

BMJ Open

A “Freeze-all” versus a “Fresh embryo transfer”-strategy in assisted reproductive technology (ART): Study protocol for a multicentre randomised controlled trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-016106
Article Type:	Protocol
Date Submitted by the Author:	27-Jan-2017
Complete List of Authors:	<p>Stormlund, Sacha; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p> <p>Løssl, Kristine; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p> <p>Zedeler, Anne; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p> <p>Bogstad, Jeanette; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p> <p>Prætorius, Lisbeth; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p> <p>Nielsen, Henriette; The Fertility Clinic, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark</p> <p>Bungum, Mona; Reproductive Medicine Centre, Skane University Hospital, Malmo, Sweden</p> <p>Skouby, Sven; The Fertility Clinic, Department of Obstetrics and Gynaecology, Herlev University Hospital, Copenhagen, Denmark</p> <p>Mikkelsen, Anne Lis; The Fertility Clinic, Department of obstetrics and Gynaecology, Holbæk University Hospital, Holbæk, Denmark</p> <p>Andersen, Anders; The Fertility Clinic, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark</p> <p>Bergh, Christina; Department of Obstetrics and Gynaecology, Institute of Clinical Sciences, Sahlgrenska Academy, Gothenburg University, Reproductive Medicine, Sahlgrenska University Hospital, SE-413 45 Gothenburg, Sweden</p> <p>Humaidan, Peter; The Fertility Clinic, Skive Regional Hospital</p> <p>Pinborg, Anja; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p>
Primary Subject Heading:	Reproductive medicine
Secondary Subject Heading:	Obstetrics and gynaecology
Keywords:	ART, FET, Freeze-all, RCT, Ongoing pregnancy rate, OPR

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



SCHOLARONE™
Manuscripts

For peer review only

TITLE PAGE

PROTOCOL ARTICLE

**A “Freeze-all” versus a “Fresh embryo transfer”-strategy in
assisted reproductive technology (ART):
Study protocol for a multicentre randomised controlled trial**

Authors

Sacha Stormlund¹, Kristine Løssl¹, Anne Zedeler¹, Jeanette Bogstad¹, Lisbeth Prætorius¹,
Henriette Svarre Nielsen², Mona Bungum³, Sven O. Skouby⁴, Anne Lis Mikkelsen⁵, Anders Nyboe
Andersen², Christina Bergh⁶, Peter Humaidan⁷, Anja Pinborg¹

Author Affiliations:

¹The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark

²The Fertility Clinic, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

³Reproductive Medicine Centre, Skane University Hospital, Malmo, Sweden

⁴ The Fertility Clinic, Department of Obstetrics and Gynaecology, Herlev University Hospital, Copenhagen, Denmark

⁵ The Fertility Clinic, Department of obstetrics and Gynaecology, Holbæk University Hospital, Holbæk, Denmark

⁶ Department of Obstetrics and Gynaecology, Institute of Clinical Sciences, Sahlgrenska Academy, Gothenburg University, Reproductive Medicine, Sahlgrenska University Hospital, SE-413 45 Gothenburg, Sweden

⁷ The Fertility Clinic, Skive Regional Hospital and Faculty of Health, Aarhus University, Aarhus, Denmark

Correspondence to: Sacha Stormlund; sacha.stormlund.01@regionh.dk

ABSTRACT

Introduction Pregnancy rates after frozen-thawed embryo transfer (FET) have improved in recent years and are now approaching or even exceeding those obtained after fresh embryo transfer.

This is partly due to improved laboratory techniques, but may also be caused by a more physiological hormonal and endometrial environment in FET cycles. Furthermore, the risk of ovarian hyperstimulation syndrome (OHSS) is practically eliminated in segmentation cycles followed by FET and the use of natural cycles in frozen-thawed embryo transfers may be beneficial for the post-implantational conditions of foetal development. However, a freeze-all strategy is not yet implemented as standard care due to limitations of large randomised trials showing a benefit of such a strategy. Thus, there is a need to test the concept against standard care in a randomised controlled design. This study aims to compare ongoing pregnancy and live birth rates between a freeze-all strategy with GnRH agonist triggering versus hCG trigger and fresh embryo transfer in a multicentre randomised controlled trial.

Methods and analysis Multicentre randomised, controlled, double-blinded trial of women undergoing ART treatment including 424 normo-ovulatory women aged 18 to 40 from Denmark and Sweden. Participants will be randomised (1:1) to one of two treatment groups of either A. hCG trigger and single blastocyst transfer in the fresh (stimulated) cycle or B. GnRH agonist trigger and single vitrified-warmed blastocyst transfer in a subsequent hCG triggered natural menstrual cycle. The primary endpoint is to compare ongoing pregnancy rates in the two treatment groups after the first single blastocyst transfer.

Ethics and dissemination

The study will be performed in accordance with the ethical principles in the Helsinki Declaration. The study is approved by the Scientific Ethical Committees in Denmark and Sweden. The results of the study will be publically disseminated.

Trial registration numbers: ClinicalTrials.gov identifier: NCT02746562; Ethical Approval, Denmark: H-1600-1116, Ethical Approval, Sweden: Dnr. 2016/654

Strengths and limitations of this study

- The design: A multicentre, randomised controlled double-blinded trial
- Superiority study powered to identify an increase in ongoing pregnancy rate in the freeze-all group compared to the conventional fresh blastocyst transfer group
- The dose and type of gonadotropin are decided and entered in the randomisation program by the doctor before randomisation is performed
- Doctors and patients are blinded to the randomisation result until the end of the controlled ovarian stimulation, which avoids bias from adjustments in gonadotropin stimulation dose
- The study includes normo-ovulatory women aged 18-39 years with a BMI < 35 thus results can be extrapolated to the majority of the normo-ovulatory infertile population
- GnRH-agonist trigger in the freeze-all group adds a concept of an OHSS-free strategy
- As both GnRH-agonist trigger and elective freeze-all are new treatment approaches, we will not be able to distinguish the two effects from each other, but compare an OHSS-free strategy to a conventional fresh transfer strategy

INTRODUCTION

The use of assisted reproductive technology (ART) is increasing and presently up to 5 % of birth cohorts in certain countries are conceived by ART.¹ In recent years, pregnancy rates following frozen embryo transfer (FET) have rapidly increased and may now be a viable and appropriate alternative to the conventional fresh embryo transfer in ART. The main reason is the introduction of vitrification, increasing post-thawing survival rates after blastocyst culture significantly as compared to previous years.²⁻³ Implantation as well as clinical and on-going pregnancy rates are correspondingly improving in frozen cycles and approaching or even exceeding those associated with fresh embryo transfer.⁴⁻⁶

A freeze-all strategy has been suggested as a way to further improve success rates in ART, arguing that the use of the best embryo in frozen cycles instead of in fresh cycles, as is conventionally done, may potentially increase pregnancy rates and live birth rates.⁶⁻⁷ The rationale is that transfer of a frozen-thawed embryo in a subsequent natural menstrual cycle has the advantage of an endometrium that has not been exposed to the supraphysiological levels of estradiol and progesterone following controlled ovarian stimulation (COS) in fresh cycles, which may negatively affect endometrial receptivity.^{5,8} Elective FET (eFET) moreover has the benefit of essentially eliminating the risk of developing late ovarian hyperstimulation syndrome (OHSS) associated with the pregnancy-related rise in human chorionic gonadotropin (hCG) levels.⁹ If ovulation is induced with a GnRH agonist instead of hCG and all embryos are subsequently frozen, even early OHSS is minimized making the overall OHSS risk extremely low.¹⁰ Freezing and thawing of embryos additionally enables an elective single embryo transfer policy with cumulative pregnancy rates similar to those seen after double embryo transfer, encouraging an elective single embryo transfer policy.¹¹⁻¹²

Despite evidence suggesting that ART outcomes may be further improved with the adaptation of a freeze-all strategy, the implementation remains a topic of ongoing debate and only one in five transfers in Europe on average was performed with frozen-thawed embryos in 2012.¹ In a large recent study, including 1508 patients with polycystic ovary syndrome comparing the freeze-all strategy with conventional fresh embryo transfer, the authors found a significantly higher frequency of live birth after the first frozen embryo transfer compared with fresh-embryo transfer (49.3% vs. 42.0%).⁷ Correspondingly, in a meta-analysis including three trials accounting for 633 cycles in women aged 27-33 years, eFET resulted in significantly higher clinical and ongoing pregnancy rates compared with fresh embryo transfer.⁶ However, the included studies showed heterogeneity and one of the included publications was later retracted due to serious methodological flaws. In addition, the vast majority of the participants were high responders (496 out of 633) accounting for

1
2
3 a highly selected group of patients, mostly consisting of PCOS patients or patients with and
4 ovarian PCO like morphology.⁶ Moreover, previous studies were performed in China, US and
5 Japan making them less generalizable to a European ART setting. According to Clinicaltrials.gov
6 there are a few ongoing European RCT's on the freeze-all strategy, however none of these studies
7 include a complete OHSS-free strategy with GnRH-agonist trigger in the freeze-all group. This
8 underlines the need for a large multicentre randomised controlled trial exploring a freeze-all
9 strategy in a broader population of ART patients. The present study will explore this approach in a
10 bi-national multicentre randomised controlled trial setting providing information on the prospect of a
11 freeze-all strategy.
12
13
14
15
16
17
18
19

20 Objectives

21 *Primary objective*

22 The primary objective of the study is to investigate if the ongoing pregnancy rate after single
23 blastocyst transfer is superior in a freeze-all and transfer later- strategy compared to the
24 conventional hCG trigger and fresh transfer strategy. Ongoing pregnancy and live birth rates will
25 be calculated per randomized patient and per transfer according to the intention-to-treat principle.
26 Additional calculations will be performed also according to the per-protocol principle. Ongoing
27 pregnancy rate is defined as an intrauterine pregnancy with a foetal heart beat at transvaginal
28 ultrasound in gestational week 7-8.
29
30
31
32
33

34 *Secondary objectives*

35 Secondary objectives include:

- 36 1. To assess cumulative live birth rates after *one complete cycle* including consecutive single
37 blastocyst transfers of all embryos deriving from that oocyte retrieval (fresh and frozen) in
38 the two study groups
- 39 2. To assess the transfer cancellation rate in the two study groups
- 40 3. To compare neonatal outcomes (preterm birth, low birth weight, SGA (small-for-gestational
41 age), LGA (large-for-gestational age) and perinatal mortality) and the incidence of
42 preeclampsia in the two study groups
- 43 4. To measure time-to-pregnancy from the date of start of COS to the date of the first ongoing
44 pregnancy in the two study groups
- 45 5. To assess quality of life for both female and male partners during the two treatment
46 protocols
- 47 6. To assess physical well-being by way of questionnaires and VAS scores regarding pain
48 and discomfort at four and 16 days after oocyte retrieval in the two study groups
49
50
51
52
53
54
55
56
57
58
59
60

METHODS AND ANALYSIS

Study design

The study is designed as a multicentre randomised, controlled double-blinded trial with seven fertility clinics in Denmark and Sweden participating. All seven clinics are part of a University Hospital setting and perform standardized treatments according to the public health care system in Denmark and Sweden. Patient enrolment started in May 2016 and completion is expected in May 2018.

Study population/Participants and recruitment

The study participants will consist of women and their partners initiating ART treatment at one of the seven participating public clinics in Denmark and Sweden. Before initiating treatment patients will attend an information meeting, where they will be informed about the standard ART procedures, treatment regimens as well as ongoing clinical studies at the treatment sites. Those patients not able to participate in the information meeting will instead be informed by a doctor at an outpatient clinic consultation. Recruitment will be carried out by the doctors and study nurses at the fertility clinics. Prior to the initiation of treatment, patient files will be browsed by investigators at the clinics to assess if the patient fulfills the immediate inclusion criteria. Screening, including ultrasound examination of the uterus and ovaries is done on menstrual cycle day two or three securing that all inclusion criteria are met. Patients fulfilling the study criteria will start COS using a GnRH antagonist co-treatment in accordance with the standard routines of the trial site.

Eligibility criteria

To participate in the study, women will be required to meet the following inclusion criteria: Female age 18 to 39 years; eligibility to initiate the first, second or third ART cycle with oocyte aspiration (IVF or ICSI); AMH level > 6.28 pmol/L (Roche Elecsys assay) corresponding to the AMH threshold level used in the Bologna criteria to characterize poor responders; regular menstrual cycle ≥ 24 days and ≤ 35 days; body mass index 18–35 kg/m²; preservation of both ovaries and capability of signing informed consent. For specific exclusion criteria see Table 1.

Randomisation and blinding

Patients who meet the inclusion criteria are randomised 1:1 to one of the two treatment groups: A. Freeze-all including GnRH agonist trigger, blastocyst vitrification and subsequent FET in a hCG triggered natural cycle or B. Traditional hCG trigger and fresh blastocyst transfer.

The randomisation is carried out by a study nurse or a non-treating doctor using a computerised randomisation program that runs a minimization algorithm, initially seeded using a random block

1
2
3 sequence for the first subjects. The minimization algorithm is balancing the following variables:
4 Female age (mean, and frequency of age ≥ 37 years), previously performed cycles (frequency of
5 0/1/2/3 cycles), nulliparous (frequency of yes/no), fertilisation method (frequency of IVF/ICSI),
6 smoking (frequency of yes/no), AMH (≤ 12 pmol/L, 13-28 pmol/L, >28 pmol/L) and mean BMI. It
7 selects with high (but less than 1.0) probability the treatment arm that provides the optimal balance
8 between the arms. It also enforces predefined maximum allowed differences in number of subjects
9 in each treatment arm at each study site (fertility clinic) and within the whole study.
10 Furthermore, the starting dose of FSH is entered into the randomisation program before
11 randomisation is performed to make sure that the FSH dose is decided upon before randomisation.
12 Both the treating consultants and patients are blinded to the randomisation results during the
13 controlled ovarian stimulation until the day when ovulation trigger is planned.
14
15
16
17
18
19

20 21 22 **Treatment arms and interventions**

23 The short GnRH antagonist protocol and blastocyst culture is applied to both treatment arms. The
24 starting dose and type of gonadotropin is decided by the doctor on stimulation day one (cycle day
25 two or three) and entered into the randomisation program prior to randomisation. Recombinant
26 follicular stimulating hormone (rFSH) or human menopausal gonadotropin (hMG) can be used
27 according to the preference of the site, but the daily dose cannot exceed 300 IU. The gonadotropin
28 stimulation will be performed according to the routine in the clinics and can be changed during the
29 treatment according to the ovarian response to stimulation evaluated through ultrasound
30 examination. GnRH antagonist co-treatment is initiated at a daily dose of 0.25 mg on stimulation
31 day five or six according to the general standards in each clinic and is continued throughout the
32 rest of the gonadotropin stimulation period.
33

34 Ultrasound examination is performed on cycle day two or three (baseline), stimulation day six or
35 seven and subsequently every second to third day until ovulation trigger is decided according to
36 the hCG/GnRH agonist trigger criterion: as soon as three follicles are ≥ 17 mm or one day later. At
37 baseline a comprehensive ultrasound examination will estimate endometrial thickness, ovarian
38 volume as well as number and size of antral follicles divided into the following three subclasses: 2-
39 4 mm, 5-7 mm and 8-10 mm. On the day of ovulation trigger endometrial thickness and
40 morphology as well as follicular development with number and size of follicles > 10 mm are
41 registered.
42

43 When ovulation trigger is decided, the result of the randomisation is disclosed to both doctors and
44 patients and ovulation and oocyte maturation is triggered with a single injection of 250 μ g of hCG
45 in the fresh embryo transfer group or a GnRH agonist trigger injection (0.5 mg Buserelin) in the
46 freeze-all group. If > 18 follicles with a diameter > 11 mm are observed in the fresh embryo
47 transfer group GnRH agonist triggering with Buserelin and vitrification of all embryos will be
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 performed to avoid severe OHSS. All fertilised oocytes are cultured to the blastocyst stage and the
4 embryos are scored and ranked according to standardised criteria ascribed to this study. The
5 ranking will assure that the blastocyst with the highest implantation potential is transferred first in
6 both groups. In the fresh transfer group, single blastocyst transfer is performed on day five after
7 oocyte retrieval if a good quality blastocyst has developed. Surplus good quality blastocysts will be
8 vitrified on day five or six. Luteal phase support is administered as vaginal progesterone according
9 to the clinics standard procedures from day two after oocyte retrieval until the day of hCG test; thus
10 luteal support is not extended into early pregnancy. In the freeze-all group all blastocysts of good
11 quality are vitrified on day five or six depending on when the blastocyst stage is reached. The
12 blastocyst with the highest rank is marked and will be the first one used in a subsequent FET hCG
13 triggered modified natural cycle. There should be *at least one completed menstrual cycle in*
14 *between the stimulation and the embryo transfer.* In FET cycles a single injection of 250 µg hCG is
15 administered, when the leading follicle is ≥ 17 mm. Blastocyst transfer is performed six or seven
16 days after the hCG injection. No luteal phase support is given.

17
18 A serum beta-hCG test is performed 11 days after blastocyst transfer. Clinical pregnancy is
19 confirmed by transvaginal ultrasound 3 to 4 weeks after a positive serum-hCG test at gestational
20 age 7-8. For overview of study design see figure 1.

31 **Data collection and management**

32 Treatment related data is collected at 1) Baseline (cycle day two or three), 2) Day of ovulation
33 trigger and 3) five days after oocyte retrieval. Data on blastocysts are collected at culture day
34 five/six. Follow-up data on all pregnancies resulting from blastocysts transferred according to the
35 study protocol will be followed from study inclusion and one year onwards. Data is transferred to
36 an online eCRF system called MediCase with an underlying Microsoft SQL server database
37 located in a guarded underground facility in Sweden. Data is backed up daily (one back-up to
38 another computer in the same physical location as the server, and a second back-up to a
39 physically separate location, also in Sweden). MediCase has a complete audit trail and is designed
40 to only contain de-identified data and is entirely based on anonymous subject ID numbers used in
41 the trial.

