 Sample size calculation

285 In order to find an effect size of a 7% increase in LBR per transfer, measured as a difference
286 in proportions between no progesterone (0.33) and any progesterone group (0.40), 1800
287 subjects are needed if allocated 1:2. In order to find a difference between progesterone 3
288 weeks and progesterone 7 weeks, as well as between no progesterone and progesterone for 3
289 weeks and 7 weeks respectively, 1200 subjects are needed if allocated 1:1. For all
290 comparisons above, except for the primary analysis, a difference between groups of 8% is
291 used. If 1800 women are allocated 1:1:1, 600 to no progesterone, 600 to progesterone 3 weeks
292 and 600 to progesterone 7 week, all four sample size calculations are fulfilled under the
293 condition of a power of 0.80, a significance level 0.05 and a two-sided Fisher's exact test.

294

295 Statistical analyses

296 The main analyses will be on the full analysis set (FAS) without imputation. Sensitivity
297 analyses will be performed on the Intention-to-treat population with imputed data.
298 Complementary analyses will be performed on the per protocol population. For unadjusted
299 comparison between two groups Fisher's exact test will be used for dichotomous variables,
300 Fisher's non-parametric permutation test will be used for continuous variables and Mantel-
301 Haenszel Chi-square test for ordered categorical variables. Mean difference with 95%
302 confidence interval (CI) will be given for all dichotomous and continuous variables. For
303 dichotomous variables relative risk and odds ratio will be given with 95% CI.
304 For adjusted analyses between two groups, multivariable logistic regression will be used for
305 dichotomous variables. If model assumption is fulfilled the corresponding model with
306 link=log will be given to present adjusted relative risk (RR) with 95% CI. For continuous
307 variables analysis of covariance (ANCOVA) will be used for adjusted analysis between two
308 groups.

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3 309 The primary efficacy analysis regarding live birth will be conducted with multiple logistic
4 regression adjusting for all stratification variables and other predefined important predictors
5 310
6 311 on the FAS population. The proportions will be given with exact 95% CI. The distribution of
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8 312 continuous variables will be given as mean, standard deviation (SD), median, first and third
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10 313 quartiles (Q1, Q3), minimum and maximum. All significance tests will be two-sided and
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12 314 conducted at the 5% significance level.

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15 316 **Monitoring**

17 317 All study participants are monitored to meet the inclusion criteria and a check is made that
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19 318 voluntarily informed consent for each study participants is obtained and documented. For all
20
21 319 study participants, the main parameters in the study are checked (live birth, clinical
22
23 320 pregnancy, miscarriage and ectopic pregnancy). The first two study participants at each center
24
25 321 will be monitored with a complete source data verification. Thereafter, a complete source data
26
27 322 verification will be performed on every fifth randomly selected study participant.

27 323

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30 325 **ETHICS AND DISSEMINATION**

31 326

34 327 The study was approved by the Swedish Ethical Review Authority (ID 2020-06774 and 2021-
35
36 328 02822) and the Swedish Medical Products Agency (ID nr 5.1-2020-102613).

37 329 The safety of participants in this study is high. As the medication/treatment with vaginal
38
39 330 progesterone is well known, SAEs or suspected unexpected serious adverse reactions
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41 331 (SUSARs) are unlikely. If, however, a participant should experience a SAE or a SUSAR the
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43 332 local investigator will contact the principal investigator with no delay and the individual
44
45 333 treatment will be stopped immediately.

46 334

48 335 The results of this trial will be presented at national as well as international scientific
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50 336 congresses and published in international scientific journals. The results of the research will
51
52 337 also be disseminated to public through broadcasts, popular science articles, and newspapers.

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55 339 **Patient and public involvement**

56 340 Development of this study protocol was done without patient involvement. The final study
57
58 341 results will be disseminated to participants on request.

59 342

DISCUSSION

The rapidly increasing use of FET worldwide and the limited evidence concerning cycle regimens for FET demands further well designed large randomized trials. Progesterone supplementation in NC-FET is widely used despite scarce evidence. Two RCTs with LBR as main outcome have been published(23, 24). In a Swedish study where mainly cleavage stage embryos and single- as well as double embryo transfer were used, a significantly higher LBR was found(23). Further, a small study from Israel, including only 59 patients, using a modified NC-FET protocol, also found a significantly higher LBR after LPS compared with no progesterone(24). The study included a mix of cleavage stage embryos and blastocysts and up to three embryos were transferred.

Available retrospective studies on LPS reveal the use of different embryo stages at embryo transfer; two-nucleus stage(25) cleavage stage embryos(26) and both cleavage embryo and blastocyst transfers(27). All these studies used human Chorionic Gonadotropin (hCG) as ovulation trigger and administration of progesterone supplementation was started at different time points after LH-surge and was administered either as intramuscular injections or as vaginal suppositories with different doses and duration of treatment.

This presented ongoing large open-label multicenter randomized clinical trial aims to investigate if vaginal LPS in NC-FET is superior to no LPS. In this set up, not only the differences in LBR and clinical pregnancy rates will be investigated, but also, the obstetrical and perinatal outcomes. This study will contribute to recommendations regarding LPS in NC-FET in the future.

The strength of this trial is the multicenter, randomized design and a large sample size of 1800 women. Broad inclusion criteria representing the patient cohort in every day practice will give a high generalizability to the results. The IVF-protocols consists only of natural cycles with no ovulation trigger. The study is not blinded to participants or investigators, which is a limitation, however less likely to introduce bias due to a robust primary outcome – live birth. By publishing the study protocol the study contributes to research-transparency. If progesterone supplementation in natural FET cycles should be shown to significantly increase the chance of live birth, the benefit for the patients, as well as for the society, would mean a) a shorter time to pregnancy, b) fewer IVF cycles needed per patient, c) reduced costs for patients and society, d) less environmental burden due to less cycles to achieve live birth

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3 377 and thus less use of hormonal IVF-treatment. On the other hand, if no beneficial effect of this
4 378 treatment can be shown, it should be abandoned and thereby implicate less financial burden
5 379 for patients as well as for society, less treatment burden for the patient and less environmental
6 380 impact associated with the use of LPS.
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10 381 **Contributors**

11 382 CB and ÅM were the primary initiators of the study, who designed and wrote the first study
12 383 protocol. AK, AS and CS contributed to the revision and editing of the study protocol. AK,
13 384 AS, CB, CS, EL, GWe, GWi, KHO, KRW, MB, MK, SL and ÅM will all be involved in the
14 385 recruitment of patients and data collection. All authors approved the final version of the study
15 386 protocol. AS and CS applied to the Swedish Ethical Review Authority. CB and ÅM applied to
16 387 the Swedish Medical Products Agency. AK and CS wrote the first draft of this manuscript
17 388 which was revised by AS, CB and ÅM. Finally all committed authors approved this protocol.
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30 394 and by the Hjalmar Svensson foundation. We will also apply for further funding.
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36 396 **Disclaimer**

37 397 Ferring Pharmaceuticals has not been involved in the design of the study protocol, nor will
38 398 they be involved in the conduct of the study or any analysis neither the reporting of the
39 399 results.
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44 401 **Competing interests**

45 402 AS has support by Ferring Pharmaceuticals. CB has support by Ferring Pharmaceuticals,
46 403 Merck A/S and Gedeon Richter. ÅM has support by Ferring Pharmaceuticals, Merck Serreno
47 404 and Gedeon Richter. All these supports are given for lectures on own research.
48 405 MB has 4 % stocks in EUGIN Sweden.
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54 407 **Patient consent for publication**

55 408 Not required.
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3 493 Legends – the ProFET trial

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5 495 Table 1: ProFET trial, inclusion and exclusion criteria. FET; Frozen embryo transfer, BMI;
6 496 Body mass index.

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8 498 Figure 1: ProFET trial, flowchart. LH; Luteinizing hormone, FET; Frozen embryo transfer,
9 499 NC-FET; Natural cycle frozen embryo transfer.