49 **Sample collection**

50 Blood samples will be collected three times during the treatment process: 1) Baseline (cycle day
51 two or three), 2) Day of ovulation induction and 3) 16 days after oocyte retrieval (day of pregnancy
52 test in the fresh embryo transfer group). For overview of samples taken see Table 2. Furthermore
53 one serum, one plasma and one fullblood sample are taken at baseline and on the day of
54 triggering and stored according to a trial specific laboratory manual in a project-specific biobank as
55
56
57
58
59
60

1
2
3 back-up for analysis of endocrine and immunological factors of relevance for pregnancy. The
4 frozen samples will be kept anonymised in the biobank with only the patient specific project ID
5 number and collection date marked on the sample. The samples will be store in the participating
6 fertility clinics and destroyed 5 years after the end of the study period if not analysed.
7
8
9

10
11 Further blood samples will be collected during the luteal phase for a smaller subgroup of 30
12 patients in each treatment group as part of a luteal phase subgroup analysis of differences in
13 hormone levels in the two groups. The following blood samples will be collected at 1) Day of
14 ovulation induction and 2) Day of ovulation induction, day of ovulation induction +7, +11, +14, +16
15 and +19: Estradiol, Inhibin-A, OH-Progesterone, Progesterone, LH and hCG.
16
17
18
19

20 **Questionnaires**

21 Women as well as male partners will be asked to fill in quality of life validated questionnaires twice
22 during the treatment process: 1) Four days after oocyte retrieval and 2) 16 days after oocyte
23 retrieval. The questionnaires consist of standardized questions specially developed to explore
24 emotional aspects as well as quality-of life related aspects of the treatment process. The women
25 will at the same time be asked to fill in questionnaires regarding physical discomfort including a
26 VAS score of physical pain in relation to the treatment.
27
28
29
30
31

32 **Statistics**

33 *Sample size calculation*

34 The trial is designed as a superiority study. Sample size calculation indicates that 424 participants
35 (n = 212 in each arm) are required to have a 80 % chance of detecting, at a significance level at
36 0.05, an increase in the primary outcome measure (ongoing pregnancy rate per transfer) from 30%
37 in the control group (fresh embryo transfer) to 43 % in the experimental group (freeze-all).
38
39
40
41
42

43 *Outcome measurements (primary and secondary)*

44 The primary endpoint is the ongoing pregnancy rate per transfer of the first blastocyst.
45 Ongoing pregnancy is defined as a pregnancy with a positive foetal heart beat at gestational week
46 7-8. Secondary endpoints are shown in Table 3.
47
48
49

50 *Statistical analyses*

51 Analyses of cumulative pregnancy rates and live birth rates after one oocyte retrieval including
52 fresh and all frozen embryo transfer cycles will be compared by Cox-regression analyses.
53 Comparisons between treatment groups will be performed primarily according to the intention-to-
54 treat (ITT) principle but per-protocol analyses will also be done. Continuous data will be compared
55
56
57
58
59
60

1
2
3 by students *t*-test or Mann-Whitney U test and Kruskal-Wallis test as appropriate. Proportions will
4 be compared with chi-square test. Predictive factors for ongoing pregnancy in the two treatment
5 groups will be tested with multivariate logistic regression analyses. A p-value of < 0.5 will be
6 considered as statistically significant.
7
8

9 10 11 **ETHICS, SAFETY AND DISSEMINATION** 12

13
14 The study has been approved by the Scientific Ethical committees in both Denmark and Sweden.
15 Following oral and written information outlining the rationale, trial design, aims and treatment
16 procedures written informed consent will be obtained from women and male partners prior to the
17 enrolment in the study.
18
19

20
21 The participants are stimulated using individualised doses of gonadotropin stimulation in
22 accordance with the clinical practice at each site. In all clinics serum AMH is considered when the
23 FSH dose is determined. All medicine used in the study is part of standard ART care.
24
25
26

27
28 The overall safety of the patients is high in both treatment groups. The risk of OHSS is expected to
29 be similar to the standard clinical protocol in the fresh embryo transfer group and lower in the
30 freeze-all group in which GnRH agonist is used for ovulation trigger. In women in the fresh embryo
31 transfer group with a risk of OHSS development (more than 18 follicles with a diameter over 11
32 mm), GnRH agonist will be used for trigger instead of hCG and all blastocysts will be vitrified and
33 the transfer postponed.
34
35
36

37
38 No financial incentive exists for the participants as all couples are reimbursed for their first three
39 ART treatments in the public health care system in the Nordic countries.
40
41
42

43 The results of the study will be publically disseminated in peer-reviewed scientific journals and
44 presented at relevant international scientific meetings such as ESHRE (European Society of
45 Human Reproduction and Embryology) and ASRM (American Society for Reproductive Medicine).
46 In addition results will be published in popular science journals and other media.
47
48
49

50 51 52 **DISCUSSION** 53

54 The increasing interest in possible benefits of a freeze-all strategy and the limitations of existing
55 randomised controlled trials comparing this strategy with conventional fresh embryo transfer
56 underline the need for additional studies. Previous studies investigating FET cycles concluded that
57
58
59
60

1
2
3 a freeze-all strategy resulted in significantly increased pregnancy rates, however these studies
4 were performed in highly selected patient populations with poor generalizability.⁶⁻⁷ Further, the
5 complete OHSS-free strategy combining GnRH agonist trigger and freeze-all has not yet been
6 investigated in a RCT setting. As GnRH agonist trigger does not hamper the yield of mature
7 oocytes¹² and reduces the risk of OHSS to an absolutely minimum, it seems rational to include
8 GnRH agonist trigger in the freeze-all concept.
9

10
11
12 The strengths of this study include the design as a multicenter randomised controlled double-
13 blinded trial as well as preregistration and publication of the study protocol for more transparency.
14 The investigation of several outcome measures related to different aspects of success parameters,
15 including quality of life may furthermore add important information as regards the future potential of
16 the freeze-all strategy in assisted reproduction.
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Appendices

Table 1. Specific exclusion criteria

Endometriosis stage III to IV
Ovarian cysts with a diameter > 30 mm at day of start of stimulation
Submucosal fibroids
Women with severe co-morbidity (IDDM (insulin dependent diabetes mellitus), NIDDM (non-insulin dependent diabetes mellitus), gastrointestinal, cardiovascular, pulmonary, liver or kidney disease)
Dysregulated thyroid disease
Non-Danish or English speaking
Contraindications or allergies to use of gonadotropins or GnRH antagonists
TESA (testicular sperm aspiration)
OD (oocyte donation)
Previous inclusion in the study

Table 2. Blood sample collection

Baseline (cycle day 2 or 3)	AMH FSH LH Estradiol Progesterone TSH TPO-antibodies Vitamin D CRP suPAR*
Day of ovulation induction	FSH LH Estradiol Progesterone CRP suPAR*
16 days after oocyte retrieval	CRP suPAR* hCG**

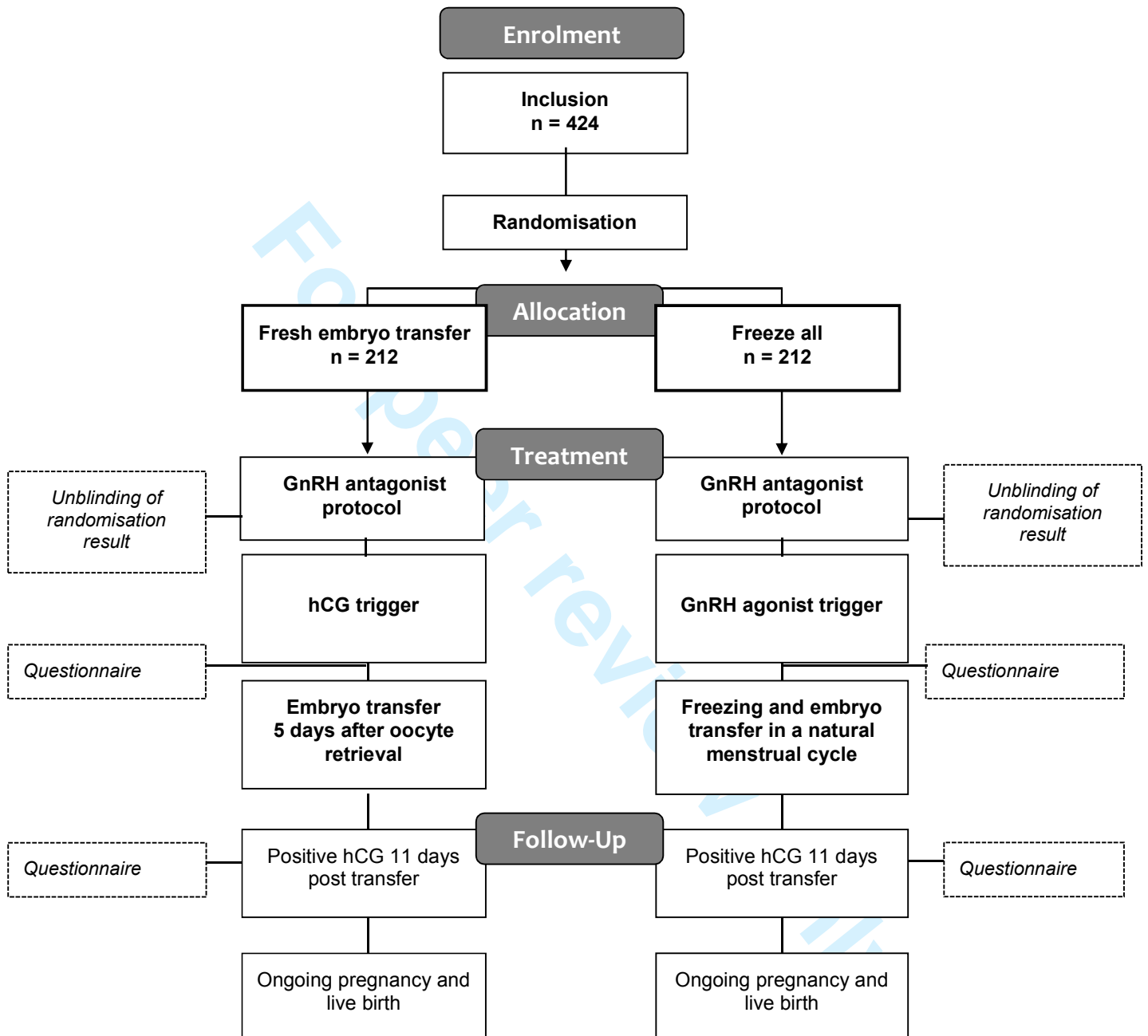
* Soluble urokinase-type plasminogen activator receptor, only measured at Hvidovre Hospital

** only fresh embryo transfer group

Table 3. Secondary endpoints

◆ Ongoing pregnancy rate per start of <i>per randomized patient, per started ovarian stimulation and per oocyte retrieval</i>
◆ Live birth rate after the first blastocyst transfer calculated <i>per randomized patient, per started ovarian stimulation, per oocyte retrieval and per transfer</i>
◆ Cumulative live birth rate after one stimulated cycle with oocyte retrieval
◆ Cumulative live birth rate after use of all frozen blastocyst or after at least 1 year of follow-up
◆ Number of cycles with no embryo transfer
◆ Time-to-pregnancy (from start of ovarian stimulation to positive hCG)
◆ Time-to-delivery
◆ Cancelled embryo transfers
◆ Ovarian hyperstimulation syndrome (OHSS)
◆ Preterm birth
◆ Low birth weight
◆ Small-for-gestational age (SGA)
◆ Large-for-gestational age (LGA)
◆ Perinatal mortality
◆ Preeclampsia
◆ Placental rupture
◆ Positive hCG 11 days post embryo transfer
◆ Miscarriage, biochemical pregnancies, ectopic pregnancies
◆ Quality of life for female and male partner
◆ Cost-effectiveness
Other outcome measurements
◆ Number of good blastocysts
◆ Number of fertilized oocytes
◆ Number of high quality embryos day 2
◆ Number of grade 1 blastocysts
◆ Number of frozen blastocyst
◆ Paraclinical data: Endocrine, genetic and immunological parameters

Figure 1. Flowchart of the Freeze-all study design



1
2
3
4
5
6
7
8
9
10
11
12
13
14 **Contributor statement** ANA, AP and SS participated in the conception, design, and writing of the
15 study protocol. KL and HSN contributed to the revision and editing of the study protocol. AP, SS,
16 KL, JB, LP, ANA, HSN, CB, PH, MB, ALM and SOS will be involved in the recruitment of patients
17 and the acquisition of data. SS wrote the first draft of this manuscript. AZ was involved in
18 developing the laboratory criteria for the study. SS, AP, ANA, PH, CB, KL, JB, LP, HSN, MB, ALM
19 and SOS will AP, ANA, PH, CB, KL, AZ, JB, LP, HSN, MB, ALM and SOS were all involved in
20 critical revision of the manuscript. All authors approved the final version of the manuscript to be
21 submitted.
22

23
24
25
26 **Competing interests** None declared

27
28 **Funding** The study is part of and fully funded by the Repronion Collaborative study, co-financed
29 by the European Union, Interreg, V ÖKS.

30
31 **Data sharing statement** This manuscript is a study protocol. Data from the final study will be
32 shared according to the coming ICJME guidelines.
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

REFERENCES

1. Calhaz-Jorge C, de Geyter C, Kupka MS, et al. Assisted reproductive technology in Europe, 2012: results generated from European registers by ESHRE. *Human reproduction* 2016;31(8):1638-52.
2. Loutradi KE, Kolibianakis EM, Venetis CA, et al. Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. *Fertility and sterility* 2008;90(1):186-93.
3. Cobo A, de los Santos MJ, Castello D, et al. Outcomes of vitrified early cleavage-stage and blastocyst stage embryos in a cryopreservation program: evaluation of 3,150 warming cycles. *Fertility and sterility* 2012;98(5):1138-46 e1.
4. Zhu D, Zhang J, Cao S, et al. Vitrified-warmed blastocyst transfer cycles yield higher pregnancy and implantation rates compared with fresh blastocyst transfer cycles--time for a new embryo transfer strategy? *Fertility and sterility* 2011;95(5):1691-5.
5. Shapiro BS, Daneshmand ST, Garner FC, et al. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfers in high responders. *Fertility and sterility* 2011;96(2):516-8.
6. Roque M, Lattes K, Serra S, et al. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. *Fertility and sterility* 2013;99(1):156-62.
7. Chen ZJ, Shi Y, Sun Y, et al. Fresh versus Frozen Embryos for Infertility in the Polycystic Ovary Syndrome. *The New England journal of medicine* 2016;375(6):523-33.
8. Kansal Kalra S, Ratcliffe SJ, Milman L, et al. Perinatal morbidity after in vitro fertilization is lower with frozen embryo transfer. *Fertility and sterility* 2011;95(2):548-53.
9. Devroey P, Polyzos NP, Blockeel C. An OHSS-Free Clinic by segmentation of IVF treatment. *Human reproduction* 2011;26(10):2593-7.
10. Youssef MA, Van der Veen F, Al-Inany HG, et al. Gonadotropin-releasing hormone agonist versus HCG for oocyte triggering in antagonist-assisted reproductive technology. *The Cochrane Database of systematic review* 2014;31;(10):CD008046.
11. Pinborg A. To transfer fresh or thawed embryos? *Seminars in reproductive medicine* 2012;30(3):230-5.
12. Engmann L, Benadiva C, Humaidan P. GnRH agonist trigger for the induction of oocyte maturation in GnRH antagonist IVF cycles: a SWOT analysis. *Reproductive BioMedicine Online* 2016 Mar;32(3):27485

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For peer review only



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

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			Page 1 of protocol
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Page 17 of protocol _____
	2b	All items from the World Health Organization Trial Registration Data Set	All accounted for see items below _____
Protocol version	3	Date and version identifier	Page 1 of protocol (header) _____
Funding	4	Sources and types of financial, material, and other support	Page 18 of protocol _____
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Page 1, 2, 3 of protocol _____

1			
2			
3		5b	Name and contact information for the trial sponsor
4			Page 1 of protocol
5			_____
6		5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and
7			interpretation of data; writing of the report; and the decision to submit the report for publication, including
8			whether they will have ultimate authority over any of these activities
9			_____
10		5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint
11			adjudication committee, data management team, and other individuals or groups overseeing the trial, if
12			applicable (see Item 21a for data monitoring committee)
13			_____
14			
15			
16			
17			
18			
19	Introduction		
20			
21	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant
22	rationale		studies (published and unpublished) examining benefits and harms for each intervention
23			Page 3, 4 5 of
24			protocol
25			_____
26		6b	Explanation for choice of comparators
27			Page 3, 4 5 of
28			protocol
29			_____
30	Objectives	7	Specific objectives or hypotheses
31			Page 5 of protocol
32			_____
33	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),
34			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)
35			Page 8-10 of
36			protocol
37			_____

37 **Methods: Participants, interventions, and outcomes**

1				
2				
3	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Page 8 List of study sites; page 1-3
4				
5				
6				
7				
8	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Page 7-8
9				
10				
11	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Page 11-13
12				
13				
14		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	Page 14-15
15				
16				
17		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Page 11 (visits)
18				
19				
20		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Not applicable
21				
22				
23				
24	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Page 5-7
25				
26				
27				
28				
29	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Page 5-13
30				
31				
32	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	Page 15
33				
34				
35	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 8
36				
37				
38				
39	Methods: Assignment of interventions (for controlled trials)			
40				
41	Allocation:			
42				
43				
44				
45				
46				
47				
48				
49				

1				
2				
3	Sequence generation	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	Page 9 _____
4				
5				
6				
7				
8	Allocation concealment mechanism	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	Page 9 _____
9				
10				
11				
12	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Page 9, 11 _____
13				
14				
15	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	Page 9 _____
16				
17				
18				
19		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	No circumstances _____
20				
21				
22				
23	Methods: Data collection, management, and analysis			
24	Data collection methods	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	Page 9, 10, 11 and 14 _____
25				
26				
27				
28				
29				
30		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	Page 13-14 _____
31				
32				
33				
34				
35				
36				
37				
38				
39				
40				
41				
42				
43				
44				
45				
46				
47				
48				
49				



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

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	Page 13,14, Page 16 Details data management can be obtained through contact to primary investigator (“projektansvarlig læge” of trial)
<hr/>			
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	Page 15 Details of planned statistical analysed can be obtained through contact to primary investigator (“projektansvarlig læge” of trial)
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Same as item 20a
	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)	Same as item 20a
<hr/>			
Methods: Monitoring			
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	No data monitoring committee used

1				
2				
3		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	No interim analyses will be performed
4				
5				
6				
7				
8	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	Not applicable
9				
10				
11	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Not available in protocol
12				
13				
14				
15				
16	Ethics and dissemination			
17				
18	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Page 17
19				
20				
21	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)	Not available in protocol
22				
23				
24				
25	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 8 of protocol
26				
27				
28		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Not applicable
29				
30				
31	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	Page 14, 16
32				
33				
34				
35	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Page 18 No competing interests exists
36				
37				
38				
39				
40				
41				
42				
43				
44				
45				
46				
47				
48				
49				

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

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	Not available in protocol
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	Not applicable
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	Not available in protocol
	31b	Authorship eligibility guidelines and any intended use of professional writers	Not available in protocol
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	No current plans
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Not available in protocol – Standard consent forms from the Danish ethical committee is used as well s participant information approved by the Ethical Committee
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	Page 10

1
2 *It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items.
3 Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons
4 "[Attribution-NonCommercial-NoDerivs 3.0 Unported](#)" license.
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45

For peer review only

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

A multicentre randomized controlled trial of a “Freeze all and transfer later”
versus a conventional “Fresh Embryo Transfer” strategy for assisted
reproductive technology (ART) in women with a regular menstrual cycle

- A Multicentre Randomized Controlled Trial of patients undergoing IVF/ICSI

Multicenter studium med syv deltagende fertilitetsklinikker i Danmark og Sverige

Projektansvarlig læge

Anja Pinborg, professor, overlæge, dr.med.