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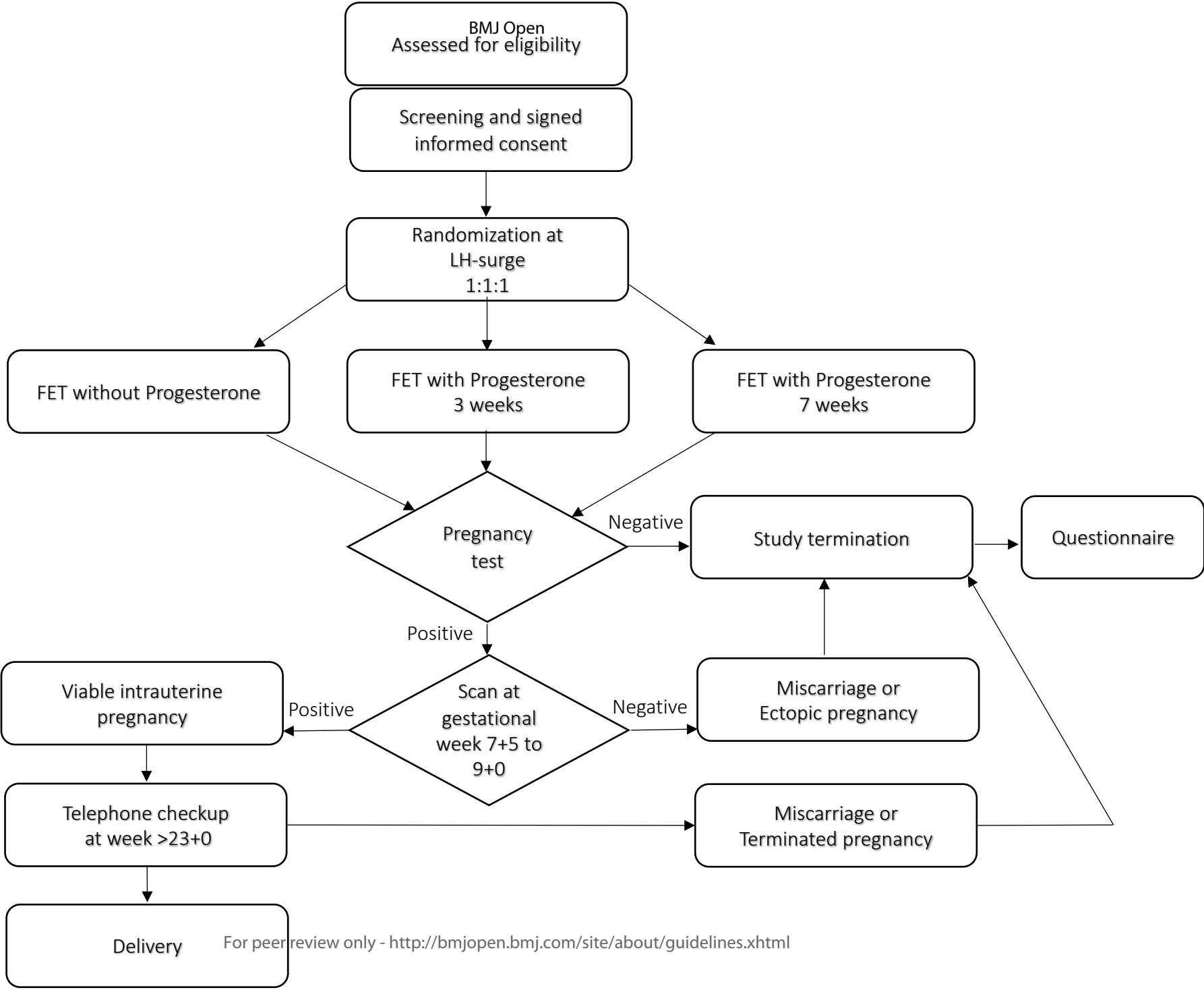
Planning
NC-FET

LH + 0 days

LH + 5-6 days

FET + 10 days

Pregnancy follow up
until delivery



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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Vaginal Progesterone as Luteal Phase Support in Natural Cycle Frozen-Thawed Embryo Transfer (ProFET): a study protocol for a multi-center open-label randomized controlled trial
Trial registration	2a	Progesterone as Luteal Support in Frozen IVF Natural Cycles (ProFET) ClinicalTrials.gov Identifier: NCT04725864
	2b	NA
Protocol version	3	Version 2.2. 2021-07-06
Funding	4	The project will be funded by grants from the Swedish state under the agreement between the Swedish government and the county councils, the ALF-agreement (ALFGBG-965526 and ALFGBG-720291), by an unrestricted grant from Ferring Pharmaceuticals and by the Hjalmar Svensson foundation.
Roles and responsibilities	5a	CB and ÅM were the primary initiators of the study, who designed and wrote the first study protocol. AK, AS and CS contributed to the revision and editing of the study protocol. AK, AS, CB, CS, EL, GWe, GWi, KHO, KRW, MB, MK, SL and ÅM will all be involved in the recruitment of patients and data collection. All authors approved the final version of the study protocol. AS and CS applied to the Swedish Ethical Review Authority. CB and ÅM applied to the Swedish Medical Products Agency. AK and CS wrote the first draft of this manuscript which was revised by AS, CB and ÅM.
	5b	Christina Bergh: christina.bergh@vregion.se
	5c	Ferring Pharmaceuticals has not been involved in the design of the study protocol, nor will they be involved in the conduct of the study or any analysis neither the reporting of the results.

5d The team at Reproductive Medicine, Sahlgrenska Hospital consisting of principal investigator ÅM, trial sponsor CB and co-workers CS, AS and AK are responsible for the study design, trial registration, ethical approvals, data collection and ongoing contact with all study sites.

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	11b	NA
	11c	NA
	11d	Page 4; table 1
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Sample size	14	Page 7
Recruitment	15	NA

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24		20c	NA
25			
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*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](#)" license.

BMJ Open

Vaginal progesterone as luteal phase support in natural cycle frozen-thawed embryo transfer (ProFET): protocol for a multicenter, open-label, randomized controlled trial

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Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Reproductive medicine
Keywords:	Reproductive medicine < GYNAECOLOGY, GYNAECOLOGY, Subfertility < GYNAECOLOGY

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Manuscripts

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3 **1 Vaginal progesterone as luteal phase support in natural cycle frozen-thawed embryo**
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5 **2 transfer (ProFET): protocol for a multicenter, open-label, randomized controlled trial**
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29 40 Keywords: luteal phase support; progesterone; natural cycle; frozen embryo transfer;
30 41 randomized controlled trial
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34 44 Word count: 3824 words
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ABSTRACT

Introduction

Vaginal progesterone supplementation is frequently given to patients receiving frozen embryo transfer (FET) in the natural cycle aiming to increase the chance of pregnancy and live birth.

To date only a few studies have investigated if progesterone supplementation is beneficial in these cycles and the level of evidence for progesterone supplementation is very low.

Methods and analysis

The ProFET trial is a multicenter, open-label, randomized controlled trial powered for this investigation, including 1800 women with regular menstrual cycles (24-35 days), aged 18-43 years planned for natural cycle frozen embryo transfer (NC-FET) receiving a single blastocyst for transfer. Participants are randomized (1:1:1) to either luteal phase progesterone for 3 weeks, luteal phase progesterone for 7 weeks or no luteal phase progesterone. The

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3 61 participating study centers consist of twelve IVF-clinics in Sweden and one in Iceland. The
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5 62 primary outcome is to investigate if luteal phase support (LPS) by vaginal progesterone
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7 63 increases the chance of a live birth per randomized patient in a natural FET cycle compared
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9 64 with no LPS.

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11 66 **Ethics and dissemination**

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13 67 The trial was approved by the Swedish Ethical Review Authority (ID 2020-06774, 2021-
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15 68 02822 and 2022-01502-02) and the Swedish Medical Products Agency (ID nr 5.1-2020-
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17 69 102613). All participants are required to provide written informed consent. The outcome of
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19 70 this study will be disseminated to the public through broadcasts, newspapers and
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21 71 presentations at scientific congresses as well as publications in international scientific
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23 72 journals.