Fertility Clinic, Gynecologic/Obstetric Department, Hvidovre Hospital

Kettegaard Allé 30, DK – Hvidovre 2650

Telephone: +45 38 62 26 56

e-mail: anja.bisgaard.pinborg.01@regionh.dk

AutorisationsID: 00QKV

Deltagende Afdelinger:

Hvidovre Hospital

Sacha Stormlund (læge 1.februar 2016, kommende PhD stud)

Kristine Løssl, overlæge, PhD,

Anne Zedeler, laboratorieleder, Ph.D.

Jeanette Wulff Bogstad, lægelig klinikleder, overlæge

Lisbeth Prætorius, overlæge

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

Herlev Hospital

Sven Skouby, professor, dr. med.

Marie Louise Grøndahl, laboratorieleder, Ph.D

Fertility Clinic, Herlev Hospital, Copenhagen University Hospital

Rigshospitalet

Henriette Svarre Nielsen, overlæge, dr.med.

Anne Loft, overlæge

Anders Nyboe Andersen, professor, overlæge, dr.med.

Fertility Clinic, Rigshospitalet, Copenhagen University Hospital

Holbæk Hospital

Anne Lis Mikkelsen, klinikleder, overlæge, dr.med.

Fertility Clinic, Region Sjælland, Holbæk Hospital

Malmö University Hospital

Aleksander Giwercman, forskningsleder, professor, overlæge, dr.med.

Fertility Centre, Malmö University Hospital, Lunds Universitet

Sahlgrenska University Hospital

Christina Bergh

Department of Obstetrics and Gynaecology, Institute of Clinical Sciences, Sahlgrenska Academy

Gothenburg University, Reproductive Medicine

Sahlgrenska University Hospital, SE-413 45 Gothenburg, Sweden

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

Fertility Clinic Skive

Peter Humaidan, professor, overlæge, dr. med

The Fertility Clinic, Skive Regional Hospital and Faculty of Health, Aarhus University, Aarhus, Denmark

Statistical advisor

Julie Forman, Cand. Scient, Associate Professor

Section of Biostatistics

University of Copenhagen

1. BACKGROUND

In recent years improved cryopreservation techniques have made frozen embryo transfer (FET) a viable and promising alternative to fresh embryo transfer in assisted reproduction (ART). The optimization of cryopreservation techniques from slow-freezing to vitrification and prolonged embryo culture from cleavage to blastocyst state encourages the use of FET as the embryo survival rate following freezing and thawing is now significantly higher reaching 95-97% (Loutradi et al., 2008). Success rates including implantation as well as clinical and on-going pregnancy rates in FET are also significantly improving and approaching or even exceeding those associated with fresh embryo transfer (Kupka et al., 2014; Roque et al., 2013; Shapiro et al., 2011; Zhu et al., 2011). This is partly due the improved laboratory techniques, but may also be due to the endometrial environment in the FET cycles, which mirrors the natural cycle. In the stimulated cycle supraphysiological levels of estradiol and progesterone are present and may cause impaired endometrial receptivity (Shapiro et al., 2011). Furthermore, obstetric and perinatal outcomes after cryopreservation of embryos have been investigated and follow-up data from children born after FET have shown lower perinatal morbidity compared with fresh embryo transfer (Kansal et al., 2011), but FET may also give rise to more large-for-gestational age babies (Pinborg et al., 2014). In addition, a recent systematic review and meta-analysis on data from 11 observational studies has shown better perinatal outcomes including lower perinatal mortality in singleton pregnancies following frozen-thawed embryo transfer

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

1
2
3
4 compared with pregnancies after fresh embryo transfer (Maheshwari et al., 2012). Moreover,
5 FET has the benefits of minimizing the risk of ovarian hyperstimulation syndrome (OHSS),
6 which is the most severe side effect of ART and potentially life threatening. Finally, improved
7 cryopreservation techniques favour an elective single embryo transfer (eSET) policy minimizing
8 multiple pregnancies after ART (Pinborg, 2012).
9
10
11
12

13
14 Despite the noticeable advantages of embryo cryopreservation, fresh embryo transfer has
15 persistently been the conventional in vitro fertilisation (IVF) procedure as only one in five
16 transfers were made using frozen-thawed embryos in Denmark in 2013
17 (www.fertilitetselskab.dk). This favour of a fresh embryo transfer strategy is however
18 reflected in other European countries including Finland, Sweden and Iceland where
19 approximately every third ART child is born after FET (Kupka et al., 2014). Some evidence
20 suggests that IVF outcomes can be further improved with the adaptation of a 'freeze-all' or
21 elective frozen embryo transfer (eFET) strategy with replacement of thawed embryos in
22 natural cycles (Evans et al., 2014; Devroey et al., 2011; Maheshwari et al., 2013; Roque et
23 al., 2013).
24
25
26
27
28
29
30
31
32

33 In a recent meta-analysis including three trials accounting for 633 cycles in women aged 27–33
34 years (Roque et al., 2013), FET resulted in significantly higher ongoing pregnancy rates (RR
35 1.32, 95% CI 1.10–1.59) and clinical pregnancy rates (RR 1.31, 95% CI 1.10–1.56). The studies
36 showed heterogeneity and only 137 of the participants were normal responders, while the rest
37 was high responders. Moreover one study included only cleavage stage embryo transfer while
38 the other two included blastocyst transfers only. The studies were performed in Japan and in
39 the US, while no European RCT has been published yet. Further, one of the included papers
40 (Aflatoonian et al., 2010) was later retracted based on findings of serious methodological flaws
41 in the study. This accentuates the need for a large multicentre, randomized controlled trial to
42 evaluate the prospect and clinical consequences of a “freeze all embryos and transfer later”
43 policy compared with conventional fresh embryo transfer.
44
45
46
47
48
49
50
51
52

53 The aim of this multicentre randomized controlled trial is to compare a “freeze-all” embryo
54 strategy with a conventional single fresh embryo transfer strategy in women 18 to 40 years of
55
56
57
58
59
60

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

age undergoing their first to third IVF/ICSI cycle women with regard to treatment outcomes, risks for mother and child, quality of life and cost-effectiveness aspects of the two treatment modalities in a short GnRH antagonist protocol with blastocyst transfer and vitrification as the freezing method.

2. STUDY AIMS

1. The primary aim is to compare ongoing pregnancy rates and live birth rates after the first single blastocyst transfer in the “freeze-all” versus “fresh embryo transfer” group.
2. To assess cumulative live birth rates after one stimulated cycle with oocyte retrieval in the two study arms.
3. To compare perinatal outcomes (preterm birth, low birth weight, small-for-gestational age, large-for-gestational-age, preeclampsia and perinatal mortality) in the two groups.
4. To measure time to pregnancy from start of ovarian stimulation and quality of life in both females and males in the two groups.
5. To explore VAS scores regarding pain and discomfort at the day of embryo transfer and 11 days post transfer in the two study arms
6. To assess female physical well-being during the two treatment modalities and to assess quality of life for both female and male partners during the two treatment protocols.

3. ENDPOINTS

Primary endpoints

- Ongoing pregnancy rate *per transfer* of the first blastocyst (pregnancy with positive fetal heart beat in gestational week 7-8)
- Ongoing pregnancy rate *per oocyte pick-up*

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

(pregnancy with positive fetal heart beat in gestational week 7-8)

- Ongoing pregnancy rate *per start of ovarian stimulation*

(pregnancy with positive fetal heart beat in gestational week 7-8)

- Ongoing pregnancy rate *per randomized patient*

(pregnancy with positive fetal heart beat in gestational week 7-8)

Secondary endpoints

- Live birth rates after the first blastocyst transfer calculated per randomized patient, per started ovarian stimulation, per oocyte pick-up and per transfer
- Cumulative live birth rate after one stimulated cycle with oocyte retrieval
- Cumulative live birth rate after use of all frozen blastocysts or after at least 1 year of follow-up.
- Number of cycles with no embryo transfer
- Time-to-pregnancy (from start of ovarian stimulation to positive hCG)
- Time-to-delivery
- Cancelled embryo transfers
- OHSS
- Preterm birth
- Low birth weight
- Small-for-gestational age (SGA)
- Large-for-gestational age (LGA)
- Perinatal mortality
- Preeclampsia
- Placental rupture
- Positive hCG 11 days post embryo transfer
- Miscarriage, biochemical pregnancies, ectopic pregnancies
- Quality of life for female and male partner
- Cost-effectiveness

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

Other outcomes

- Number of good quality blastocyst
- Number of fertilized oocytes
- Number of high quality embryos day 2
- Number of grade 1 blastocysts?
- Number of frozen blastocysts
- Para-clinical data: Endocrine, genetic and immunological parameters influencing pregnancy

4. STUDY POPULATION

Inclusion criteria

- Women > 6.28 pmol/L with the Roche Elecsys assay* (AMH > 1.1 ng/ml ~ 7.85 pmol/L old assay).
This is according to the Bologna criteria for POR; AMH < 0.5–1.1 ng/ml (3.57-7,85 pmol/l (old assay) ~ 2,86 – 6,28 pmol/l Elecsys)(Ferraretti et al., 2011)
- Female age 18 year to less than 40 years
- 1.-3. IVF/ICSI cycle with oocyte aspiration
- Regular menstrual cycle ≥ 24 days and ≤ 35 days
- BMI ≥ 18 or < 35 kg/m²
- Two ovaries
- Can and will sign the informed content
- **Exclusion criteria**
 - Women who do not fulfil the inclusion criteria
 - Endometriosis stage III to IV
 - Ovarian cysts with diameter > 30 mm at day of start of stimulation
 - Submucosal fibroids
 - Women with severe co-morbidity (IDDM, NIDDM, gastrointestinal, cardio-vascular, pulmonary, liver or kidney disease)
 - Dysregulation of thyroid disease
 - Not Danish or English speaking women

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

- Contraindications or allergies to use of gonadotrophins or GnRH antagonists
- TESA (testicular sperm aspiration)
- OD (oocyte donation)
- Previous inclusion in the study

5. METHODS

Inclusion of patients

- All couples or single/lesbian women starting IVF/ICSI treatment participate in a standard information meeting arranged by the clinic. During this 2 hour meeting patients and their partners are informed about the normal IVF/ICSI procedures, treatments and research in the clinic as well as this study. If patients are not attending the information meeting, they will be informed about the study at their first outpatient visit at the clinic.
- Few patients do not participate in the information meeting and they will have an appointment at the outpatient clinic in the Fertility clinic, where they will receive information about the IVF/ICSI treatment and be informed about this study.
- Patient files are browsed by one of the investigators, who decide if the patient is eligible. After the information meeting all patients receives a phone call from a doctor/study nurse, where they are informed about the treatment plan. If the inclusion criteria are fulfilled, the couples will receive oral information about the study and asked if they are interested in participating in the study, if so, the written patient information is sent to the couples by email. If the couples are interested, a visit is planned on menstrual cycle day 2-4. The couple is informed that they can bring an assessor to the oral information visit. The written information is send by email, which leaves possibility of reading and reflection.

Informed consent

At the fertility clinic the patient will be seen by one of the investigators. Patients will be informed about the aim of the project and risks in accordance with the guidelines from the Scientific Ethical

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

Committee. In case of questions, these will be answered. If the patients need more time for reflection, a new visit will be arranged. After signing the informed consent, the patient will be screened.

Screening – cycle day 2-4

- Medical and gynaecological history inclusive reproductive history including menstrual cycle length, smoking (yes/no), years of infertility
- Transvaginal ultrasound examination including ovarian volume, antral follicle count (AFC), and endometrial thickness and morphology and exclusion of pathology
- Height and weight
- Blood samples: AMH, FSH, LH, estradiol, progesterone, TSH, TPO antibodies, vitamin D, CRP and suPAR* (*only done at Hvidovre Hospital)
- One full blood, one plasma and one serum sample is cryopreserved as back-up and for analysis of endocrine and immunological factors of relevance for pregnancy

Screening should be performed no later than 3 months before randomization.

Randomization

When the patient has signed the informed consent, has been screened and it is confirmed that the inclusion criteria are met, the patient is randomized to one of the two arms:

- I. hCG arm with traditional hCG triggering and fresh blastocyst transfer
- II. GnRH agonist triggering arm with blastocyst cryopreservation and subsequent transfer in a natural cycle.

Computerized randomization is performed according to the 1) Trial site and to 2) Female age ≤ 37 years or >37 years. The gonadotrophin stimulation dose is decided upon before randomization and entered into the database before randomization. The doctor and patient are blinded to the randomization until the day of hCG or GnRH agonist triggering.

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

Blood samples

Blood samples are collected at

- Baseline before the first gonadotrophin injection (cycle day 2-4): AMH, FSH, LH, estradiol, progesterone, TSH, TPO-antibodies, vitamin D CRP and suPAR* (*only done at Hvidovre Hospital)
- Day of trigger-injection: FSH, LH, estradiol, progesterone, CRP and suPAR* (*only done at Hvidovre Hospital)
- Day 16 after oocyte pick-up: hCG (only in the fresh embryo transfer group), CRP and suPAR* (*only done at Hvidovre Hospital)

At baseline and at day of trigger an extra full blood, plasma and serum sample is collected and stored according to a trial-specific laboratory manual. This will be stored in the freezer as back-up and for analysis of endocrine and immunological factors of relevance for this study.

Subgroup analyses in the luteal phase

During the luteal phase with embryo transfer of the stimulated fresh cycle and non-stimulated FER cycle blood samples are taken on day of hCG injection, hCG injection day+7, +11, +14, +16 and +19 for patients included at Hvidovre Hospital until 30 patient in each arm has been achieved. The following blood samples are collected; Estradiol, Inhibin-A, OH-progesterone, Progesterone, LH and hCG.

All blood samples are confidential. The frozen samples are anonymous, so no person identifiable date is left on the sample. Only the patient project ID number and the collection date identifies the sample. The study will be approved by the Danish Data Protection Agency and the Scientific Ethical Committee of the Capital Region, Region Zealand and the Region Skåne in Sweden. The blood samples will be stored in the participating fertility clinics and if not used then five years after end of the study, at December 1st, 2023 blood samples will be definitively destroyed.

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

Gonadotropin stimulation treatment

The dose of gonadotrophin is decided and entered into the computer programme before randomization. The doctor and patient are blinded to the randomization until the day of hCG or GnRH agonist triggering. The study nurse is not blinded.

The ovarian stimulation with recombinant follicular stimulating hormone (rFSH) or human menopausal gonadotrophin (hMG) can start immediately after randomization in a short GnRH antagonist protocol. The gonadotrophin stimulation is performed according to the general standards in each of the clinics and can be altered according to the ovarian response. The GnRH antagonist is initiated at a daily dose of 0.25 mg at stimulation day 5 or 6 according to the clinical standards and continued throughout the rest of the gonadotropin stimulation period. The gonadotrophin dose cannot exceed a daily dose of 300 IU. Both groups are treated according to the short GnRH-antagonist protocol, where a higher dose of gonadotropin than 300 IU has been shown to be of no added value for further follicular growth. The maximum stimulation period is 20 days.

The medication for the study is bought by the patients themselves according to general prescription rules.

Ultrasound

Ultrasound examination is performed on cycle day 2-3 (Stim1), Stim 5-8 and thereafter every 2-3 days until ovulation trigger is decided. At the start of stimulation comprehensive sonography is performed with details on each ovary, including ovarian volume, number of antral follicle in the following subclasses: 2-4mm, 5-7mm and 8-10mm.

The following parameters are measured on the day of ovulation trigger or the day before:
Follicular development with size and number of follicles >10 mm, endometrial thickness and echogenicity and uterine pathology.

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

Ovulation induction

As soon as three follicles of ≥ 17 mm are observed or one day after a single injection of 250 μg of human chorionic gonadotrophin (hCG) is administered in the “fresh transfer“-arm, while GnRH agonist triggering with GnRH agonist Buserelin 0.5 mg is administered in the “freeze-all” arm.

Triggering of ovulation with a GnRH agonist Buserelin 0.5 mg is allowed in the fresh embryo transfer arm in case of risk of severe OHSS after the following the criteria: In the fresh embryo arm: If > 18 follicles with a diameter > 11 mm are observed on the day of triggering, GnRH agonist triggering should be used and all blastocysts frozen (Papanikolaou et al., 2011). Coasting / surfing cannot be used.

Oocyte retrieval

Oocyte retrieval is performed 36 ± 2 hours after hCG or GnRH agonist administration.

IVF/ICSI

Oocytes are fertilised by either IVF or ICSI and embryos are cultured individually according to the normal procedure in the clinics.

Embryo transfer

I. “Fresh embryo transfer” group

Single blastocyst transfer is always performed on day five after oocyte pick-up if a blastocyst is developed. Surplus good quality blastocyst are vitrified on day five or six.

Luteal phase support is administered as vaginal progesterone (vaginal gel (Crinone) 90 mg/dose x 1 daily or vaginal tablets 100 mg x 3 daily (Lutinus)) according to the standard procedure in each of the individual clinics from day 2 after oocyte retrieval and to confirmation of pregnancy or

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

negative hCG 11 days post transfer. In case of a positive pregnancy test an ultrasound scan is performed three to four weeks later to confirm an intrauterine pregnancy with a live foetus.

Triggering of ovulation with a GnRH agonist Buserelin 0.5 mg is allowed in the fresh embryo transfer arm in case of risk of severe OHSS, following the criteria: If > 18 follicles with a diameter > 11 mm are observed, GnRH agonist triggering should be used and all blastocysts frozen (Papanikolaou et al., 2011). Coasting / surfing cannot be used.

II. "Freeze all and transfer later" group

For patients in this group all embryos of a good quality are vitrified at the blastocyst stage day 5 in the stimulated cycle. Criteria for freezing of blastocyst are according to the criteria in the specific clinic. The "best" embryo (i.e. of the highest quality is selected after predefined strict criteria according to the specific trial laboratory manual) is marked and is the first one to be warmed after at least one menstrual cycle that is considered as a wash out period.

In the menstrual cycle with blastocyst transfer, an hCG injection of 6500 units is given when the leading follicle is ≥ 17 mm. Embryo transfer is performed 6-7 days after hCG injection. No luteal phase support is needed.

Pregnancy test

A serum beta-hCG test is performed 11 days after blastocyst transfer. Clinical pregnancy is confirmed by transvaginal ultrasound 3 to 4 weeks after a positive serum-hCG.

Follow-up both groups

A follow-up of all pregnancies will be performed within three months after delivery or termination of pregnancy on predefined information sheets.

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

Information on background data, pregnancies and deliveries are returned to Hvidovre Fertility clinic on predefined case report forms (CRF) including pregnancy and delivery information sheets.

All pregnancies resulting from blastocyst retrieved and thawed according to this study protocol will be followed from study inclusion (Stim day 1) and one year onwards.

All data are anonymized by encryption in the database with no personal identifiable data.

We will retrieve data from the patient clinical files and clinical databases with information regarding previous diseases, hospital admissions, former and current fertility treatment, pregnancy and delivery data on pregnancies related to this study. Both females and males will be informed about this in the patient information. This collected information will be used to characterize the populations and to minimize risk of bias.

We will also gain information regarding the coming child and the female and male will sign a separate informed consent regarding this.

VAS-score and physical discomfort questionnaire

Women in both arms will be requested to fill-in their level of pain and discomfort on a VAS-score scale and a physical discomfort questionnaire as well as a quality of life questionnaire at the day of oocyte pick-up +4 and the day of oocyte pick up + 16.

Visits

Every visit is registered on a standardized stimulation scheme made specifically for this study. The schemes are normally used as standards in the clinics.

Criteria for withdrawal

A patient can be withdrawn from the study at any time, if the patient wishes to do so or if there is a medical indication decided by the investigator. The patient participation in the study can be interrupted, if one of the following criteria are present:

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

- The patients general condition contraindicates participation
- Protocol violation, which the investigator assess to have influence on the treatment
- Safety

Patients will be carefully monitored from stimulation start, at Stim6 and thereafter every 2-3 day in the clinic. The treatment will be monitored by transvaginal ultrasound of the ovaries. After each visit the patients will receive thorough information on the drug dosage and administration. This will follow the normal procedure in the clinic. If a patient is taking the wrong dosage, it will be documented on the stimulation scheme. This is not dangerous to the patient as the treatment is monitored by ultrasound scans hence a risk of OHSS will be discovered there.

In case of risk of OHSS in the “fresh-embryo-transfer”-arm, the ovulation trigger will not be induced by hCG but with Buserelin 0.5 mg. The further treatment of this patient will be handled according to the routine of the clinic.

In case of OHSS the patient is monitored at the clinic until recovery. Overall the safety of the patients is high in both the fresh embryo transfer and the freeze all group as the gonadotrophin stimulation corresponds to the normal program for patients at risk of OHSS. Furthermore patients with irregular cycles i.e. as part of polycystic ovarian syndrome, who in general have a higher risk of OHSS, are not included in this study.

6. STATISTICS AND SAMPLE SIZE

Superiority study

In all 424 (n= 212 in each arm) patients are required to have an 80% chance of detecting, as significant at the 5% level, an increase in the primary outcome measure from 30% in the control group to 43% in the experimental group. A difference of 15% was found in a randomized controlled trial by Shapiro et al, 2011 between the “freeze-all” arm and the fresh transfer group.

The statistical analyses will be performed by investigator together with statistical experts at Hvidovre Hospital and associate professor Julie Forman, Department of Biostatistics, Faculty of Health and Medical Sciences, University of Copenhagen.

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

7. STUDY MEDICATION

All medicine used in this study is normally used as standard care for the patients in the short GnRH antagonist protocol. Patients will have prescriptions on all the medicine and will take all the medicine at home as is the routine in the clinics.

Dosage and administration

Treatment dose at the first day and during the ovarian stimulation is planned by the investigator and the patient is further instructed by a nurse, so that the patient is confident in self administration at home according to the normal clinical routine.

Side effects

Most side effects are mild and related to the medication during the stimulation. Unwanted OHSS is a risk in all IVF treatment but is considered low in this project as a standard IVF/ICSI protocol is used with individualized dosing. Further, in the freeze-all group the risk of OHSS is expected to be lower than in the standard care group as all blastocyst transfers are postponed to cycles without ovarian stimulation.

8. DATA SECURITY AND ETHICAL ASPECTS

Data security

One full blood and one serum sample at baseline and at the day of ovulation induction will be collected and stored according to the trial specific laboratory manual on all women included in this trial for future analyses of endocrine, immunological and protein markers. All data will be collected in a single database including all project subjects with an identification code thus data on each subject will be anonymous, when entered into the database.

Ethical aspects

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

The study will be performed according to the Danish Law and ethical principles in the Helsinki Declaration. This covers that study subjects receive both oral and written information and the opportunity of time for reflection and that they can discuss their participation with a third person.

The participants will be given a individualized dose of gonadotropin according to their serum AMH level, which is standard for patients at all five Fertility Clinics in Denmark and Sweden. The risk of OHSS will be similar to the standard clinical protocol and lower in the “freeze-all” group.

With a “freeze-all embryo and transfer later” protocol in ART, the risk of OHSS in women undergoing IVF/ICSI will be minimized and the embryo development will benefit from an endometrium less influenced by supra-physiological levels of estradiol and progesterone in the fresh embryo transfer cycle. This may also be beneficial for the children born after the treatments.

The study is approved by the Scientific Ethical Committee in the Capital Region (H-1600-1116) and by the Scientific Ethical Committee in Region Skåne in Sweden (Dnr. 2016/654)

The study will be approved by the Data Protection Agencies in Denmark and Sweden.

9. TIME SCHEDULE AND PUBLICATION

Protocol will be send to the Scientific Ethical Committee in the Capital Region, Denmark in January 2016 and inclusion of patients will start as soon as the approval from SEC has been obtained. The inclusion of patients will run from March 2016 to February 2018. Statistical analyses, writing and preparing manuscripts will go on from February 2018 to January 2019.

The results of the study will be presented at national as well as international scientific congresses and published in high impact international scientific journals in reproductive medicine such as Human Reproduction or Fertility and Sterility. Further results of public interest will be reported in the public press.

10.FINANCING

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

1
2
3
4 The project is initiated by Professor Anja Pinborg. This project is part of the Reprounion program,
5
6 which has been supported by the Interreg-program for Öresund-Kattegat-Skagerak from EU,
7
8 Capital Region of Denmark, Region Skåne and Ferring Pharmaceutical Company. The project has
9
10 been financed with 450.000 euro (3.375.000 dkk) by a grant from Interreg/EU.

11
12 Patients included in this study and the Scientific Ethical Committee will be informed if further
13
14 funding is obtained for this study. Funding will be transferred to a research account in the bank of
15
16 Hvidovre Hospital, Capital Region of Denmark.
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

11. REFERENCES

1. Aflatoonian et al. Can fresh embryo transfers be replaced by cryopreserved-thawed embryo transfers in assisted reproductive cycles? A randomized controlled trial. *J Assist Reprod Genet* 2010;27:357–63.
2. Devroey P, Polyzos NP, Blockeel C. An OHSS-Free Clinic by segmentation of IVF treatment. *Hum Reprod* 2011;26:2593-7.
3. Evans J, Hannan NJ, Edgell TA, Vollenhoven BJ, Lutjen PJ, Osianlis T, Salamonsen LA, Rombauts LJ. Fresh versus frozen embryo transfer: backing clinical decisions with scientific and clinical evidence. *Hum Reprod Update* 2014;20:808-21.
4. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L; ESHRE working group on Poor Ovarian Response Definition. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. *Hum Reprod* 2011;26:1616-24.
5. Goswami M, Murdoch AP, Haimes E. To freeze or not to freeze embryos: clarity, confusion and conflict. *Hum Fertil (Camb)*. 2015;18:113-20.
6. Kansal Kalra S, Ratcliffe SJ, Milman L, Gracia CR, Coutifaris C, Barnhart KT. Perinatal morbidity after in vitro fertilization is lower with frozen embryo transfer. *Fertil Steril* 2011;95:548–553.
7. Kupka MS, Ferraretti AP, de Mouzon J, Erb K, D'Hooghe T, Castilla JA, Calhaz-Jorge C, De Geyter C, Goossens V; European IVF-Monitoring Consortium, for the European Society of Human Reproduction and Embryology. The European IVF-monitoring programme (EIM), for the European Society of Human Reproduction and Embryology (ESHRE). Assisted reproductive technology in Europe, 2010: results generated from European registers by ESHRE. *Hum Reprod* 2014;29:2099-113.
8. Loutradi et al. *Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis*. *Fertil Steril* 2008;90:186-93.
9. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and Perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in-vitro fertilisation treatment. A systematic review and meta-analysis. *Fertil Steril* 2012;98:368–377.
10. Maheshwari A and Bhattacharya S. Elective frozen embryo transfer cycles for all: ready for prime time? *Hum Reprod* 2013;28:6-9.

November 17th, 2016

Protokol_Freeze all and transfer later_V2_17112016

11. Papanikolaou EG, Humaidan P, Polyzos N, Kalantaridou S, Kol S, Benadiva C, Tournaye H, Tarlatzis B. New algorithm for OHSS prevention. *Reprod Biol Endocrinol* 2011;9:147.
12. Pinborg A. To transfer Fresh or Thawed embryos? *Semin Reprod Med* 2012;30:230-235.
13. Roque M, Lattes K, Serra S, Solà I, Geber S, Carreras R, Checa MA. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. *Fertil steril* 2013; 99:156–62.
14. Roque M. Freeze-all policy: is it time for that? *J Assist Reprod Genet* 2015;32:171-6.
15. Roque M, Valle M, Guimarães F, Sampaio M, Geber S. Freeze-all policy: fresh vs. frozen-thawed embryo transfer. *Fertil Steril* 2015;103:1190-3.
16. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. *Fertil Steril* 2011;96:344–348.
17. Zhu D, Zhang J, Cao S, Zhang J, Heng BC, Huang M et al. Vitrified-warmed blastocyst transfer cycles yield higher pregnancy and implantation rates compared with fresh blastocyst transfer cycles – time for a new embryo transfer strategy?. *Fertil Steril* 2011;95:1691–95.

BMJ Open

**Comparison of a “freeze all” strategy including GnRH agonist trigger versus a “fresh transfer” strategy including hCG trigger in assisted reproductive technology (ART)
– A study protocol for a randomised controlled trial**

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-016106.R1
Article Type:	Protocol
Date Submitted by the Author:	28-Mar-2017
Complete List of Authors:	<p>Stormlund, Sacha; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p> <p>Løssl, Kristine; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p> <p>Zedeler, Anne; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p> <p>Bogstad, Jeanette; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p> <p>Prætorius, Lisbeth; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p> <p>Nielsen, Henriette; The Fertility Clinic, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark</p> <p>Bungum, Mona; Reproductive Medicine Centre, Skane University Hospital, Malmö, Sweden</p> <p>Skouby, Sven; The Fertility Clinic, Department of Obstetrics and Gynaecology, Herlev University Hospital, Copenhagen, Denmark</p> <p>Mikkelsen, Anne Lis; The Fertility Clinic, Department of obstetrics and Gynaecology, Holbæk University Hospital, Holbæk, Denmark</p> <p>Andersen, Anders; The Fertility Clinic, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark</p> <p>Bergh, Christina; Department of Obstetrics and Gynaecology, Institute of Clinical Sciences, Sahlgrenska Academy, Gothenburg University, Reproductive Medicine, Sahlgrenska University Hospital, SE-413 45 Gothenburg, Sweden</p> <p>Humaidan, Peter; The Fertility Clinic, Skive Regional Hospital</p> <p>Pinborg, Anja; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p>
Primary Subject Heading:	Reproductive medicine
Secondary Subject Heading:	Obstetrics and gynaecology

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Keywords:	ART, FET, Freeze-all, RCT, Ongoing pregnancy rate, OPR

SCHOLARONE™
Manuscripts

For peer review only

1
2
3
4 1
5
6
7 2
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60TITLE PAGE
PROTOCOL ARTICLE

3 *Comparison of a “freeze all” strategy including GnRH agonist trigger*
4 *versus a “fresh transfer” strategy including hCG trigger in assisted*
5 *reproductive technology (ART)*
6 *– A study protocol for a randomised controlled trial*

7 **Authors**

8 Sacha Stormlund¹, Kristine Løssl¹, Anne Zedeler¹, Jeanette Bogstad¹, Lisbeth Prætorius¹,
9 Henriette Svarre Nielsen², Mona Bungum³, Sven O. Skouby⁴, Anne Lis Mikkelsen⁵, Anders Nyboe
10 Andersen², Christina Bergh⁶, Peter Humaidan⁷, Anja Pinborg¹

11 **Author Affiliations:**

12 ¹The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital,
13 Hvidovre, Copenhagen, Denmark

14 ²The Fertility Clinic, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

15 ³Reproductive Medicine Centre, Skane University Hospital, Malmö, Sweden

16 ⁴ The Fertility Clinic, Department of Obstetrics and Gynaecology, Herlev University Hospital,
17 Copenhagen, Denmark

18 ⁵ The Fertility Clinic, Department of obstetrics and Gynaecology, Holbæk University Hospital,
19 Holbæk, Denmark

20 ⁶ Department of Obstetrics and Gynaecology, Institute of Clinical Sciences, Sahlgrenska Academy,
21 Gothenburg University, Reproductive Medicine, Sahlgrenska University Hospital, SE-413 45
22 Gothenburg, Sweden

23 ⁷ The Fertility Clinic, Skive Regional Hospital and Faculty of Health, Aarhus University, Aarhus,
24 Denmark

25 **Correspondence to:** Sacha Stormlund; sacha.stormlund.01@regionh.dk

1
2
3 26 **ABSTRACT**
4
5

6 27 **Introduction** Pregnancy rates after frozen-thawed embryo transfer (FET) have improved in recent
7
8 28 years and are now approaching or even exceeding those obtained after fresh embryo transfer.

9
10 29 This is partly due to improved laboratory techniques, but may also be caused by a more
11
12 30 physiological hormonal and endometrial environment in FET cycles. Furthermore, the risk of
13
14 31 ovarian hyperstimulation syndrome (OHSS) is practically eliminated in segmentation cycles
15
16 32 followed by FET and the use of natural cycles in frozen-thawed embryo transfers may be beneficial
17
18 33 for the post-implantational conditions of foetal development. However, a freeze-all strategy is not
19
20 34 yet implemented as standard care due to limitations of large randomised trials showing a benefit of
21
22 35 such a strategy. Thus, there is a need to test the concept against standard care in a randomised
23
24 36 controlled design. This study aims to compare ongoing pregnancy and live birth rates between a
25
26 37 freeze-all strategy with GnRH agonist triggering versus hCG trigger and fresh embryo transfer in a
27
28 38 multicentre randomised controlled trial.

29 39 **Methods and analysis** Multicentre randomised, controlled, double-blinded trial of women
30
31 40 undergoing ART treatment including 424 normo-ovulatory women aged 18 to 39 from Denmark
32
33 41 and Sweden. Participants will be randomised (1:1) either A. GnRH agonist trigger and single
34
35 42 vitrified-warmed blastocyst transfer in a subsequent hCG triggered natural menstrual cycle or B.
36
37 43 hCG trigger and single blastocyst transfer in the fresh (stimulated) cycle. The primary endpoint is
38
39 44 to compare ongoing pregnancy rates per randomised patient in the two treatment groups after the
40
41 45 first single blastocyst transfer.