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25 74 **Trial registration number**

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27 75 ClinicalTrials.gov (NCT04725864) and EudraCT (2020-005552-38).
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30 31 77 **Strengths and limitations of this study**

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34 79 • The trial has a randomized design, powered to evaluate if luteal support with vaginal
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36 80 progesterone will improve live birth rate in natural cycle frozen embryo transfers (NC-
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38 81 FETs) when a single blastocyst is transferred.
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40 82 • The trial is conducted in women planning FET in natural cycles without exogenous
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42 83 ovulation trigger.
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44 84 • If overall superiority of progesterone is demonstrated, the sample size will allow
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46 85 evaluation of whether treatment duration of 7 weeks is superior to 3 weeks.
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48 86 • The broad inclusion criteria of women with regular menstrual cycles will ensure high
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50 87 generalizability of the results.
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52 88 • The study is open label, blinded neither to participants nor to treating physicians,
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54 89 which is a limitation; however, this limitation is countered by the use of a robust
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56 90 primary outcome (live birth).

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59 **INTRODUCTION**

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106 In recent years, there has been a dramatic increase in the use of frozen-thawed embryo transfers (FET) cycles in in-vitro fertilization (IVF) all over the world. The FET rate in the United States has doubled since 2015, accounting for 78.8 % of all embryo transfers using non-donor Assisted Reproductive Technology (ART) in 2019(1). Similar changes are taking place in Europe(2) and in Sweden where the FET rate now accounts for 48 % of all IVF-cycles(3). The main reason for this increase is the improved embryo survival and high pregnancy/LBR after transfer of vitrified/thawed blastocysts compared to the previously used technique with transfer of thawed slow-frozen cleavage stage embryos(4, 5). Furthermore, high embryo survival rate facilitates the practice of single embryo transfer(6), reducing multiple pregnancy rate and thereby decreasing the risk of adverse perinatal outcomes.

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107 Recently, the freeze-all concept has gained high popularity all over the world. Several large trials, comparing freeze-all vs fresh transfer, have shown similar live birth rates in ovulatory patients(7-10) while freeze-all has been shown to be beneficial in anovulatory patients(11). The freeze-all concept is also widely used when pending risk of ovarian hyperstimulation syndrome (OHSS), and has almost eliminated the risk of OHSS, a potentially life-threatening condition(12-14).

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114 The most efficient protocol for FET is still not known. A Cochrane review, including 18 RCTs, comparing different cycle regimens for FET, comprising a total of 3815 women did not support one treatment modality over another when investigating LBR, however, with low certainty of evidence(15).

118

119 Safety aspects in ART are of great importance in treatment decision. Recently, interest has risen concerning the role of the corpus luteum (CL) in frozen cycles and studies evaluating the risks of altered vascular adaptation associated with pregnancies following FET according to the presence or absence of CL have been published(16). The CL, developing after ovulation, is known to produce estrogen and progesterone, but also relaxin, a hormone that regulates the maternal cardiovascular and renal systems and hence mediates the hemodynamic changes occurring during pregnancy. In a prospective cohort study including almost 700 women, programmed cycles (artificial cycles using estrogen and progesterone for endometrial preparation) in FET with no CL present were associated with an almost three-fold increased risk of preeclampsia compared with modified natural cycles (natural cycles triggered by

1
2
3 129 human chorionic gonadotrophin (hCG), for ovulation) with one CL present(16). Furthermore,
4
5 130 in a recent Swedish large registry study, including almost 10 000 pregnancies/deliveries after
6
7 131 FET, doubled rates of both hypertensive disorders of pregnancy and postpartum hemorrhage
8
9 132 were found in programmed cycles compared to natural cycles(17). These studies thus support
10
11 133 FET in natural cycles.

12 134
13 135 Luteal phase support (LPS) in fresh IVF cycles has been proven mandatory(18). Less is
14
15 136 known regarding the role of LPS with progesterone in natural FET cycles. A natural ovulatory
16
17 137 cycle would suggest that no supplementation needs to be given. However, the luteinizing
18
19 138 hormone peak – used as a urine sample to detect ovulation - does not guarantee a subsequent
20
21 139 ovulation. Furthermore, several studies have shown that corpus luteum deficiency with
22
23 140 midluteal serum progesterone levels <10ng/ml could be a reason to support implantation and
24
25 141 early pregnancy with LPS, even in a cycle where ovulation has occurred(19, 20). A study
26
27 142 from 2018(21) showed that low but also high levels of progesterone were associated with a
28
29 143 reduction in clinical pregnancy rate and LBR compared to normal levels. This has also been
30
31 144 confirmed in a more recent study(22). Not only the doses, but also the duration of luteal phase
32
33 145 support is widely discussed and differ between studies.

34 146
35 147 A systematic review and meta-analysis from 2020, including one RCT and three retrospective
36
37 148 studies, found no evidence of an improved clinical pregnancy rate after progesterone support
38
39 149 in NC-FET(23). A more recent systematic and meta-analysis, showed a benefit of
40
41 150 progesterone as luteal phase support in NC-FET for LBR(24). However, the two meta-
42
43 151 analyses included a mix of RCTs and observational studies and had a wide heterogeneity
44
45 152 regarding progesterone treatment regimens. The authors concluded that further large,
46
47 153 randomized studies are needed to improve the certainty of evidence.

48 154
49 155 In view of the limited knowledge concerning a possible advantage of progesterone as luteal
50
51 156 phase support in NC-FET, the aim of this large RCT is to investigate if progesterone as luteal
52
53 157 phase support increases LBR compared with no progesterone. In addition, assessment of
54
55 158 perinatal and obstetric outcomes will be performed. Furthermore, the trial will investigate if
56
57 159 the duration of progesterone support matters and assess the association between serum
58
59 160 progesterone levels in early luteal phase and IVF outcome(20, 25, 26).

60 161
62 **OBJECTIVES**

163

Primary objective

To investigate if LPS by vaginal progesterone increases the chance of a live birth after FET in a natural cycle compared with no LPS. If progesterone support is superior to no treatment, we will further investigate if 7 weeks of treatment is more effective than 3 weeks.

168

Secondary objectives

170

1. To compare study groups regarding secondary outcomes including biochemical, clinical and ongoing pregnancy, as well as miscarriage.
2. To compare perinatal and obstetrical outcomes.
3. To compare self-reported side effects in women receiving and not receiving LPS with vaginal progesterone.
4. Investigate the association between serum progesterone levels before FET and LBR .

177

METHODS AND ANALYSIS

179

Study design

This multicenter, open-label, randomized, controlled Phase IV trial includes the participation of twelve fertility clinics in Sweden and one in Iceland. All clinics perform standardized treatment according to the public healthcare system guidelines in Sweden and Iceland. Patient enrollment began in May 2021 and is planned to continue until June 2024.

185

A total of 1800 women undergoing NC-FET after conventional IVF or intracytoplasmic sperm injection (ICSI) treatment at one of the nine participating clinics will be recruited. As a clinical routine, patients scheduled for NC-FET contact their fertility clinic on the first day of the menstrual bleeding to schedule the treatment. Subsequently, a study nurse or doctor will identify and contact patients who fulfill the inclusion criteria to ask for interest in participating. Study information is sent to the patient by regular mail or through a secured website. Signed written or digitally informed consent is returned to the clinic either by regular mail or by contact through the website. (supplemental file 1)

194

Eligibility criteria

Inclusion and exclusion criteria are specified in Table 1.

Table 1. ProFET trial inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Natural cycle FET with blastocyst	Oocyte donor cycles
Regular menstrual cycle (24-35 days)	Uterine malformations: cervical anomalies, submucosal uterine fibroid or endometrial polyps requiring surgery.
Age 18-43 years	Hypersensitivity to vaginal progesterone
BMI 18.5-35 (kg/m ²)	Medical contraindication to progesterone treatment
Understand written and spoken Swedish, English or Arabic and have signed a written informed consent.	Serious concomitant disease contraindicating ART and pregnancy
	Preimplantation genetic testing (PGT)
	Previously included in the ProFET study
	Participation in another study with an investigational product within the last 30 days

197 FET; Frozen embryo transfer, BMI; Body mass index.

198

199 **Treatment and intervention**

200 At the endogenous surge of LH (luteinizing hormone ; a hormone that naturally rises to
201 trigger ovulation), study participants are randomized 1:1:1 to one of three groups:

202

203 A. No vaginal progesterone

204 B. Vaginal progesterone for 3 weeks

205 C. Vaginal progesterone for 7 weeks

206

207 Patients randomized to luteal phase progesterone are instructed to administrate 100 mg
208 vaginal progesterone (Lutinus[®]; Ferring Pharmaceuticals, Saint-Prex, Switzerland) three
209 times daily starting 3 days after the LH-surge. Participants are asked to leave a blood sample
210 for analysis of serum progesterone regardless of group-allocation. A blood sample will be
211 drawn in the morning 3 days after the LH-surge, before any start of progesterone. The result
212 will not be available to the patient, neither to the treating clinician until the end of the study.

1
2
3 213 On day 5-6 after LH-surge, a blastocyst is transferred according to standard embryo transfer
4 214 procedure. Patients randomized to vaginal progesterone will continue administration of
5 215 progesterone until a pregnancy test. In the case of a positive pregnancy test patients will
6 216 continue with vaginal progesterone for a total of 3 or 7 weeks respectively. In the case of a
7 217 negative pregnancy test or miscarriage later on the patient will stop progesterone treatment.
8
9 218 See figure 1.

13 219

15 220 **Randomization**

16
17 221 Study data are recorded in an electronic case report file (e-CRF) designed by Medicase
18 222 (Sahlgrenska Science Park, Gothenburg, Sweden) which also includes a randomization
19 223 program. Randomization is stratified for:
20
21 224

22 224

- 23 225 - Previous ET not resulting in positive pregnancy test, number (0-2, ≥ 3)
- 24 226 - Parity 0/ ≥ 1
- 25 227 - Age (<35/ ≥ 35 years)
- 26 228 - Treatment site

29 229

32 230 **Blinding procedure**

33 231 The trial is not blinded, neither to patients nor to treating physicians. Analyses are done by a
34 232 statistician, blinded to group allocation.
35
36 233

37 233

39 234 **Data collection**

40
41 235 Patient-related data are collected and variables are registered in the e-CRF program at the
42 236 following time points:
43
44 237

45 237

- 46 238 1) screening before LH-surge
- 47 239 2) randomization at LH-surge
- 48 240 3) FET at LH + 5 or 6 days
- 49 241 4) result of pregnancy-test (urine sample)
- 50 242 5) early pregnancy scan (7 weeks + 5 days to 9 weeks + 0 days) in case of positive
51 243 pregnancy test
- 52 244 6) through a follow up (by telephone) after gestational week 23 + 0 days
- 53 245 7) from the patient's and the newborn's medical records after delivery

56 246

247 **Sample collection**

248 Blood samples will be collected at LH + 3 days, whenever possible. A blood sample of 5 ml is
249 analyzed for serum progesterone level. The blood samples will be sent to and analyzed at the
250 Swedish certified laboratory Unilabs and are then discarded.

251

252 **Transvaginal ultrasound scans**

253 If the patient conceives, an early transvaginal pregnancy scan will be made at gestational age
254 7 weeks + 5 days to 9 weeks + 0 days, for estimation of number of gestational sacs, number of
255 fetuses, crown-rump length and viability.

256

257 **Questionnaires**

258 The participants will be asked to fill out a questionnaire regarding registration of possible
259 study medication side effects. The form is filled out regardless of group allocation and
260 submitted in connection with vaginal ultrasound at gestational age 7 weeks + 5 days to 9
261 weeks + 0 days – or earlier in the case of a negative pregnancy test or miscarriage. Specified
262 reported symptoms will be recorded as adverse events (AEs) in the e-CRF. Serious adverse
263 events (SAEs) will be followed until two weeks after delivery.

264

265 **Data management**

266 Data is transferred to an online e-CRF; Medicase. The Medicase database is based on coded
267 subject ID numbers used in the trial. Data are stored on a server located at Sahlgrenska
268 University Hospital, Gothenburg, Sweden, with a daily backup. Only research staff at the
269 Sahlgrenska University Hospital will have access to the final dataset. Ownership of data is
270 determined by co-operation agreements as well as data processing agreements between
271 Sahlgrenska University Hospital and the participating clinics.

272

273 **STATISTICS**

274

275 **Outcome measurements**

276 Primary outcome is live birth. Secondary outcomes include biochemical and clinical
277 pregnancy rates, miscarriage rates and obstetric and neonatal outcomes in the study groups.
278 For a complete list of secondary outcomes, see supplemental file 2. Self-reported side effects
279 will be reported as mild, moderate or severe. Progesterone levels 3 days after LH-surge will
280 be measured in units of nmol/L.

281

282 Sample size calculation

283 In order to find an effect size of a 7% increase in LBR per randomized patient, measured as a
284 difference in proportions between no progesterone (0.33) and any progesterone group (0.40),
285 1800 subjects are needed if allocated 1:2. In order to find a difference between no
286 progesterone (0.33) and progesterone for 3 weeks (0.41) and 7 weeks (0.41) respectively,
287 1200 subjects are needed if allocated 1:1. Also, for the comparison between the progesterone
288 groups, 1200 subjects are needed if allocated 1:1, to detect a difference of 8%, (0.38 for 3
289 weeks of progesterone vs 0.46 for 7 weeks. For all comparisons above, except for the primary
290 analysis, a difference between groups of 8% is used. If 1800 women are allocated 1:1:1, 600
291 to no progesterone, 600 to progesterone 3 weeks and 600 to progesterone 7 week, all four
292 sample size calculations are fulfilled under the condition of a power of 0.80, a significance
293 level 0.05 and a two-sided Fisher's exact test.

294

295 We thus have two primary superiority analyses in this study. The first is the comparison of
296 LBR between no progesterone and the combined group of any progesterone with Fisher's
297 exact test on significance level 0.05. If this test is significant the probability mass of 5% will
298 be transferred to the second comparison of live birth between progesterone for 3 weeks
299 compared with progesterone for 7 weeks. If the first test is significant, we have been able to
300 show that any progesterone gives significantly higher LBR than in women without
301 progesterone. If in the second comparison 7 weeks shows significantly higher LBR than 3
302 weeks, we have also confirmed superiority regarding 7 weeks over 3 weeks. If the first
303 analysis is non-significant, we have not been able to show any confirmative results in this
304 study. The comparisons between no progesterone and 3 weeks progesterone and between no
305 progesterone and 7 weeks progesterone is performed to calculate mean difference with 95%
306 CI between these groups.

307

308 Statistical analyses

309 The main analyses will be on the full analysis set (FAS) without imputation. Complementary
310 analyses will be performed on the per protocol population. The primary efficacy analysis
311 regarding live birth will be conducted with multivariable logistic regression adjusting for all
312 stratification variables on the FAS population. The first sensitivity primary analysis will be
313 the same analysis also adjusted for the following other predefined important predictors:

314

- 315 - body-mass index
- 316 - smoking status
- 317 - duration of subfertility
- 318 - previous miscarriage (yes/no)
- 319 - blastocyst (day 5/day 6 at cryopreservation)
- 320 - number of embryos transferred

321

322 The second sensitivity primary analysis will be the same analysis as the primary efficacy
323 analysis but performed on the intention-to-treat population with multiple imputation based on
324 100 datasets. Both primary outcome and stratified variables will be imputed. For adjusted
325 analyses between two groups, multivariable logistic regression will be used for dichotomous
326 variables. If model assumption is fulfilled the corresponding model with link=log will be
327 given to present adjusted relative risk (RR) with 95% CI. For continuous variables analysis of
328 covariance (ANCOVA) will be used for adjusted analysis between two groups.

329

330 Explorative unadjusted mean difference between the two groups with 95% confidence
331 interval (CI) will be given for dichotomous variables and continuous variables together with
332 effect sizes. For continuous variables these 95% CI will be based on T-test or Fisher's non-
333 parametric permutation test. For dichotomous variables relative risk and odds ratio will be
334 given with 95% CI. Proportions will be given with exact 95% CI.

335

336 For comparison between two groups Mantel – Haenszel Chi square test will be used for
337 ordered categorical variables and Fisher's exact test for dichotomous variables.