42 46 **Ethics and dissemination**

43 47 The study will be performed in accordance with the ethical principles in the Helsinki Declaration.
44
45 48 The study is approved by the Scientific Ethical Committees in Denmark and Sweden. The results
46
47 49 of the study will be publically disseminated.

48 50 **Trial registration numbers:** ClinicalTrials.gov identifier: NCT02746562; Ethical Approval,
49
50 51 Denmark: H-1600-1116, Ethical Approval, Sweden: Dnr. 2016/654
51
52
53
54
55
56
57
58
59
60

1
2
3 52 **Strengths and limitations of this study**
4
5

6 53 Strengths
7

- 8 54 • The design: A multicentre, randomised controlled double-blinded trial powered to identify
9 an increase in ongoing pregnancy rate in the freeze-all group compared to the conventional
10 55 fresh blastocyst transfer group
11 56
12
13 57 • The study includes normo-ovulatory women aged 18-39 years with a BMI < 35 thus results
14 can be extrapolated to the majority of the normo-ovulatory infertile population
15 58
16
17 59 • GnRH-agonist trigger in the freeze-all group adds a concept of an OHSS-free strategy
18
19

20 60 Limitations
21

- 22
23 61 • As both GnRH-agonist trigger and elective freeze-all are new treatment approaches, we will
24 not be able to distinguish the two effects from each other, but compare an OHSS-free
25 62 strategy to a conventional fresh transfer strategy
26 63
27
28 64 • The study is powered to detect a 12 % difference in ongoing pregnancy between the two
29 groups, thus smaller but yet clinically relevant differences may be overlooked
30 65
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

66 INTRODUCTION

67 The use of assisted reproductive technology (ART) is increasing and presently up to 5 % of birth
68 cohorts in certain countries are conceived by ART.¹ In recent years, pregnancy rates following
69 frozen embryo transfer (FET) have rapidly increased and may now be a viable and appropriate
70 alternative to the conventional fresh embryo transfer in ART. The main reason is the introduction of
71 vitrification, increasing post-thawing survival rates after blastocyst culture significantly as compared
72 to previous years.²⁻³ Implantation as well as clinical and ongoing pregnancy rates are
73 correspondingly improving in frozen cycles and approaching or even exceeding those associated
74 with fresh embryo transfer.⁴⁻⁶

75 A freeze-all strategy has been suggested as a way to further improve success rates in ART,
76 arguing that the use of the best embryo in frozen cycles instead of in fresh cycles may potentially
77 increase pregnancy rates and live birth rates.⁶⁻⁷ The rationale is that transfer of a frozen-thawed
78 embryo in a subsequent natural menstrual cycle has the advantage of an endometrium that has
79 not been exposed to the supraphysiological levels of estradiol and progesterone following
80 controlled ovarian stimulation (COS) in fresh cycles, which may negatively affect endometrial
81 receptivity.⁵⁻⁸ Elective FET (eFET) moreover has the benefit of essentially eliminating the risk of
82 developing late ovarian hyperstimulation syndrome (OHSS) associated with the pregnancy-related
83 rise in human chorionic gonadotropin (hCG) levels.⁹ If ovulation is induced with a GnRH agonist
84 instead of hCG and all embryos are frozen, even early OHSS is minimized making the overall
85 OHSS risk extremely low.¹⁰ Freezing and thawing of embryos additionally encourages an elective
86 single embryo transfer policy with cumulative pregnancy rates similar to those seen after double
87 embryo transfer.¹¹⁻¹²

88 Despite evidence suggesting that ART outcomes may be further improved with the adaptation of a
89 freeze-all strategy, the implementation remains a topic of ongoing debate and only one in five
90 transfers in Europe on average was performed with frozen-thawed embryos in 2012.¹ In a large
91 recent study, including 1508 patients with polycystic ovary syndrome comparing the freeze-all
92 strategy with conventional fresh embryo transfer, the authors found a significantly higher frequency
93 of live birth after the first frozen embryo transfer compared with fresh embryo transfer (49.3% vs.
94 42.0%).⁷ Correspondingly, in a meta-analysis including three trials accounting for 633 cycles in
95 women aged 27-33 years, eFET resulted in significantly higher clinical and ongoing pregnancy
96 rates compared with fresh embryo transfer.⁶ However, the included studies showed heterogeneity
97 and one of the included publications was later retracted due to serious methodological flaws. In
98 addition, the vast majority of the participants were high responders (496 out of 633) accounting for
99 a highly selected group of patients, mostly consisting of PCOS patients or patients with and

1
2
3 100 ovarian PCO like morphology.⁶ Moreover, previous studies were performed in China, US and
4 101 Japan making them less generalizable to a European ART setting. According to Clinicaltrials.gov
5 102 there are a few ongoing European RCT's on the freeze-all strategy, however none of these studies
6 103 investigate an almost complete OHSS-free strategy including GnRH-agonist trigger in the freeze-
7 104 all group.
8 105 OHSS is one of the most severe side effects of ART and is potentially life threatening. The present
9 106 protocol describes a randomised trial assessing a new ART treatment strategy, where OHSS can
10 107 be almost completely avoided. The results are very important as the majority of our patients could
11 108 avoid the OHSS risk by applying the "GnRH agonist and freeze-all" strategy, maybe even with a
12 109 higher chance of pregnancy. This concept has not been assessed before, and should relevantly be
13 110 considered when planning studies investigating the freeze-all strategy underlining the need for
14 111 large multicentre randomised controlled trials exploring the GnRH agonist and freeze-all strategy in
15 112 a broad population of ART patients. The present study will explore this approach in a bi-national
16 113 multicentre randomised controlled trial setting providing information on the prospect of a freeze-all
17 114 strategy.

115 **Objectives**

116 *Primary objective*

117 The primary objective of the study is to investigate if the ongoing pregnancy rate per randomised
118 patient after the first potential single blastocyst transfer is superior in a freeze-all and transfer later-
119 strategy compared to the conventional hCG trigger and fresh transfer strategy.

120 Ongoing pregnancy rate is defined as an intrauterine pregnancy with a foetal heart beat at
121 transvaginal ultrasound in gestational week 7-8.

122 Ongoing pregnancy rate per first blastocyst transfer is also considered as a primary aim of the
123 study addressing possible differences in endometrial receptivity between the two groups.

124 *Secondary objectives*

125 Secondary objectives include:

- 126 1. To assess cumulative live birth rates after *one complete cycle* including consecutive single
127 blastocyst transfers of all embryos deriving from that oocyte retrieval (fresh and frozen) in
128 the two study groups
- 129 2. To assess the transfer cancellation rate in the two study groups
- 130 3. To assess the prevalence of OHSS in the two study groups
- 131 4. To compare neonatal outcomes (preterm birth, low birth weight, SGA (small-for-gestational
132 age), LGA (large-for-gestational age) and perinatal mortality) and the incidence of
133 preeclampsia in the two study groups

- 1
2
3 134 5. To measure time-to-pregnancy from the date of start of COS to the date of the first ongoing
4 pregnancy in the two study groups
5 135
6 136 6. To assess quality of life for both female and male partners during the two treatment
7 protocols
8 137
9 138 7. To assess physical well-being by way of questionnaires and VAS scores regarding pain
10 and discomfort at four and 16 days after oocyte retrieval in the two study groups
11 139
12
13

14 140 **METHODS AND ANALYSIS**

17 141 **Study design**

18 142 The study is designed as a multicentre randomised, controlled double-blinded trial with seven
19 fertility clinics in Denmark and Sweden participating. All seven clinics are part of a University
20 Hospital setting and perform standardized treatments according to the public health care system in
21 Denmark and Sweden. Patient enrolment started in May 2016 and the last patients are expected to
22 be included in the study in May 2018 with the primary outcome measure, ongoing pregnancy rate,
23 being known for these patients approximately four months later for the patients allocated to the
24 freeze-all group.
25
26
27
28
29
30

31 149 **Study population/Participants and recruitment**

32 150 The study participants will consist of women and their partners initiating ART treatment at one of
33 the seven participating public clinics in Denmark and Sweden. Before initiating treatment patients
34 will attend an information meeting, where they will be informed about the standard ART
35 procedures, treatment regimens as well as ongoing clinical studies at the treatment sites. Those
36 patients not able to participate in the information meeting will instead be informed by a doctor at an
37 outpatient clinic consultation. Recruitment will be carried out by the doctors and study nurses at the
38 fertility clinics. Prior to the initiation of treatment, patient files will be browsed by investigators at the
39 clinics to assess if the patient fulfills the immediate inclusion criteria. Screening, including
40 ultrasound examination of the uterus and ovaries is done on menstrual cycle day two or three
41 securing that all inclusion criteria are met. Patients fulfilling the study criteria will start COS using a
42 GnRH antagonist co-treatment in accordance with the standard routines of the trial site.
43
44
45
46
47
48
49

50 161 **Eligibility criteria**

51
52 162 To participate in the study, women will be required to meet the following inclusion criteria: Female
53 age 18 to 39 years; eligibility to initiate the first, second or third ART cycle with oocyte aspiration
54 (IVF or ICSI); AMH level > 6.28 pmol/L (Roche Elecsys assay) corresponding to the AMH
55 threshold level used in the Bologna criteria to characterize poor responders; regular menstrual
56
57
58
59
60

166 cycle \geq 24 days and \leq 35 days; body mass index 18–35 kg/m²; preservation of both ovaries and
 167 capability of signing informed consent. For specific exclusion criteria see Table 1.

Table 1. Specific exclusion criteria

Endometriosis stage III to IV
Ovarian cysts with a diameter > 30 mm at day of start of stimulation
Submucosal fibroids
Women with severe co-morbidity (IDDM (insulin dependent diabetes mellitus), NIDDM (non-insulin dependent diabetes mellitus), gastrointestinal, cardiovascular, pulmonary, liver or kidney disease)
Dysregulated thyroid disease
Non-Danish or English speaking
Contraindications or allergies to use of gonadotropins or GnRH antagonists
TESA (testicular sperm aspiration)
OD (oocyte donation)
Previous inclusion in the study

168 **Randomisation and blinding**

169 Patients who meet the inclusion criteria are randomised 1:1 to one of the two treatment groups: A.
 170 Freeze-all including GnRH agonist trigger, blastocyst vitrification and subsequent FET in an hCG
 171 triggered natural cycle or B. Traditional hCG trigger and fresh blastocyst transfer.
 172 The randomisation is carried out by a study nurse or a non-treating doctor using a computerised
 173 randomisation program that runs a minimization algorithm, initially seeded using a random block
 174 sequence for the first subjects. The minimization algorithm is balancing the following variables:
 175 Female age (mean, and frequency of age \geq 37 years), previously performed cycles (frequency of
 176 0/1/2 cycles), nulliparous (frequency of yes/no), fertilisation method (frequency of IVF/ICSI),
 177 smoking (frequency of yes/no), AMH (\leq 12 pmol/L, 13-28 pmol/L, >28 pmol/L) and mean BMI. It
 178 selects with high (but less than 1.0) probability the treatment arm that provides the optimal balance
 179 between the arms. It also enforces predefined maximum allowed differences in number of subjects
 180 in each treatment arm at each study site (fertility clinic) and within the whole study.
 181 Furthermore, the starting dose of FSH is entered into the randomisation program before
 182 randomisation is performed to make sure that the FSH dose is decided upon before randomisation.
 183 Both the treating consultants and patients are blinded to the randomisation results during the
 184 controlled ovarian stimulation until the day when ovulation trigger is planned.

1
2
3
4
5 185 **Treatment arms and interventions**

6 186 The short GnRH antagonist protocol and blastocyst culture is applied in both treatment arms. The
7 187 starting dose and type of gonadotropin is decided by the doctor on stimulation day one (cycle day
8 188 two or three) and entered into the randomisation program prior to randomisation. Individualized
9 189 gonadotropin dosing based on AMH, age, weight, previous COH cycles are applied. Recombinant
10 190 follicular stimulating hormone (rFSH) or human menopausal gonadotropin (hMG) can be used
11 191 according to the preference of the site, but the daily dose cannot exceed 300 IU. The gonadotropin
12 192 stimulation will be performed according to the routine in the clinics and can be changed during the
13 193 treatment according to the ovarian response to stimulation evaluated through ultrasound
14 194 examination. GnRH antagonist co-treatment is initiated at a daily dose of 0.25 mg on stimulation
15 195 day five or six according to the general standards in each clinic and is continued throughout the
16 196 rest of the gonadotropin stimulation period.

17 197 Ultrasound examination is performed on cycle day two or three (baseline), stimulation day six or
18 198 seven and subsequently every second to third day until ovulation trigger is decided according to
19 199 the hCG/GnRH agonist trigger criterion: as soon as three follicles are ≥ 17 mm or one day later. At
20 200 baseline a comprehensive ultrasound examination will estimate endometrial thickness, ovarian
21 201 volume as well as number and size of antral follicles divided into the following three subclasses: 2-
22 202 4 mm, 5-7 mm and 8-10 mm. On the day of ovulation trigger endometrial thickness and
23 203 morphology as well as follicular development with number and size of follicles > 10 mm are
24 204 registered.

25 205 When ovulation trigger is decided, the result of the randomisation is disclosed to both doctors and
26 206 patients and ovulation and oocyte maturation is triggered with a GnRH agonist trigger injection (0.5
27 207 mg Buserelin) in the freeze-all group or a single injection of 250 μg of hCG in the fresh embryo
28 208 transfer group. If >18 follicles with a diameter >11 mm are observed in the fresh embryo transfer
29 209 group GnRH agonist triggering with Buserelin and vitrification of all embryos will be performed to
30 210 avoid severe OHSS. All fertilised oocytes are cultured to the blastocyst stage and the embryos are
31 211 scored and ranked according to standardised criteria ascribed to this study. The ranking will assure
32 212 that the blastocyst with the highest implantation potential is transferred first in both groups. In the
33 213 fresh transfer group, single blastocyst transfer is performed on day five after oocyte retrieval if a
34 214 good quality blastocyst has developed. Surplus good quality blastocysts will be vitrified on day five
35 215 or six. Luteal phase support is administered as vaginal progesterone according to the clinics
36 216 standard procedures from day two after oocyte retrieval until the day of hCG test; thus luteal
37 217 support is not extended into early pregnancy. In the freeze-all group all blastocysts of good quality
38 218 are vitrified on day five or six depending on when the blastocyst stage is reached. The blastocyst
39 219 with the highest rank is marked and will be the first one used in a subsequent hCG triggered

40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 220 modified natural cycle FET. There should be *at least one completed menstrual cycle in between*
4 221 *the stimulation and the embryo transfer*. In FET cycles a single injection of 250 µg hCG is
5 222 administered, when the leading follicle is ≥ 17 mm. Blastocyst transfer is performed six or seven
6 223 days after the hCG injection. No luteal phase support is given.

7
8 224 A plasma hCG test is performed 11 days after blastocyst transfer. Ongoing clinical pregnancy is
9 225 defined as foetal heart beat at gestational age 7-8 confirmed by transvaginal ultrasound 3 to 4
10 226 weeks after a positive plasma-hCG test.

11 12 13 14 15 227 **Data collection and management**

16 228 Treatment related data is collected at 1) Baseline (cycle day two or three), 2) Day of ovulation
17 229 trigger and 3) five days after oocyte retrieval. Data on blastocysts are collected at culture day
18 230 five/six. Follow-up data on all pregnancies resulting from blastocysts transferred according to the
19 231 study protocol will be followed from study inclusion and one year onwards. Data is transferred to
20 232 an online eCRF system called MediCase with an underlying Microsoft SQL server database
21 233 located in a guarded underground facility in Sweden. Data is backed up daily (one back-up to
22 234 another computer in the same physical location as the server, and a second back-up to a
23 235 physically separate location, also in Sweden). MediCase has a complete audit trail and is designed
24 236 to only contain de-identified data and is entirely based on anonymous subject ID numbers used in
25 237 the trial.

26 27 28 29 30 31 32 33 34 238 **Sample collection**

35 239 Blood samples will be collected three times during the treatment process: 1) Baseline (cycle day
36 240 two or three), 2) Day of ovulation trigger and 3) 16 days after oocyte retrieval (day of pregnancy
37 241 test in the fresh embryo transfer group). For overview of samples see Table 2. Furthermore one
38 242 serum, plasma and fullblood sample are drawn at baseline and on the day of triggering and stored
39 243 according to a trial specific laboratory manual in a project-specific biobank as back-up for analysis
40 244 of endocrine and immunological factors of relevance for pregnancy. The frozen samples will be
41 245 kept anonymised in the biobank with only the patient specific project ID number and collection date
42 246 marked on the sample. The samples will be store in the participating fertility clinics and destroyed 5
43 247 years after the end of the study period if not analysed.

44
45
46
47
48
49
50 248 Further blood samples will be collected during the luteal phase for a smaller subgroup of 30
51 249 patients in each treatment group as part of a luteal phase subgroup analysis of differences in
52 250 hormone levels in the two groups. The following blood samples will be collected at 1) Day of
53 251 ovulation induction and 2) Day of ovulation trigger, day of ovulation trigger +7, +11, +14, +16 and
54 252 +19: Estradiol, Inhibin-A, OH-Progesterone, Progesterone, LH and hCG.