338 The distribution of continuous variables will be given as mean, standard deviation (SD),
339 median, first and third quartiles (Q1, Q3), minimum and maximum. All significance tests will
340 be two-sided and conducted at the 5% significance level.

341

342 **Monitoring**

343 All study participants are monitored to meet the inclusion criteria and a check is made that
344 voluntarily informed consent for each study participants is obtained and documented. For all
345 study participants, the main parameters in the study are monitored (live birth, clinical
346 pregnancy, miscarriage and ectopic pregnancy). The first two study participants at each center
347 will be monitored with a complete source data verification. Thereafter, a complete source data
348 verification will be performed on every fifth randomly selected study participant.

349

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3 350 **Patient and public involvement**

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5 351 Development of this study protocol was done without patient or public involvement. The final
6
7 352 study results will be disseminated to participants on request.

8
9 353

10 354 **ETHICS AND DISSEMINATION**

11
12 355

13 356 The study was approved by the Swedish Ethical Review Authority (ID 2020-06774 and 2021-
14 357 02822) and the Swedish Medical Products Agency (ID nr 5.1-2020-102613). All participants
15 358 are required to sign a written informed consent form before study entry (supplemental file 1).
16
17 359 The safety of participants in this study is high. As the medication/treatment with vaginal
18 360 progesterone is well known, SAEs or suspected unexpected serious adverse reactions
19 361 (SUSARs) are unlikely. If, however, a participant should experience a SAE or a SUSAR the
20 362 local investigator will contact the principal investigator with no delay and the individual
21 363 treatment will be stopped immediately.

22
23 364

24 365 The results of this trial will be presented at national as well as international scientific
25 366 congresses and published in international scientific journals. The results of the research will
26 367 also be disseminated to public through broadcasts, popular science articles, and newspapers.

27
28 368

29 369 **DISCUSSION**

30
31 370

32 371 The rapidly increasing use of FET worldwide and the limited evidence concerning cycle
33 372 regimens for FET demands further well designed large randomized trials. Progesterone
34 373 supplementation in NC-FET is widely used despite scarce evidence. Two RCTs with LBR as
35 374 main outcome have been published(27, 28). In a Swedish study where mainly cleavage stage
36 375 embryos and single- as well as double embryo transfer were used, a significantly higher LBR
37 376 was found(27). Further, a small study from Israel, including only 59 patients, using a
38 377 modified NC-FET protocol, also found a significantly higher LBR after LPS compared with
39 378 no progesterone(28). The study included a mix of cleavage stage embryos and blastocysts and
40 379 up to three embryos were transferred.

41
42 380

43 381 Available retrospective studies on LPS reveal the use of different embryo stages at embryo
44 382 transfer; two-nucleus stage(29) cleavage stage embryos(30) and both cleavage embryo and
45 383 blastocyst transfers(31). All these studies used human Chorionic Gonadotropin (hCG) as

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2
3 384 ovulation trigger and administration of progesterone supplementation was started at different
4
5 385 time points after LH-surge and was administrated either as intramuscular injections or as
6
7 386 vaginal suppositories with different doses and duration of treatment.
8
9 387

10 388 This presented ongoing large open-label multicenter randomized clinical trial aims to
11
12 389 investigate if vaginal LPS in NC-FET is superior to no LPS. In this set up, not only the
13
14 390 differences in LBR and clinical pregnancy rates will be investigated, but also, the obstetrical
15
16 391 and perinatal outcomes. This study will contribute to recommendations regarding LPS in NC-
17
18 392 FET in the future.
19 393

20 394 The strength of this trial is the multicenter, randomized design and a large sample size of
21
22 395 1800 women. Broad inclusion criteria representing the patient cohort in everyday practice will
23
24 396 ensure a high generalizability. The IVF protocols consist only of natural cycles with no
25
26 397 ovulation trigger. The study is not blinded to participants or investigators, which is a
27
28 398 limitation, however, the use of a robust primary outcome (live birth) makes this less likely to
29
30 399 introduce bias.
31 400

32
33 401 If progesterone supplementation in natural FET cycles should be shown to significantly
34
35 402 increase the chance of live birth, the benefit for the patients, as well as for the society, would
36
37 403 mean a) a shorter time to pregnancy, b) fewer IVF cycles needed per patient, c) reduced costs
38
39 404 for patients and society, d) less environmental burden due to less cycles to achieve live birth
40
41 405 and thus less use of hormonal IVF-treatment. On the other hand, if no beneficial effect of this
42
43 406 treatment can be shown, it should be abandoned and thereby implicate less financial burden
44
45 407 for patients as well as for society, less treatment burden for the patient and less environmental
46
47 408 impact associated with the use of LPS.

48 409 **Contributors**

49
50 410 CB and ÅM were the primary initiators of the study, who designed and wrote the first version
51
52 411 of the study protocol. AK, AS and CS contributed to the revision and editing of the study
53
54 412 protocol. AK, AS, CB, CS, EL, GWe, GWi, KHO, KRW, MB, MK, SL and ÅM will all be
55
56 413 involved in the recruitment of patients and data collection. All authors approved the final
57
58 414 version of the study protocol. AS and CS applied to the Swedish Ethical Review Authority.
59
60 415 CB and ÅM applied to the Swedish Medical Products Agency. AK and CS wrote the first

1
2
3 416 draft of this manuscript which was revised by AS, CB and ÅM. All authors approved this
4
5 417 protocol.

6 418

7
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9
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11
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13
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15
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17
18 424 and by the Hjalmar Svensson foundation. We will also apply for further funding.

19 425

20 426 **Disclaimer**

21
22 427 Ferring Pharmaceuticals has not been involved in the design of the study protocol, nor will
23
24 428 they be involved in the conduct of the study or any analysis or reporting of the results.

25 429

26
27 430 **Competing interests**

28
29 431 CB and ÅM declare support from Ferring Pharmaceuticals, Merck Sereno and Gedeon
30
31 432 Richter. MB has 4 % stocks in EUGIN Sweden. None of the other authors had conflicts of
32
33 433 interest.

34 434

35
36 435 **Patient consent for publication**

37 436 Not required.

38
39 437

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- 59 531
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2
3 532 FIGURE LEGENDS

4 533

5 534 **Figure 1: ProFET trial flowchart**

6 535 LH; Luteinizing hormone, FET; Frozen embryo transfer, NC-FET; Natural cycle frozen
7 536 embryo transfer.