Table 2. Blood sample collection

Baseline (cycle day 2 or 3)	AMH FSH LH Estradiol Progesterone TSH TPO-antibodies Vitamin D CRP suPAR*
Day of ovulation induction	FSH LH Estradiol Progesterone CRP suPAR*
16 days after oocyte retrieval	CRP suPAR* hCG**

* Soluble urokinase-type plasminogen activator receptor, only measured at Hvidovre Hospital

** only fresh embryo transfer group

253 Questionnaires

254 Women as well as male partners will be asked to fill in quality of life validated questionnaires twice
 255 during the treatment process: 1) Four days after oocyte retrieval and 2) 16 days after oocyte
 256 retrieval. The questionnaires consist of standardized questions specially developed to explore
 257 emotional aspects as well as quality-of life related aspects of the treatment process. The women
 258 will at the same time be asked to fill in questionnaires regarding physical discomfort including a
 259 VAS score of physical pain in relation to the treatment.

260 Statistics

261 *Sample size calculation*

262 The trial is designed as a superiority study. Sample size calculation indicates that 424 participants
 263 (n = 212 in each arm) are required to have a 80 % chance of detecting, at a significance level at
 264 0.05, an increase in the primary outcome measure (ongoing pregnancy rate per randomised after
 265 first potential blastocyst transfer) from 30% in the control group (fresh embryo transfer) to 43 % in
 266 the experimental group (freeze-all).

1
2
3 267 *Outcome measurements (primary and secondary)*
4

5 268 The primary endpoint is the ongoing pregnancy rate per randomised patient after the transfer of
6 269 the first potential blastocyst. Ongoing pregnancy is defined as a pregnancy with a positive foetal
7
8 270 heart beat at gestational week 7-8.

9 271 Other endpoints explored in the study contribute to the assessment of other relevant aspects of the
10 272 freeze-all strategy including ongoing pregnancy rates per transfer, per started stimulation and per
11 273 oocyte pick-up (percentage of participants with an ultrasound confirmation of foetal heart beat at
12 274 gestational age 7-8) as well as live birth rate and cumulative live birth rates (percentage of
13 275 participants with 1 live born neonate after 1 year of follow-up). The study furthermore aims to
14 276 document the prevalence of OHSS assessed by the number of patients admitted to hospital under
15 277 this diagnosis and the number of patients having ascites puncture. In addition, it is planned to
16 278 evaluate pregnancy related complications as well as neonatal outcomes in both groups. For
17 279 complete overview of all secondary endpoint measures see Table 3.
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 3. Secondary endpoints

◆ Ongoing pregnancy rate per start of <i>per started ovarian stimulation and per oocyte retrieval</i>
◆ Live birth rate after the first blastocyst transfer calculated <i>per randomized patient, per started ovarian stimulation, per oocyte retrieval and per transfer</i>
◆ Cumulative live birth rate after one stimulated cycle with oocyte retrieval
◆ Cumulative live birth rate after use of all frozen blastocyst or after at least 1 year of follow-up
◆ Number of cycles with no embryo transfer
◆ Time-to-pregnancy (from start of ovarian stimulation to positive hCG)
◆ Time-to-delivery
◆ Cancelled embryo transfers
◆ Ovarian hyperstimulation syndrome (OHSS)
◆ Preterm birth
◆ Low birth weight
◆ Small-for-gestational age (SGA)
◆ Large-for-gestational age (LGA)
◆ Perinatal mortality
◆ Preeclampsia
◆ Placental rupture
◆ Positive hCG 11 days post embryo transfer
◆ Miscarriage, biochemical pregnancies, ectopic pregnancies
◆ Quality of life for female and male partner
◆ Cost-effectiveness
Other outcome measurements
◆ Number of good blastocysts
◆ Number of fertilized oocytes
◆ Number of high quality embryos day 2
◆ Number of grade 1 blastocysts
◆ Number of frozen blastocyst
◆ Paraclinical data: Endocrine, genetic and immunological parameters

1
2
3 280 *Statistical analyses*

4
5 281 Analyses of cumulative pregnancy rates and live birth rates after one oocyte retrieval including
6
7 282 fresh and all frozen embryo transfer cycles will be compared by Cox-regression analyses.
8
9 283 Comparisons between treatment groups will be performed primarily according to the intention-to-
10
11 284 treat (ITT) principle but per-protocol analyses will also be done. Continuous data will be compared
12
13 285 by students *t*-test or Mann-Whitney U test and Kruskal-Wallis test as appropriate. Proportions will
14
15 286 be compared with chi-square test. Predictive factors for ongoing pregnancy in the two treatment
16
17 287 groups will be tested with multivariate logistic regression analyses. A p-value of < 0.5 will be
18
19 288 considered as statistically significant.

20
21 289 *Patients in fresh embryo transfer group with GnRH agonist triggering*

22
23 290 Patients allocated to the fresh transfer group who end up receiving GnRH agonist trigger and
24
25 291 vitrification of all blastocysts due to risk of OHSS (>18 follicles with a diameter >11 mm on trigger
26
27 292 day) will still be analysed as part of the fresh transfer group according to the intention-to-treat
28
29 293 principle. Their first blastocyst transfer will derive from their first FET cycle and ongoing
30
31 294 pregnancies from these first transfers will be included in the numerator together with ongoing
32
33 295 pregnancies derived from the majority of patients with first blastocyst transfer in the fresh cycle.
34
35 296 The denominator will be all randomised patients.

36
37 297 **ETHICS, SAFETY AND DISSEMINATION**

38
39 298 The study has been approved by the Danish regional committee on Health Research Ethics of the
40
41 299 Capital Region and the Swedish national council on medical ethics.
42
43 300 Following oral and written information outlining the rationale, trial design, aims and treatment
44
45 301 procedures written informed consent will be obtained from women and male partners prior to the
46
47 302 enrolment in the study.

48
49 303 The participants are stimulated using individualised doses of gonadotropin stimulation in
50
51 304 accordance with the clinical practice at each site. In all clinics serum AMH is considered when the
52
53 305 FSH dose is determined. All medicine used in the study is part of standard ART care.

54
55 306 The overall safety of the patients is high in both treatment groups. The risk of OHSS is expected to
56
57 307 be similar to the standard clinical protocol in the fresh embryo transfer group and lower in the
58
59 308 freeze-all group in which GnRH agonist is used for ovulation trigger. In women in the fresh embryo
60
309 transfer group with a risk of OHSS development (more than 18 follicles with a diameter over 11

310 mm), GnRH agonist will be used for trigger instead of hCG and all blastocysts will be vitrified and
311 the transfer postponed.

312 No financial incentive exists for the participants as all couples are reimbursed for their first three
313 ART treatments in the public health care system in the Nordic countries.

314 The results of the study will be publically disseminated in peer-reviewed scientific journals and
315 presented at relevant international scientific meetings such as ESHRE (European Society of
316 Human Reproduction and Embryology) and ASRM (American Society for Reproductive Medicine).
317 In addition results will be published in popular science journals and other media.

318 **DISCUSSION**

319 The increasing interest in possible benefits of a freeze-all strategy and the limitations of existing
320 randomised controlled trials comparing this strategy with conventional fresh embryo transfer
321 underline the need for additional studies. The few previous RCT's have demonstrated significantly
322 increased pregnancy- and delivery rates with freeze-all, however these studies were performed in
323 highly selected patient populations with poor generalizability.⁶⁻⁷ Further, the treatment strategy
324 combining GnRH agonist trigger and freeze-all minimizing the risk of severe OHSS development
325 has not yet been investigated in a RCT setting. As GnRH agonist trigger does not hamper the yield
326 of mature oocytes¹² and reduces the risk of OHSS to an absolutely minimum, it seems rational to
327 include GnRH agonist trigger in the freeze-all concept. Evidently, we are unable to distinguish
328 between the effect of the GnRH-agonist trigger and the effect of elective freeze-all, when both are
329 included in the freeze-all treatment arm. The present study therefore compares an 'OHSS-free'
330 freeze-all strategy including GnRH agonist trigger with a fresh transfer strategy with hCG trigger. In
331 both treatment arms individualized gonadotropin dosing is used with the possibility of conversion to
332 GnRH agonist trigger and segmentation in case of risk of OHSS development in the fresh embryo
333 transfer group. Individualized gonadotropin dosing based on female age and weight, antral follicle
334 count, AMH and results of previous COH cycles is applied, as this is the standard treatment
335 approach used routinely in all of the participating clinics. The AMH cut-off value at 6.28 pmol/L
336 (Roche Elecsys assay) corresponding to the Bologna criteria for poor ovarian response was
337 chosen to have a reasonable chance of the patient ending up with at least one usable blastocyst
338 after aspiration. It could be argued that an open randomisation, rather than a double-blinded study
339 design, would allow a better exploration of the concept as higher gonadotropin doses and more
340 oocytes could be safely aimed for in the freeze-all group. However, as this is the first RCT of a
341 freeze-all strategy including GnRH agonist trigger, a double-blinded design was chosen to

1
2
3 342 minimize differences between the two treatment arms and gonadotropin dosing is decided upon
4 343 independently of allocation to treatment group, as this is done prior to randomisation. In addition,
5 344 even though a strategy combining GnRH agonist trigger and freeze-all is near OHSS free,
6 345 increasing gonadotropin dosing would nonetheless add a potential risk of early OHSS in the
7 346 patients.

8 347 The primary endpoint of this study is to investigate ongoing pregnancy rates per randomised
9 348 patient after the first potential blastocyst transfer. Cumulative rates are additionally planned to be
10 349 calculated, but as the number of aspirated oocytes is expected to be the same in both treatment
11 350 groups due to gonadotropin dosing being decided upon independently of allocated treatment
12 351 group, the effect of the freeze all strategy on the results of the first transfer may be diluted with the
13 352 inclusion of additional FET's.

14 353 The strengths of this study include the design as a multicenter randomised controlled double-
15 354 blinded trial as well as preregistration and publication of the study protocol for more transparency.
16 355 The investigation of several outcome measures related to different aspects of success parameters,
17 356 including quality of life may furthermore add important information as regards the future potential of
18 357 the freeze-all strategy in assisted reproduction.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

FIGURE LEGEND Figure 1:

Figure 1. Flowchart of the Freeze-all study design

For peer review only

1
2
3 **Contributor statement** ANA, AP and SS participated in the conception, design, and writing of the
4 study protocol. KL, HSN and CB contributed to the revision and editing of the study protocol. AP,
5 SS, KL, JB, LP, ANA, HSN, CB, PH, MB, ALM and SOS will be involved in the recruitment of
6 patients and the acquisition of data. SS wrote the first draft of this manuscript. AZ was involved in
7 developing the laboratory criteria for the study. SS, AP, ANA, PH, CB, KL, JB, LP, HSN, MB, ALM
8 and SOS will AP, ANA, PH, CB, KL, AZ, JB, LP, HSN, MB, ALM and SOS were all involved in
9 critical revision of the manuscript. All authors approved the final version of the manuscript to be
10 submitted.
11

12 **Competing interests** None declared

13 **Funding** The study is part of and fully funded by the Repronion Collaborative study, co-financed
14 by the European Union, Interreg, V ÖKS.
15

16 **Data sharing statement** This manuscript is a study protocol. Data from the final study will be
17 shared according to the coming ICJME guidelines.
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

REFERENCES

1. Calhaz-Jorge C, de Geyter C, Kupka MS, et al. Assisted reproductive technology in Europe, 2012: results generated from European registers by ESHRE. *Human reproduction* 2016;31(8):1638-52.
2. Loutradi KE, Kolibianakis EM, Venetis CA, et al. Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. *Fertility and sterility* 2008;90(1):186-93.
3. Cobo A, de los Santos MJ, Castello D, et al. Outcomes of vitrified early cleavage-stage and blastocyst stage embryos in a cryopreservation program: evaluation of 3,150 warming cycles. *Fertility and sterility* 2012;98(5):1138-46 e1.
4. Zhu D, Zhang J, Cao S, et al. Vitrified-warmed blastocyst transfer cycles yield higher pregnancy and implantation rates compared with fresh blastocyst transfer cycles--time for a new embryo transfer strategy? *Fertility and sterility* 2011;95(5):1691-5.
5. Shapiro BS, Daneshmand ST, Garner FC, et al. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfers in high responders. *Fertility and sterility* 2011;96(2):516-8.
6. Roque M, Lattes K, Serra S, et al. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. *Fertility and sterility* 2013;99(1):156-62.
7. Chen ZJ, Shi Y, Sun Y, et al. Fresh versus Frozen Embryos for Infertility in the Polycystic Ovary Syndrome. *The New England journal of medicine* 2016;375(6):523-33.
8. Kansal Kalra S, Ratcliffe SJ, Milman L, et al. Perinatal morbidity after in vitro fertilization is lower with frozen embryo transfer. *Fertility and sterility* 2011;95(2):548-53.
9. Devroey P, Polyzos NP, Blockeel C. An OHSS-Free Clinic by segmentation of IVF treatment. *Human reproduction* 2011;26(10):2593-7.
10. Youssef MA, Van der Veen F, Al-Inany HG, et al. Gonadotropin-releasing hormone agonist versus HCG for oocyte triggering in antagonist-assisted reproductive technology. *The Cochrane Database of systematic review* 2014;31;(10):CD008046.
11. Pinborg A. To transfer fresh or thawed embryos? *Seminars in reproductive medicine* 2012;30(3):230-5.
12. Engmann L, Benadiva C, Humaidan P. GnRH agonist trigger for the induction of oocyte maturation in GnRH antagonist IVF cycles: a SWOT analysis. *Reproductive BioMedicine Online* 2016 Mar;32(3):27485

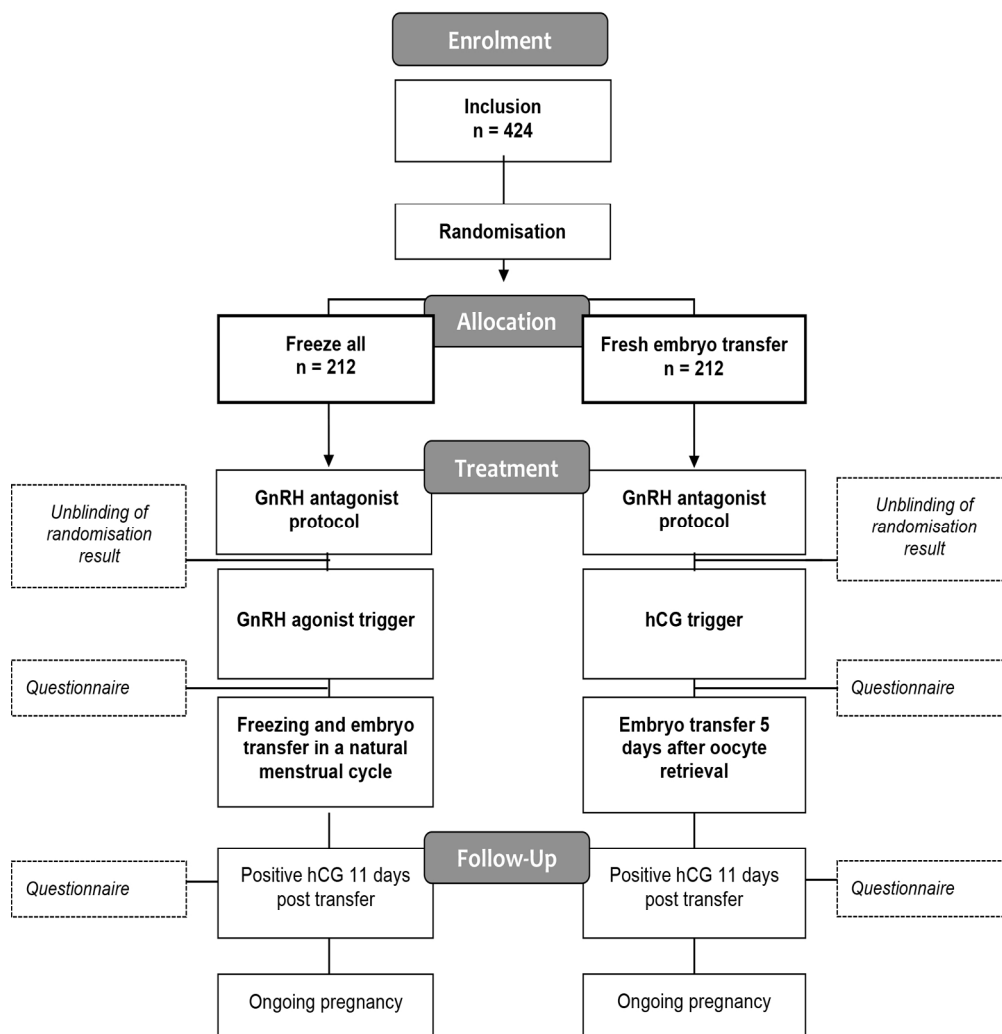


Figure 1. Flowchart of the Freeze-all study design

173x186mm (300 x 300 DPI)



February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

A multicentre randomized controlled trial of a “Freeze all and transfer later”
versus a conventional “Fresh Embryo Transfer” strategy for assisted
reproductive technology (ART) in women with a regular menstrual cycle

- A Multicentre Randomized Controlled Trial of patients undergoing IVF/ICSI

Multicenter studium med otte deltagende fertilitetsklinikker i Danmark og Sverige

Projektansvarlig læge

Anja Pinborg, professor, overlæge, dr.med.

Fertility Clinic, Gynecologic/Obstetric Department, Hvidovre Hospital

Kettegaard Allé 30, DK – Hvidovre 2650

Telephone: +45 38 62 26 56

e-mail: anja.bisgaard.pinborg.01@regionh.dk

AutorisationsID: 00QKV

Deltagende Afdelinger:

Hvidovre Hospital

Sacha Stormlund (læge 1.februar 2016, kommende PhD stud)

Kristine Løssl, overlæge, PhD,

Anne Zedeler, laboratorieleder, Ph.D.

Jeanette Wulff Bogstad, lægelig klinikleder, overlæge

Lisbeth Prætorius, overlæge

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

Herlev Hospital

Sven Skouby, professor, dr. med.

Marie Louise Grøndahl, laboratorieleder, Ph.D

Fertility Clinic, Herlev Hospital, Copenhagen University Hospital

Rigshospitalet

Henriette Svarre Nielsen, overlæge, dr.med.

Anne Loft, overlæge

Anders Nyboe Andersen, professor, overlæge, dr.med.

Fertility Clinic, Rigshospitalet, Copenhagen University Hospital

Holbæk Hospital

Anne Lis Mikkelsen, klinikleder, overlæge, dr.med.

Fertility Clinic, Region Sjælland, Holbæk Hospital

Malmö University Hospital

Aleksander Giwercman, forskningsleder, professor, overlæge, dr.med.

Fertility Centre, Malmö University Hospital, Lunds Universitet

Sahlgrenska University Hospital

Christina Bergh

Department of Obstetrics and Gynaecology, Institute of Clinical Sciences, Sahlgrenska Academy

Gothenburg University, Reproductive Medicine

Sahlgrenska University Hospital, SE-413 45 Gothenburg, Sweden

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

Fertility Clinic Skive

Peter Humaidan, professor, overlæge, dr. med

The Fertility Clinic, Skive Regional Hospital and Faculty of Health, Aarhus University, Aarhus, Denmark

Storklinik,

Nina la Cour Freisleben, speciallæge, phd

Vivaneo Copenhagen, Storklinik, Store Kongesgade 40 G, 1264-Copenhagen K, Denmark

Statistical advisor

Julie Forman, Cand. Scient, Associate Professor

Section of Biostatistics

University of Copenhagen

1. BACKGROUND

In recent years improved cryopreservation techniques have made frozen embryo transfer (FET) a viable and promising alternative to fresh embryo transfer in assisted reproduction (ART). The optimization of cryopreservation techniques from slow-freezing to vitrification and prolonged embryo culture from cleavage to blastocyst state encourages the use of FET as the embryo survival rate following freezing and thawing is now significantly higher reaching 95-97% (Loutradi et al., 2008). Success rates including implantation as well as clinical and on-going pregnancy rates in FET are also significantly improving and approaching or even exceeding those associated with fresh embryo transfer (Kupka et al., 2014; Roque et al., 2013; Shapiro et al., 2011; Zhu et al., 2011). This is partly due the improved laboratory techniques, but may also be due to the endometrial environment in the FET cycles, which mirrors the natural cycle. In the stimulated cycle supraphysiological levels of estradiol and progesterone are present and may cause impaired endometrial receptivity (Shapiro et al., 2011). Furthermore, obstetric and perinatal outcomes after cryopreservation of embryos have been investigated and follow-up data from children born after FET have shown lower perinatal morbidity compared with fresh

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 embryo transfer (Kansal et al., 2011), but FET may also give rise to more large-for-gestational
5 age babies (Pinborg et al., 2014). In addition, a recent systematic review and meta-analysis on
6 data from 11 observational studies has shown better perinatal outcomes including lower
7 perinatal mortality in singleton pregnancies following frozen-thawed embryo transfer
8 compared with pregnancies after fresh embryo transfer (Maheshwari et al., 2012). Moreover,
9 FET has the benefits of minimizing the risk of ovarian hyperstimulation syndrome (OHSS),
10 which is the most severe side effect of ART and potentially life threatening. Finally, improved
11 cryopreservation techniques favour an elective single embryo transfer (eSET) policy minimizing
12 multiple pregnancies after ART (Pinborg, 2012).
13
14
15
16
17
18
19

20
21 Despite the noticeable advantages of embryo cryopreservation, fresh embryo transfer has
22 persistently been the conventional in vitro fertilisation (IVF) procedure as only one in five
23 transfers were made using frozen-thawed embryos in Denmark in 2013
24 (www.fertilitetsselskab.dk). This favour of a fresh embryo transfer strategy is however
25 reflected in other European countries including Finland, Sweden and Iceland where
26 approximately every third ART child is born after FET (Kupka et al., 2014). Some evidence
27 suggests that IVF outcomes can be further improved with the adaptation of a 'freeze-all' or
28 elective frozen embryo transfer (eFET) strategy with replacement of thawed embryos in
29 natural cycles (Evans et al., 2014; Devroey et al., 2011; Maheshwari et al., 2013; Roque et
30 al., 2013).
31
32
33
34
35
36
37
38
39

40 In a recent meta-analysis including three trials accounting for 633 cycles in women aged 27–33
41 years (Roque et al., 2013), FET resulted in significantly higher ongoing pregnancy rates (RR
42 1.32, 95% CI 1.10–1.59) and clinical pregnancy rates (RR 1.31, 95% CI 1.10–1.56). The studies
43 showed heterogeneity and only 137 of the participants were normal responders, while the rest
44 was high responders. Moreover one study included only cleavage stage embryo transfer while
45 the other two included blastocyst transfers only. The studies were performed in Japan and in
46 the US, while no European RCT has been published yet. Further, one of the included papers
47 (Aflatoonian et al., 2010) was later retracted based on findings of serious methodological flaws
48 in the study. This accentuates the need for a large multicentre, randomized controlled trial to
49
50
51
52
53
54
55
56
57
58
59
60

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 evaluate the prospect and clinical consequences of a “freeze all embryos and transfer later”
5
6 policy compared with conventional fresh embryo transfer.
7

8
9 The aim of this multicentre randomized controlled trial is to compare a “freeze-all” embryo
10 strategy with a conventional single fresh embryo transfer strategy in women 18 to 40 years of
11 age undergoing their first to third IVF/ICSI cycle women with regard to treatment outcomes,
12 risks for mother and child, quality of life and cost-effectiveness aspects of the two treatment
13 modalities in a short GnRH antagonist protocol with blastocyst transfer and vitrification as the
14 freezing method.
15
16
17
18
19
20
21
22

23 **2. STUDY AIMS**

- 24
25
26 1. The primary aim is to compare ongoing pregnancy rates per randomized patient and ongoing
27 pregnancy rates per transfer in the “freeze-all” versus “fresh embryo transfer” group.
28
29
- 30
31 2. To assess live birth rates per randomized patient and per transfer in the “freeze-all” versus
32 “fresh embryo transfer” group
33
34
- 35
36 3. To assess cumulative live birth rates after one stimulated cycle with oocyte retrieval in the two
37 study arms.
38
39
- 40
41 4. To compare perinatal outcomes (preterm birth, low birth weight, small-for-gestational age,
42 large-for-gestational-age, preeclampsia and perinatal mortality) in the two groups.
43
44
- 45
46 5. To measure time to pregnancy from start of ovarian stimulation and quality of life in both
47 females and males in the two groups.
48
- 49
50 6. To explore VAS scores regarding pain and discomfort at the day of embryo transfer and 11 days
51 post transfer in the two study arms
52
- 53
54 7. To assess female physical well-being during the two treatment modalities and to assess
55 quality of life for both female and male partners during the two treatment protocols.
56
57
58
59
60

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

3. ENDPOINTS

Primary endpoints

- Ongoing pregnancy rate *per randomized patient*
(pregnancy with positive fetal heart beat in gestational week 7-8)
- Ongoing pregnancy rate *per transfer* of the first blastocyst
(pregnancy with positive fetal heart beat in gestational week 7-8)
- Ongoing pregnancy rate *per oocyte pick-up*
(pregnancy with positive fetal heart beat in gestational week 7-8)
- Ongoing pregnancy rate *per start of ovarian stimulation*
(pregnancy with positive fetal heart beat in gestational week 7-8)

Secondary endpoints

- Live birth rates calculated per randomized patient, per started ovarian stimulation, per oocyte pick-up and per transfer
- Cumulative live birth rate after one stimulated cycle with oocyte retrieval
- Cumulative live birth rate after use of all frozen blastocysts or after at least 1 year of follow-up.
- Number of cycles with no embryo transfer
- Time-to-pregnancy (from start of ovarian stimulation to positive hCG)
- Time-to-delivery
- Cancelled embryo transfers
- OHSS
- Preterm birth
- Low birth weight
- Small-for-gestational age (SGA)
- Large-for-gestational age (LGA)
- Perinatal mortality

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

- Preeclampsia
- Placental rupture
- Positive hCG 11 days or according to the local routine post embryo transfer
- Miscarriage, biochemical pregnancies, ectopic pregnancies
- Quality of life for female and male partner
- Cost-effectiveness

Other outcomes

- Number of good quality blastocyst
- Number of fertilized oocytes
- Number of high quality embryos day 2 (defined by the study laboratory manual)
- Number of grade 1 blastocysts (defined by the study laboratory manual)
- Number of frozen blastocysts
- Para-clinical data: Endocrine, genetic and immunological parameters influencing pregnancy

4. STUDY POPULATION

Inclusion criteria

- Women > 6.28 pmol/L with the Roche Elecsys assay* (AMH > 1.1 ng/ml ~ 7.85 pmol/L old assay).
This is according to the Bologna criteria for POR; AMH < 0.5–1.1 ng/ml (3.57-7,85 pmol/l (old assay) ~ 2,86 – 6,28 pmol/l Elecsys)(Ferraretti et al., 2011)
- Female age 18 year to less than 40 years
- 1.-3. IVF/ICSI cycle with oocyte aspiration
- Regular menstrual cycle ≥ 24 days and ≤ 35 days
- BMI ≥ 18 or < 35 kg/m²
- Two ovaries
- Can and will sign the informed content
- **Exclusion criteria**
- Women who do not fulfil the inclusion criteria

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

- Endometriosis stage III to IV
- Ovarian cysts with diameter > 30 mm at day of start of stimulation
- Submucosal fibroids
- Women with severe co-morbidity (i.e Insulin Dependent Diabetes Mellitus (IDDM), Non-Insulin Dependent Diabetes Mellitus (NIDDM), gastrointestinal, cardio-vascular, pulmonary, liver or kidney disease)
- Dysregulation of thyroid disease
- Not Danish, Swedish or English speaking women
- Contraindications or allergies to use of gonadotrophins or GnRH antagonists
- TESA (testicular sperm aspiration)
- OD (oocyte donation)
- Previous inclusion in the study

5. METHODS

Inclusion of patients

- All couples or single/lesbian women starting IVF/ICSI treatment participate in a standard information meeting arranged by the clinic. During this 2 hour meeting patients and their partners are informed about the normal IVF/ICSI procedures, treatments and research in the clinic as well as this study. If patients are not attending the information meeting, they will be informed about the study at their first outpatient visit at the clinic.
- Few patients do not participate in the information meeting and they will have an appointment at the outpatient clinic in the Fertility clinic, where they will receive information about the IVF/ICSI treatment and be informed about this study.
- Patient files are browsed by one of the investigators, who decide if the patient is eligible. After the information meeting all patients receives a phone call from a doctor/study nurse, where they are informed about the treatment plan. If the inclusion criteria are fulfilled, the couples will receive oral information about the study and asked if they are interested in participating in the study, if so, the written patient information is sent to the couples by

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 email. If the couples are interested, a visit is planned on menstrual cycle day 2-4. The
5 couple is informed that they can bring an assessor to the oral information visit. The written
6 information is send by email, which leaves possibility of reading and reflection.
7
8
9

10 11 12 **Informed consent**

13
14
15 At the fertility clinic the patient will be seen by one of the investigators. Patients will be informed
16 about the aim of the project and risks in accordance with the guidelines from the Scientific Ethical
17 Committee. In case of questions, these will be answered. If the patients need more time for
18 reflection, a new visit will be arranged. After signing the informed consent, the patient will be
19 screened.
20
21
22
23
24

25 **Screening – cycle day 2-4**

- 26
27
- 28 • Medical and gynaecological history inclusive reproductive history including menstrual
29 cycle length, smoking (yes/no), years of infertility
 - 30 • Transvaginal ultrasound examination including ovarian volume, antral follicle count (AFC),
31 and endometrial thickness and morphology and exclusion of pathology
 - 32 • Height and weight
 - 33 • Blood samples: AMH, FSH, LH, estradiol, progesterone, TSH, TPO antibodies, vitamin D,
34 CRP and suPAR* (*only done at Hvidovre Hospital)
 - 35 • One full blood, one plasma and one serum sample is cryopreserved as back-up and for
36 analysis of endocrine and immunological factors of relevance for pregnancy
- 37
38
39
40
41
42
43
44

45 Screening should be performed no later than 3 months before randomization.
46
47
48

49 **Randomization**

50
51
52 When the patient has signed the informed consent, has been screened and it is confirmed that the
53 inclusion criteria are meet, the patient is randomized to one of the two arms:
54
55

- 56
57 I. hCG arm with traditional hCG triggering and fresh blastocyst transfer
58
59
60

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 II. GnRH agonist triggering arm with blastocyst cryopreservation and subsequent
5 transfer in a natural cycle.
6
7
8
9

10 Computerized randomization is performed according to the 1) Trial site and to 2) Female age ≤ 37
11 years or >37 years. The gonadotrophin stimulation dose is decided upon before randomization and
12 entered into the database before randomization. The doctor and patient are blinded to the
13 randomization until the day of hCG or GnRH agonist triggering.
14
15
16
17
18
19

20 **Blood samples**
21

22 Blood samples are collected at
23

- 24
25
26
 - Baseline before the first gonadotrophin injection (cycle day 2-4): AMH, FSH, LH,
27 estradiol, progesterone, TSH, TPO-antibodies, vitamin D CRP and suPAR* (*only
28 done at Hvidovre Hospital)
 - Day of trigger-injection: FSH, LH, estradiol, progesterone, CRP and suPAR* (*only
29 done at Hvidovre Hospital)
 - Day 16 after oocyte pick-up: hCG (only in the fresh embryo transfer group), CRP and
30 suPAR* (*only done at Hvidovre Hospital)

31
32
33
34
35
36
37
38
39
40

41 At baseline and at day of trigger an extra full blood, plasma and serum sample is collected and
42 stored according to a trial-specific laboratory manual. This will be stored in the freezer as back-up
43 and for analysis of endocrine and immunological factors of relevance for this study.
44
45
46

47 *Subgroup analyses in the luteal phase*
48

49 During the lutealphase with embryo transfer of the stimulated fresh cycle and non-stimulated FER
50 cycle blood samples are taken on day of hCG injection, hCG injection day+7, +11, +14, +16 and +19
51 for patients included at Hvidovre Hospital until 30 patient in each arm has been achieved. The
52 following blood samples are collected; Estradiol, Inhibin-A, OH-progesterone, Progesterone, LH
53 and hCG.
54
55
56
57
58
59
60

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 All blood samples are confidential. The frozen samples are anonymous, so no person identifiable
5 date is left on the sample. Only the patient project ID number and the collection date identifies
6 the sample. The study will be approved by the Danish Data Protection Agency and the Scientific
7 Ethical Committee of the Capital Region, Region Zealand and the Region Skåne in Sweden. The
8 blood samples will be stored in the participating fertility clinics and if not used then five years after
9 end of the study, at December 1st, 2023 blood samples will be definitively destroyed.
10
11
12
13
14

15 16 17 18 **Gonadotropin stimulation treatment**

19
20 The dose of gonadotrophin is decided and entered into the computer programme before
21 randomization. The doctor and patient are blinded to the randomization until the day of hCG or
22 GnRH agonist triggering. The study nurse is not blinded.
23
24
25

26
27 The ovarian stimulation with recombinant follicular stimulating hormone (rFSH) or human
28 menopausal gonadotrophin (hMG) can start immediately after randomization in a short GnRH
29 antagonist protocol. The gonadotrophin stimulation is performed according to the general
30 standards in each of the clinics and can be altered according to the ovarian response. The GnRH
31 antagonist is initiated at a daily dose of 0.25 mg at stimulation day 5 or 6 according to the clinical
32 standards and continued throughout the rest of the gonadotropin stimulation period. The
33 gonadotrophin dose cannot exceed a daily dose of 300 IU. Both groups are treated according to
34 the short GnRH-antagonist protocol, where a higher dose of gonadotropin than 300 IU has been
35 shown to be of no added value for further follicular growth. The maximum stimulation period is 20
36 days.
37
38
39
40
41
42
43

44
45 The medication for the study is bought by the patients themselves according to general
46 prescription rules.
47
48
49

50 51 52 **Ultrasound**

53
54
55 Ultrasound examination is performed on cycle day 2-3 (Stim1), Stim 5-8 and thereafter every 2-3
56 days until ovulation trigger is decided. At the start of stimulation comprehensive sonography is
57
58
59
60

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 performed with details on each ovary, including ovarian volume, number of antral follicle in the
5 following subclasses: 2-4mm, 5-7mm and 8-10mm.
6
7

8 The following parameters are measured on the day of ovulation trigger or the day before:

9
10 Follicular development with size and number of follicles >10 mm, endometrial thickness and
11 echogenicity and uterine pathology.
12
13

14 15 16 17 **Ovulation induction**

18
19 As soon as three follicles of ≥ 17 mm are observed or one day after a single injection of 250 μ g of
20 human chorionic gonadotrophin (hCG) is administered in the “fresh transfer“-arm, while GnRH
21 agonist triggering with GnRH agonist Buserelin 0.5 mg is administered in the “freeze-all” arm.
22
23

24
25 Triggering of ovulation with a GnRH agonist Buserelin 0.5 mg is allowed in the fresh embryo
26 transfer arm in case of risk of severe OHSS after the following the criteria: In the fresh embryo
27 arm: If > 18 follicles with a diameter > 11 mm are observed on the day of triggering, GnRH agonist
28 triggering should be used and all blastocysts frozen (Papanikolaou et al., 2011). Coasting / surfing
29 cannot be used.
30
31
32
33
34
35
36
37

38 **Oocyte retrieval**

39
40 Oocyte retrieval is performed 36 ± 2 hours after hCG or GnRH agonist administration.
41
42
43
44

45 **IVF/ICSI**

46
47
48 Oocytes are fertilised by either IVF or ICSI and embryos are cultured individually according to the
49 normal procedure in the clinics.
50
51
52
53
54