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For peer review only

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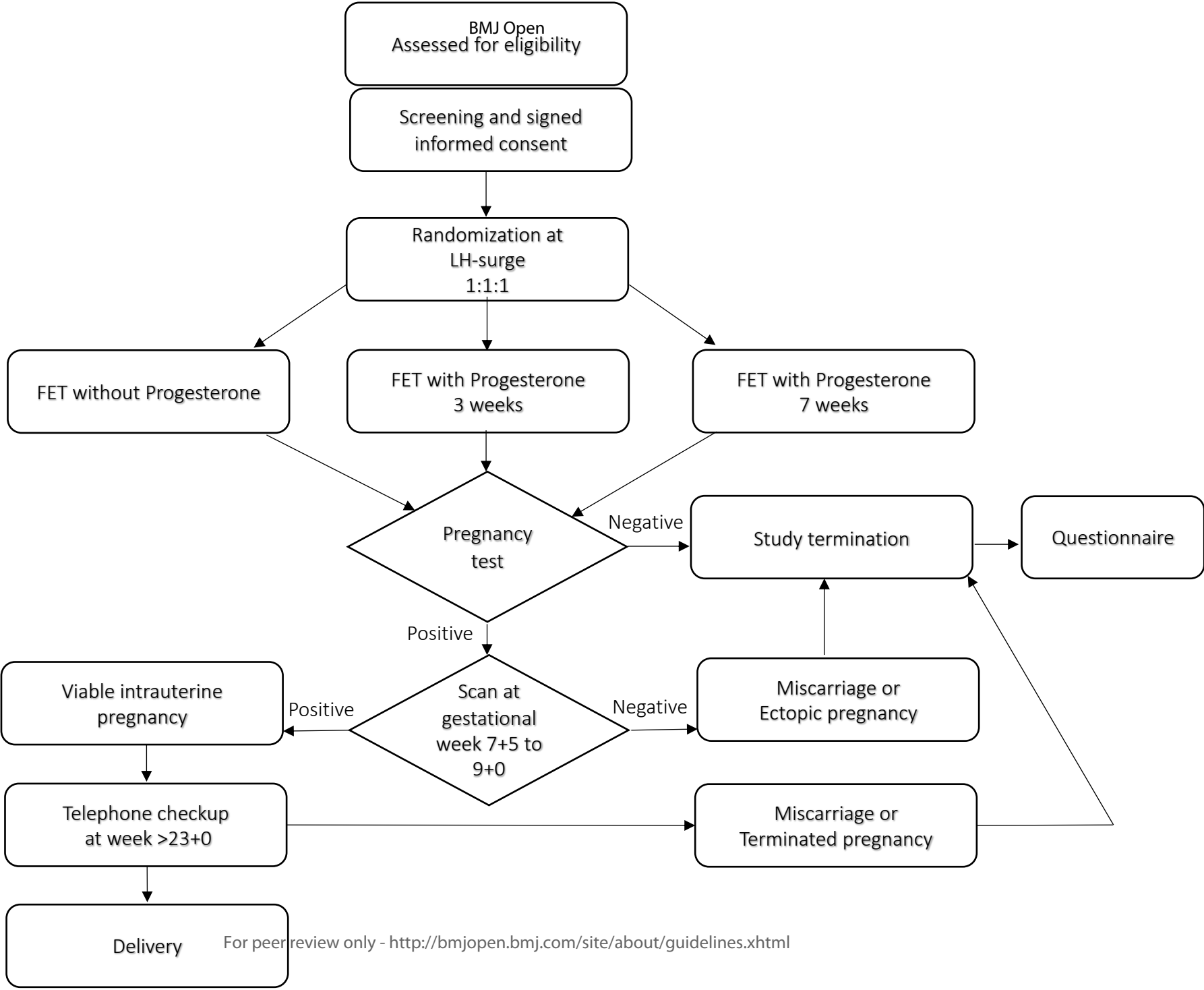
Planning
NC-FET

LH + 0 days

LH + 5-6 days

FET + 10 days

Pregnancy follow up
until delivery



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Study information ProFET

Treatment with Progesterone after IVF with Frozen Embryo Transfer in a natural cycle with ovulation

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The ProFET trial has its name from Progesterone, and FET, short for Frozen Embryo Transfer.

21

Information to participants in the trial

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We hereby ask for your participation in the ProFET study. In this document we provide information about the project and what participation may entail.

26

What kind of research project is this? Why are we asking you to participate?

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In IVF treatment (In Vitro Fertilisation) it has become increasingly common to transfer a frozen/thawed embryo. Frozen embryo transfer accounts for 46 % of all IVF treatments in Sweden (www.qivf.se, Annual report 2020). The increased use of frozen embryo transfer is due to improved results after the introduction of new freezing procedures and embryo culture methods.

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The corpus luteum, occurring in the ovary after ovulation, produces progesterone to support an early pregnancy. In IVF-cycles, where no ovulation has occurred, extra progesterone is needed and provided as medication after embryo transfer. In the same way, treatment with progesterone is given to all patients undergoing IVF stimulation with transfer of a fresh embryo, as the own hormone production during these treatments is suppressed.

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Today, it is not known whether progesterone treatment after a frozen embryo transfer in a natural ovulatory cycle, improves the chance of live birth. Nevertheless, this treatment is sometimes given, despite its lack of known benefits to the patient.

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The ProFET trial aims to find out if the addition of progesterone after a frozen embryo transfer in a natural cycle increases the chances of live birth. Each participant will be randomly assigned to one out of three groups. Group A will undergo frozen embryo transfer without the addition of progesterone. This group corresponds to normal clinical routine. Group B will be treated with progesterone, taken as a vaginal tablet, three times daily under three weeks. Group C will be treated with progesterone, taken as a vaginal tablet, three times daily during seven weeks. Some women will be asked for an additional blood sample, to measure their blood progesterone levels, before frozen embryo transfer.

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5 You are asked to participate because you are currently undergoing IVF treatment with a
6 planned frozen embryo transfer in a natural cycle. You are between 18 to 43 years of age,
7 have a BMI (body mass index) between 18.5 – 35 kilogram/m², a regular menstrual cycle, and
8 understand Swedish, English or Arabic.
9

10
11 The research principal for the project is the Reproductive Medicine unit at the Sahlgrenska
12 University Hospital, Region Västra Götaland.
13
14

15 **Research design**

16
17
18 The section below describes the participation in the trial.
19

- 20 1. You will be in contact with a doctor or nurse, in order to plan your frozen embryo
21 transfer. If you meet the criteria for participation and wish to participate, you are asked
22 to sign a consent form at the clinic or a digital consent form via 1177. You will also
23 receive a questionnaire where you will keep notes on any symptoms after embryo
24 transfer. You will receive this form even if you belong to the group that does not take
25 any medicine.
26
27
- 28 2. Once you have a positive ovulation test, we ask you to contact your clinic according to
29 ordinary routines and schedule an appointment for a frozen embryo transfer. One of the
30 study doctors or nurses will randomly assign you to one of the three groups. The
31 participants in Group A undergo a frozen embryo transfer without any additional
32 treatment. Group B is prescribed vaginal tablets containing progesterone three times
33 daily for three weeks. Group C is prescribed vaginal tablets containing progesterone
34 three times daily for seven weeks. It is not possible, as a participant in the clinical trial,
35 to ask to be placed in a particular group. The group allocation is computerised. If you are
36 allocated to one of the groups treated with progesterone, you commence your treatment
37 three times daily, starting three days after the positive ovulation test. You can pick up
38 the medicine at any pharmacy, e-prescriptions are sent by the responsible doctor.
39
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46 When possible, we will draw a blood sample three days after the positive ovulation test,
47 but before starting the progesterone treatment. A blood sample is taken even if you
48 belong to the group that does not receive progesterone. The blood sample is drawn from
49 a vein in the arm. Approximately 5 ml, roughly the amount of a teaspoon is needed. The
50 test is called S-Progesterone and measures the level of progesterone (corpus luteum
51 hormone) in the blood.
52
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56 3. Frozen embryo transfer.
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5 4. A urinary pregnancy test is taken at home according to routine instructions given after
6 a frozen embryo transfer. Contact via phone with the doctor/nurse responsible for the
7 trial, regarding outcomes/results:
8

9 Not pregnant: Your participation in the trial ends. Questionnaire is handed in. If you
10 belong to one of the groups treated with progesterone (Groups B or C), your treatment
11 with the drug ends.
12

13
14
15 Pregnant: If you belong to Groups B or C (treated with progesterone) you continue
16 with your treatment for three or seven weeks, respectively, based on what group you
17 were assigned to. If you belong to the group that will take progesterone tablets for
18 seven weeks, a new e-prescription will be sent for prolonged treatment.
19 A transvaginal ultrasound is scheduled for gestational week eight or nine to confirm
20 pregnancy. Questionnaire is handed in. Routine ultrasounds during pregnancy will be
21 offered, as standard for all pregnant women. After gestational week 22 you will receive
22 a phone call by the study nurse, who will ask about how your pregnancy proceeds.
23
24
25

26
27 Participants in the trial are required to fill out a questionnaire regarding unexpected
28 symptoms, which can be attributed to the administered drug, or to other causes. Participation
29 may require self-administration of a vaginal tablet three times per day for a period of three or
30 seven weeks.
31
32

33
34 Expenses for progesterone tablets, will be reimbursed financially by the ProFET trial at the
35 Reproductive Medicine unit at the Sahlgrenska University Hospital.
36

37
38 We will also collect data from national registries (Pregnancy Register, Swedish Neonatal
39 Quality Register, Statistics Sweden) and from medical records on antenatal care and delivery,
40 as well as your child's records, regarding your child's condition.
41
42
43

44 **Possible outcomes and risks**

45
46 The treatment does not involve any risk unless you have a hypersensitivity to the drug or have
47 any of the diseases contraindicating the use of the study drug. Patient with any of these
48 conditions will be allowed to participate in the trial.
49

50 Participation in the trial does not entail discomfort or pain. Some patients experience the
51 vaginal suppository as smudgy. There are no known long-term side effects related to
52 treatment with vaginal progesterone suppositories, that could lead to injury or risk.
53
54

55 Should you experience discomfort or have any questions, you are welcome to contact the
56 study nurse during office hours. In case of acute gynaecological problems outside office
57 hours, you should contact a gynaecological emergency department. If you become ill in some
58 way, are hospitalised or on sick leave, you must report this to the study nurse or the doctor
59
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5 responsible for the trial, as all illness during an ongoing trial must be reported in accordance
6 with current rules.
7

8 **Information about your stored data**

9
10 The project will collect data and keep relevant records about you. The IVF treatment will be
11 recorded in the clinic's regular medical record system and is protected by confidentiality. If a
12 blood sample is drawn, the test result will be entered in your medical chart and in a specific
13 research database. Collected research data will be stored without name or social security
14 number, but instead under a study code number, protected in a designated research data base.
15 All personal data is confidential, and no unauthorized person will be able to access it.
16
17

18
19 When the collected data is analysed, no individual can be identified. The same also applies
20 when the trial and its result are reviewed by an independent safety committee, and when
21 results from the trial is published in scientific journals.
22

23
24 Data will be archived for fifteen years, in accordance with research regulations. Data analysis
25 is solely for research purposes, and the legal basis is public interest/research in accordance
26 with EU:s data protection regulation for the treatment.
27
28

29
30 The collected data is the responsibility of the board of the Sahlgrenska University Hospital. In
31 accordance with EU:s data protection regulation you are entitled – without cost – to view your
32 own trial records. You are also entitled to have any potentially false data corrected. You are
33 also entitled to request your records being erased or limited in access. If you want to review
34 your records, please contact the Principal Investigator, dr Åsa Magnusson,
35 Reproduktionsmedicin, Sahlgrenska Universitetssjukhuset, e-mail:
36 asa.magnusson@vgregion.se Telephone: 031-342 10 00. Data protection officer is reachable
37 at: Sahlgrenska Universitetssjukhuset, Dataskyddombudet, 413 45 Göteborg. Telephone 031-
38 343 27 15. sahlgrenska.universitetssjukhuset.dso@vgregion.se. Any complaint with how
39 your personal data is handled, may be submitted to the Integrity Protection Authority which
40 is supervisory authority.
41
42
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44

45 **How do I get information about the results from the trial?**

46
47 The research will be published in international scientific journals. The research team
48 encourages all participating IVF clinics to present the results on their respective websites.
49
50

51 **Insurance and compensation**

52
53 IVF treatment is covered by the Swedish patient injury insurance. Participation in the trial
54 does not involve any additional costs, and therefore no compensation is offered for
55 participation.
56

57 **Participation is voluntary**

58
59 Your participation is entirely voluntary, and you may at any time withdraw your consent
60 without giving an explanation. A withdrawal will not impact on your future care or treatment.

Should you wish to stop your participation, please contact the responsible parties for the trial.
(See information below.)

Responsible for the trial

The main investigators are:

- Dr. Åsa Magnusson, e-mail asa.magnusson@vgregion.se telephone 031-342 10 00
 - Dr. Caroline Stadelmann, e-mail caroline.stadelmann@vgregion.se telephone 031-343 67 59
- both at Reproductive Medicine, Sahlgrenska University Hospital, Gothenburg.

The following sections are edited for each clinic. Locally responsible for the trial are at:

Livio Fertility Center, Gothenburg

- Dr. Göran Westlander, e-mail goran.westlander@livio.se telephone 031 710 46 30

Nordic IVF, Gothenburg

- Dr. Eva Lundborg, e-mail eva.lundborg@nordicivf.se telephone 031-333 09 70

Carl von Linnékliniken, Uppsala

- Dr. Thomas Brodin, e-mail thomas.brodin@linne.se telephone 018-55 13 02

Stockholm IVF

- Prof. Mats Brännström, e-mail mats.brannstrom@obgyn.gu.se telephone 08-420 036 09

University Hospital in Linköping

- Dr. Susanne Liffner, e-mail susanne.m.liffner@regionostergotland.se telephone 010-103 00 00

The Fertility Unit, University Hospital in Örebro

- Dr. Gabriella Widlund, e-mail gabriella.widlund@regionorebrolan.se telephone 019-602 30 86

Reproductive Medicine Center (RMC), Malmö

- Dr. Margareta Kitlinski, e-mail margareta.kitlinski@skane.se telephone 040-33 21 64

Livio Reykjavik

- Dr. Snorri Einarsson, e-mail snorri.einarsson@livio.is telephone +35 4 430 40 00

Reproductive Medicine, Karolinska University Hospital

- Prof. Kenny Rodriguez-Wallberg, e-mail kenny.rodriquez-wallberg@ki.se telephone 08-585 87 506

Livio Fertility Center Umeå

- Dr. Sofia De Sousa Soares, e-mail sofia.desousasoares@livio.se telefon 090 785 69 41

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5 IVF-gruppen vid Sophiahemmet AB

6 -Dr Arthur Aanesen, e-mail arthur.aanesen@livio.se telefon 0706 717701

7
8 Livio Falun

9 -Dr Bo Claesson, e-mail bo.claesson@livio.se telefon 023 17324

10
11 Livio Gärdet

12 -Dr Camilla Stenfelt, e-mail camilla.stenfelt@livio.se telefon 08-58612000

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24 **Consent to participate in the study**

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27 I have received oral and written information about the trial and have had the opportunity to
28 ask questions. I may keep the written information.

29
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31
32
33 I agree to participate in the study "ProFET". At the same time, I agree that information
34 about me is processed in the manner described in the research study information and
35 that data from described records and registers may be obtained.
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41 **Study participant**

42
43 Social security number:

Place and date	Signature
	Name clarification

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59 Doctor who receives consent:
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Place and date	Signature
	Name clarification

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Supplemental file 2

The ProFET trial – a complete list of secondary outcomes

1. Number of participants with biochemical pregnancy [Time Frame: 2-3 weeks after embryo transfer.]

A pregnancy diagnosed only by the detection of beta hCG in serum or urine.

2. Number of participants with clinical pregnancy [Time Frame: 4-8 weeks after embryo transfer.]

A pregnancy diagnosed by ultrasonographic visualization of one or more gestational sacs.

3. Number of participants with ongoing pregnancy [Time Frame: 5-7 weeks after embryo transfer.]

An intrauterine pregnancy with one or more fetuses with heartbeats measured in gestational week 7+5 to 9+0 with vaginal ultrasound.

4. Number of participants with miscarriage [Time Frame: Up to 20 weeks after embryo transfer.]

The spontaneous loss of an intra-uterine pregnancy prior to 22 completed weeks of gestational age. Also, the outcome will be reported according to Core Outcome Measure for Infertility Trials (Duffy et al., 2020) in a separate appendix.

5. Number of participants with ectopic pregnancy [Time Frame: Up to 20 weeks after embryo transfer.]

A pregnancy outside the uterine cavity, diagnosed by ultrasound, surgical visualization, or histopathology.

6. Number of participants with termination of pregnancy [Time Frame: Up to 20 weeks after embryo transfer.]

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2
3 Defined as the termination of a clinical pregnancy, by deliberate interference that
4 takes place before 22 completed weeks of gestational age. Also, the outcome will be
5 reported according to Core Outcome Measure for Infertility Trials (Duffy et al.,
6 2020) in a separate appendix.
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11 7. Birth weight [Time Frame: Up to 41 weeks after embryo transfer.]
12

13 Defined as weight in grams at birth.
14
15

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18 8. Gestational age at delivery [Time Frame: Up to 41 weeks after embryo transfer.]
19

20 The gestational age at FET is calculated by adding the number of culture days to
21 ovulation (ovulation=day 14). Gestational age at delivery is then calculated by
22 adding the number of days since FET.
23
24
25

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27
28 9. Preterm birth [Time Frame: Up to 35 weeks after embryo transfer.]
29

30 Defined as a child born alive before 37 completed weeks of pregnancy.
31
32

33
34 10. Very preterm birth [Time Frame: Up to 30 weeks after embryo transfer.]
35

36 Defined as a child born alive before 32 completed weeks of pregnancy.
37
38
39

40
41 11. Low birth weight [Time Frame: Up to 41 weeks after embryo transfer.]
42

43 Birth weight less than 2500 g.
44
45
46

47
48 12. Very low birth weight [Time Frame: Up to 41 weeks after embryo transfer.]
49

50 Birth weight less than 1500 g.
51
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53

54
55 13. Stillbirth [Time Frame: Up to 41 weeks after embryo transfer.]
56

57 The death of a fetus prior to the complete expulsion or extraction from its mother,
58 after and including 22 completed weeks of gestational age. Also, the outcome will
59
60

1
2
3 be reported according to Core Outcome Measure for Infertility Trials (Duffy et al.,
4 2020) in a separate appendix.
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9 14. Perinatal death [Time Frame: Up to 41 weeks after embryo transfer and 7 days after
10 birth.]
11

12 Fetal or neonatal death occurring during late pregnancy (at 22 completed weeks of
13 gestational age and later), during childbirth, or up to seven days after birth. Also,
14 the outcome will be reported according to Core Outcome Measure for Infertility
15 Trials (Duffy et al., 2020) in a separate appendix.
16
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19

20 15. Number of children with birth defects [Time Frame: Up to 41 weeks after embryo
21 transfer.]
22

23 Congenital birth defects were defined according the International Statistical
24 Classification of Diseases and Related Health Problems (ICD-10). And further
25 defined according to the EUROCAT classification system.
26
27
28
29
30

31 16. Number of children admitted to Neonatal Intensive Care Unit (NICU)
32 [Time Frame: Up to 41 weeks after embryo transfer and 7 days after birth.]
33

34 Defined as children that were admitted to NICU after birth.
35
36
37
38

39 17. Number of participants with hypertensive disorders of pregnancy [Time Frame: Up to
40 41 weeks after embryo transfer including the postpartum period before discharge of
41 mother.]
42

43 Hypertensive disorders of pregnancy defined as high blood pressure disorders
44 including preeclampsia, gestational hypertension and chronic hypertension.
45
46
47
48

49 18. Number of participants with placenta previa [Time Frame: Up to 41 weeks after
50 embryo transfer.]
51

52 Defined as a placenta covering the internal os of the cervix, at time of delivery.
53
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57 19. Number of participants with placenta abruption [Time Frame: Up to 41 weeks after
58 embryo transfer.]
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3 Defined as the premature separation of a normally located placenta from the uterine
4 wall that occurs before delivery of the fetus.
5
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- 8
9 20. Number of participants with postpartum hemorrhage [Time Frame: Up to 41 weeks
10 after embryo transfer.]
11

12 Defined as a cumulative blood loss of greater than 1,000 mL or blood loss
13 accompanied by signs or symptoms of hypovolemia within 24 hours after the birth
14 process.
15
16

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18
19 21. Number of participants with Cesarean section [Time Frame: Up to 41 weeks after
20 embryo transfer.]
21

22 Defined as a surgical procedure used to deliver a baby through incisions in the
23 abdomen and uterus.
24
25

- 26
27
28 22. Number of participants with thromboembolic events [Time Frame: Up to 41 weeks
29 after embryo transfer including the postpartum period before discharge of mother.]
30

31 Defined as formation in a blood vessel of a clot (thrombus) that breaks loose and is
32 carried by the blood stream to plug another vessel.
33
34

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37 23. Maternal mortality [Time Frame: Up to 41 weeks after embryo transfer including the
38 postpartum period before discharge of mother.]
39

40 Defined as female deaths from any cause related to or aggravated by pregnancy or
41 its management (excluding accidental or incidental causes) during pregnancy and
42 childbirth.
43
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46
47 24. Number of participants with treatment related side effects [Time Frame: Up to 8
48 weeks after embryo transfer.]
49

50 Side effects reported according to study specific questionnaire. Questions are
51 answered with yes or no. If yes, symptoms are described, but not by using a scale.
52
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56 25. Number of participants with adverse events [Time Frame: Up to 8 weeks after
57 embryo transfer.]
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3 Any untoward medical occurrence in symptom or disease temporally associated
4 with the use of the medicinal (investigational) product, whether or not related to the
5 medicinal product.
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10 26. Cost effectiveness [Time Frame: After study completion, an average of 1 year.]
11

12 Comparison between groups regarding the total costs for the intervention divided by
13 treatment efficacy (live birth).
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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	___ 1 ___
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	___ 3 ___
	2b	All items from the World Health Organization Trial Registration Data Set	___ NA ___
Protocol version	3	Date and version identifier	___ NA ___
Funding	4	Sources and types of financial, material, and other support	___ 15 ___
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	___ 14-15 ___
	5b	Name and contact information for the trial sponsor	___ 15 ___
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	___ 15 ___
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	___ 14-15 ___

1	Introduction			
2				
3	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant	_____ 1-6 _____
4	rationale		studies (published and unpublished) examining benefits and harms for each intervention	
5				
6		6b	Explanation for choice of comparators	_____ NA _____
7				
8	Objectives	7	Specific objectives or hypotheses	_____ 6 _____
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),	
11			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	_____ 6-10 _____
12				
13				
14	Methods: Participants, interventions, and outcomes			
15				
16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	_____ 6 _____
17			be collected. Reference to where list of study sites can be obtained	
18				
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	_____ 7; table 1 _____
20			individuals who will perform the interventions (eg, surgeons, psychotherapists)	
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be	_____ 7-8 _____
23			administered	
24				
25		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose	_____ NA _____
26			change in response to harms, participant request, or improving/worsening disease)	
27				
28		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence	_____ NA _____
29			(eg, drug tablet return, laboratory tests)	
30				
31		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	_____ 7; table 1 _____
32				
33	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood	_____ 11 _____
34			pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation	
35			(eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen	
36			efficacy and harm outcomes is strongly recommended	
37				
38	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits	_____ 6 _____
39			for participants. A schematic diagram is highly recommended (see Figure)	
40				
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1	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	_____ 11 _____
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	_____ NA _____
5				

6 **Methods: Assignment of interventions (for controlled trials)**

7 Allocation:

8				
9				
10	Sequence	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	_____ 8 _____
11	generation			
12				
13				
14				
15				
16	Allocation	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	_____ 8 _____
17	concealment			
18	mechanism			
19				
20				
21	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	_____ 7 _____
22				
23				
24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	_____ 8 _____
25				
26				
27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	_____ NA _____
28				
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31 **Methods: Data collection, management, and analysis**

32				
33	Data collection	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	_____ 9 _____
34	methods			
35				
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39		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	_____ NA _____
40				
41				
42				

1	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	_____ 10 _____
2				
3				
4				
5	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	_____ 11-12 _____
6				
7				
8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_____ 11-12 _____
9				
10		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	_____ NA _____
11				
12				
13				

Methods: Monitoring

14				
15				
16	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_____ 12-13 _____
17				
18				
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21				
22		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	_____ NA _____
23				
24				
25	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_____ 13 _____
26				
27				
28	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_____ NA _____
29				
30				
31				

Ethics and dissemination

32				
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	_____ 13 _____
35				
36				
37	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	_____ NA _____
38				
39				
40				
41				
42				

1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	___7,13___
2				
3				
4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	___NA___
5				
6				
7	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	___10___
8				
9				
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	___15___
11				
12				
13	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	___10___
14				
15				
16	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	___NA___
17				
18				
19				
20	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	___10___
21				
22				
23				
24		31b	Authorship eligibility guidelines and any intended use of professional writers	___NA___
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	___NA___
27				
28				
29	Appendices			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	___3,7___
32				
33				
34	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	___8-9___
35				
36				

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons [“Attribution-NonCommercial-NoDerivs 3.0 Unported”](https://creativecommons.org/licenses/by-nc-nd/3.0/) license.