55 **Embryo transfer**

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 **I. “Fresh embryo transfer” group**
5

6 Single blastocyst transfer is always performed on day five after oocyte pick-up if a blastocyst is
7 developed. Surplus good quality blastocyst are vitrified on day five or six.
8

9
10 *Luteal phase support* is administered as vaginal progesterone (vaginal gel (Crinone) 90 mg/dose x 1
11 daily or vaginal tablets 100 mg x 3 daily (Lutinus)) according to the standard procedure in each of
12 the individual clinics from day 2 after oocyte retrieval and to confirmation of pregnancy or
13 negative hCG 11-15 days post transfer. In case of a positive pregnancy test an ultrasound scan is
14 performed three to four weeks later to confirm an intrauterine pregnancy with a live foetus.
15
16

17
18 Triggerring of ovulation with a GnRH agonist Buserelin 0.5 mg is allowed in the fresh embryo
19 transfer arm in case of risk of severe OHSS, following the criteria: If > 18 follicles with a diameter >
20 11 mm are observed, GnRH agonist triggering should be used and all blastocysts frozen
21 (Papanikolaou et al., 2011). Coasting / surfing cannot be used.
22
23
24
25
26
27
28
29
30

31 **II. “Freeze all and transfer later” group**
32

33 For patients in this group all embryos of a good quality are vitrified at the blastocyst stage day 5 in
34 the stimulated cycle. Criteria for freezing of blastocyst are according to the criteria in the specific
35 clinic. The “best” embryo (i.e. of the highest quality is selected after predefined strict criteria
36 according to the specific trial laboratory manual) is marked and is the first one to be warmed after
37 at least one menstrual cycle that is considered as a wash out period.
38
39
40
41
42

43 In the menstrual cycle with blastocyst transfer, an hCG injection of 6500 units is given when the
44 leading follicle is ≥ 17 mm. Embryo transfer is performed 6-7 days after hCG injection. No luteal
45 phase support is needed.
46
47
48
49
50

51
52 **Pregnancy test**
53
54
55
56
57
58
59
60

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 A serum beta-hCG test is performed 11 days after blastocyst transfer or according to local routine.
5
6 Clinical pregnancy is confirmed by transvaginal ultrasound 3 to 4 weeks after a positive serum-
7
8 hCG.
9

10 11 12 **Follow-up both groups**

13
14
15 A follow-up of all pregnancies will be performed within three months after delivery or termination
16
17 of pregnancy on predefined information sheets.
18

19
20 Information on background data, pregnancies and deliveries are returned to Hvidovre Fertility
21
22 clinic on predefined case report forms (CRF) including pregnancy and delivery information sheets.
23
24 All pregnancies resulting from blastocyst retrieved and thawed according to this study protocol
25
26 will be followed from study inclusion (Stim day 1) and one year onwards.
27

28 All data are anonymized by encryption in the database with no personal identifiable data.
29

30 We will retrieve data from the patient clinical files and clinical databases with information
31
32 regarding previous diseases, hospital admissions, former and current fertility treatment,
33
34 pregnancy and delivery data on pregnancies related to this study. Both females and males will be
35
36 informed about this in the patient information. This collected information will be used to
37
38 characterize the populations and to minimize risk of bias.

39 We will also gain information regarding the coming child and the female and partner will sign a
40
41 separate informed consent in Denmark and in Sweden a single consent form for the couple
42
43 regarding this.
44

45 46 **VAS-score and physical discomfort questionnaire**

47
48 Women in both arms will be requested to fill-in their level of pain and discomfort on a VAS-score
49
50 scale and a physical discomfort questionnaire as well as a quality of life questionnaire at the day of
51
52 oocyte pick-up +4 and the day of oocyte pick up + 16.
53
54

55 56 57 **Visits** 58 59 60

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 Every visit is registered on a standardized stimulation scheme made specifically for this study. The
5 schemes are normally used as standards in the clinics.
6
7
8
9

10 **Criteria for withdrawal**

11
12
13 A patient can be withdrawn from the study at any time, if the patient wishes to do so or if there is
14 a medical indication decided by the investigator. The patient participation in the study can be
15 interrupted, if one of the following criteria is present:
16
17

- 18 • The patients general condition contraindicates participation
- 19 • Protocol violation, which the investigator assess to have influence on the treatment
- 20 • Safety

21
22
23
24
25
26 Patients will be carefully monitored from stimulation start, at Stim6 and thereafter every 2-3 day
27 in the clinic. The treatment will be monitored by transvaginal ultrasound of the ovaries. After each
28 visit the patients will receive thorough information on the drug dosage and administration. This
29 will follow the normal procedure in the clinic. If a patient is taking the wrong dosage, it will be
30 documented on the stimulation scheme. This is not dangerous to the patient as the treatment is
31 monitored by ultrasound scans hence a risk of OHSS will be discovered there.
32
33
34
35
36

37
38 In case of risk of OHSS in the “fresh-embryo-transfer”-arm, the ovulation trigger will not be
39 induced by hCG but with Buserelin 0.5 mg. The further treatment of this patient will be handled
40 according to the routine of the clinic.
41
42

43
44 In case of OHSS the patient is monitored at the clinic until recovery. Overall the safety of the
45 patients is high in both the fresh embryo transfer and the freeze all group as the gonadotrophin
46 stimulation corresponds to the normal program for patients at risk of OHSS. Furthermore patients
47 with irregular cycles i.e. as part of polycystic ovarian syndrome, who in general have a higher risk
48 of OHSS, are not included in this study.
49
50
51
52
53
54

55 **6. STATISTICS AND SAMPLE SIZE**

56
57
58
59
60

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

Superiority study

In all 424 (n= 212 in each arm) patients are required to have an 80% chance of detecting, as significant at the 5% level, an increase in the primary outcome measure ongoing pregnancy rate per randomised patient and per transfer from 30% in the control group to 43% in the experimental group. A difference of 15% was found in a randomized controlled trial by Shapiro et al, 2011 between the “freeze-all” arm and the fresh transfer group.

The statistical analyses will be performed by investigator together with statistical experts at Hvidovre Hospital and associate professor Julie Forman, Department of Biostatistics, Faculty of Health and Medical Sciences, University of Copenhagen.

A Statistical Analysis Plan (SAP) will be presented before closing of the database and before any statistical analyses are performed.

7. STUDY MEDICATION

All medicine used in this study is normally used as standard care for the patients in the short GnRH antagonist protocol. Patients will have prescriptions on all the medicine and will take all the medicine at home as is the routine in the clinics.

Dosage and administration

Treatment dose at the first day and during the ovarian stimulation is planned by the investigator and the patient is further instructed by a nurse, so that the patient is confident in self administration at home according to the normal clinical routine.

Side effects

Most side effects are mild and related to the medication during the stimulation. Unwanted OHSS is a risk in all IVF treatment but is considered low in this project as a standard IVF/ICSI protocol is used with individualized dosing. Further, in the freeze-all group the risk of OHSS is expected to be lower than in the standard care group as all blastocyst transfers are postponed to cycles without ovarian stimulation.

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

8. DATA SECURITY AND ETHICAL ASPECTS

Data security

One full blood and one serum sample at baseline and at the day of ovulation induction will be collected and stored according to the trial specific laboratory manual on all women included in this trial for future analyses of endocrine, immunological and protein markers. All data will be collected in a single database including all project subjects with an identification code thus data on each subject will be anonymous, when entered into the database.

Ethical aspects

The study will be performed according to the Danish Law and ethical principles in the Helsinki Declaration. This covers that study subjects receive both oral and written information and the opportunity of time for reflection and that they can discuss their participation with a third person.

The participants will be given a individualized dose of gonadotropin according to their serum AMH level, which is standard for patients at all five Fertility Clinics in Denmark and Sweden. The risk of OHSS will be similar to the standard clinical protocol and lower in the “freeze-all” group.

With a “freeze-all embryo and transfer later” protocol in ART, the risk of OHSS in women undergoing IVF/ICSI will be minimized and the embryo development will benefit from an endometrium less influenced by supra-physiological levels of estradiol and progesterone in the fresh embryo transfer cycle. This may also be beneficial for the children born after the treatments.

The study is approved by the Scientific Ethical Committee in the Capital Region (H-1600-1116) and by the Scientific Ethical Committee in Region Skåne in Sweden (Dnr. 2016/654)

The study will be approved by the Data Protection Agencies in Denmark and Sweden.

9. TIME SCHEDULE AND PUBLICATION

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 Protocol will be send to the Scientific Ethical Committee in the Capital Region, Denmark in January
5
6 2016 and inclusion of patients will start as soon as the approval from SEC has been obtained. The
7
8 inclusion of patients will run from March 2016 to February 2018. Statistical analyses, writing and
9
10 preparing manuscripts will go on from February 2018 to January 2019.

11
12 The results of the study will be presented at national as well as international scientific congresses
13
14 and published in high impact international scientific journals in reproductive medicine such as
15
16 Human Reproduction or Fertility and Sterility. Further results of public interest will be reported in
17
18 the public press.

21 22 **10.FINANCING**

23
24
25 The project is initiated by Professor Anja Pinborg. This project is part of the Reprounion program,
26
27 which has been supported by the Interreg-program for Öresund-Kattegat-Skagerak from EU,
28
29 Capital Region of Denmark, Region Skåne and Ferring Pharmaceutical Company. The project has
30
31 been financed with 450.000 euro (3.375.000 dkk) by a grant from Interreg/EU.

32
33 Patients included in this study and the Scientific Ethical Committee will be informed if further
34
35 funding is obtained for this study. Funding will be transferred to a research account in the bank of
36
37 Hvidovre Hospital, Capital Region of Denmark.

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

11. REFERENCES

1. Aflatoonian et al. Can fresh embryo transfers be replaced by cryopreserved-thawed embryo transfers in assisted reproductive cycles? A randomized controlled trial. *J Assist Reprod Genet* 2010;27:357–63.
2. Devroey P, Polyzos NP, Blockeel C. An OHSS-Free Clinic by segmentation of IVF treatment. *Hum Reprod* 2011;26:2593-7.
3. Evans J, Hannan NJ, Edgell TA, Vollenhoven BJ, Lutjen PJ, Osianlis T, Salamonsen LA, Rombauts LJ. Fresh versus frozen embryo transfer: backing clinical decisions with scientific and clinical evidence. *Hum Reprod Update* 2014;20:808-21.
4. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L; ESHRE working group on Poor Ovarian Response Definition. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. *Hum Reprod* 2011;26:1616-24.
5. Goswami M, Murdoch AP, Haines E. To freeze or not to freeze embryos: clarity, confusion and conflict. *Hum Fertil (Camb)*. 2015;18:113-20.
6. Kansal Kalra S, Ratcliffe SJ, Milman L, Gracia CR, Coutifaris C, Barnhart KT. Perinatal morbidity after in vitro fertilization is lower with frozen embryo transfer. *Fertil Steril* 2011;95:548–553.
7. Kupka MS, Ferraretti AP, de Mouzon J, Erb K, D'Hooghe T, Castilla JA, Calhaz-Jorge C, De Geyter C, Goossens V; European IVF-Monitoring Consortium, for the European Society of Human Reproduction and Embryology. The European IVF-monitoring programme (EIM), for the European Society of Human Reproduction and Embryology (ESHRE). Assisted reproductive technology in Europe, 2010: results generated from European registers by ESHRE. *Hum Reprod* 2014;29:2099-113.
8. Loutradi et al. *Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis*. *Fertil Steril* 2008;90:186-93.
9. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and Perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in-vitro fertilisation treatment. A systematic review and meta-analysis. *Fertil Steril* 2012;98:368–377.
10. Maheshwari A and Bhattacharya S. Elective frozen embryo transfer cycles for all: ready for prime time? *Hum Reprod* 2013;28:6-9.

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

11. Papanikolaou EG, Humaidan P, Polyzos N, Kalantaridou S, Kol S, Benadiva C, Tournaye H, Tarlatzis B. New algorithm for OHSS prevention. *Reprod Biol Endocrinol* 2011;9:147.
12. Pinborg A. To transfer Fresh or Thawed embryos? *Semin Reprod Med* 2012;30:230-235.
13. Roque M, Lattes K, Serra S, Solà I, Geber S, Carreras R, Checa MA. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. *Fertil steril* 2013; 99:156–62.
14. Roque M. Freeze-all policy: is it time for that? *J Assist Reprod Genet* 2015;32:171-6.
15. Roque M, Valle M, Guimarães F, Sampaio M, Geber S. Freeze-all policy: fresh vs. frozen-thawed embryo transfer. *Fertil Steril* 2015;103:1190-3.
16. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. *Fertil Steril* 2011;96:344–348.
17. Zhu D, Zhang J, Cao S, Zhang J, Heng BC, Huang M et al. Vitrified-warmed blastocyst transfer cycles yield higher pregnancy and implantation rates compared with fresh blastocyst transfer cycles – time for a new embryo transfer strategy?. *Fertil Steril* 2011;95:1691–95.



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

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			Page 1 of protocol
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Page 17 of protocol _____
	2b	All items from the World Health Organization Trial Registration Data Set	All accounted for see items below _____
Protocol version	3	Date and version identifier	Page 1 of protocol (header) _____
Funding	4	Sources and types of financial, material, and other support	Page 18 of protocol _____
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Page 1, 2, 3 of protocol _____

1			
2			
3		5b	Name and contact information for the trial sponsor
4			Page 1 of protocol
5			_____
6		5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and
7			interpretation of data; writing of the report; and the decision to submit the report for publication, including
8			whether they will have ultimate authority over any of these activities
9			_____
10		5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint
11			adjudication committee, data management team, and other individuals or groups overseeing the trial, if
12			applicable (see Item 21a for data monitoring committee)
13			_____
14			
15			
16			
17			
18			
19	Introduction		
20			
21	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant
22	rationale		studies (published and unpublished) examining benefits and harms for each intervention
23			Page 3, 4 5 of
24			protocol
25			_____
26		6b	Explanation for choice of comparators
27			Page 3, 4 5 of
28			protocol
29			_____
30	Objectives	7	Specific objectives or hypotheses
31			Page 5 of protocol
32			_____
33	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),
34			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)
35			Page 8-10 of
36			protocol
37			_____

37 **Methods: Participants, interventions, and outcomes**

1				
2				
3	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Page 8 List of study sites; page 1-3
4				
5				
6				
7				
8	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Page 7-8
9				
10				
11	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Page 11-13
12				
13				
14		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	Page 14-15
15				
16				
17		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Page 11 (visits)
18				
19				
20		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Not applicable
21				
22				
23				
24	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Page 5-7
25				
26				
27				
28				
29	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Page 5-13
30				
31				
32	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	Page 15
33				
34				
35	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 8
36				
37				
38				

Methods: Assignment of interventions (for controlled trials)

Allocation:

1				
2				
3	Sequence	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any	Page 9
4	generation		factors for stratification. To reduce predictability of a random sequence, details of any planned restriction	_____
5			(eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants	
6			or assign interventions	
7				
8	Allocation	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered,	Page 9
9	concealment		opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	_____
10	mechanism			
11				
12	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to	Page 9, 11
13			interventions	_____
14				
15	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome	Page 9
16			assessors, data analysts), and how	_____
17				
18		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's	No circumstances
19			allocated intervention during the trial	_____
20				
21				
22				
23	Methods: Data collection, management, and analysis			
24	Data collection	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related	Page 9, 10, 11 and
25	methods		processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of	14
26			study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known.	_____
27			Reference to where data collection forms can be found, if not in the protocol	
28				
29		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be	Page 13-14
30			collected for participants who discontinue or deviate from intervention protocols	_____
31				
32				
33				
34				
35				
36				
37				
38				
39				
40				
41				
42				
43				
44				
45				
46				
47				
48				
49				

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

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

Page 13,14, Page 16
Details data management can be obtained through contact to primary investigator (“projektansvarlig læge” of trial

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

Page 15
Details of planned statistical analysed can be obtained through contact to primary investigator (“projektansvarlig læge” of trial

20b

Methods for any additional analyses (eg, subgroup and adjusted analyses)

Same as item 20a

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)

Same as item 20a

Methods: Monitoring

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

No data monitoring committee used

1				
2				
3		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	No interim analyses will be performed
4				
5				
6				
7				
8	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	Not applicable
9				
10				
11	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Not available in protocol
12				
13				
14				
15				
16	Ethics and dissemination			
17				
18	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Page 17
19				
20				
21	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)	Not available in protocol
22				
23				
24				
25	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 8 of protocol
26				
27				
28		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Not applicable
29				
30				
31	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	Page 14, 16
32				
33				
34				
35	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Page 18 No competing interests exists
36				
37				
38				
39				
40				
41				
42				
43				
44				
45				
46				
47				
48				
49				

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

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	Not available in protocol
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	Not applicable
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	Not available in protocol
	31b	Authorship eligibility guidelines and any intended use of professional writers	Not available in protocol
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	No current plans
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Not available in protocol – Standard consent forms from the Danish ethical committee is used as well s participant information approved by the Ethical Committee
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	Page 10

1
2 *It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items.
3
4 Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons
5 [“Attribution-NonCommercial-NoDerivs 3.0 Unported”](#) license.
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

For peer review only

BMJ Open

Comparison of a “freeze all” strategy including GnRH agonist trigger versus a “fresh transfer” strategy including hCG trigger in assisted reproductive technology (ART) – A study protocol for a randomised controlled trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-016106.R2
Article Type:	Protocol
Date Submitted by the Author:	01-May-2017
Complete List of Authors:	<p>Stormlund, Sacha; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p> <p>Løssl, Kristine; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p> <p>Zedeler, Anne; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p> <p>Bogstad, Jeanette; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p> <p>Prætorius, Lisbeth; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p> <p>Nielsen, Henriette; The Fertility Clinic, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark</p> <p>Bungum, Mona; Reproductive Medicine Centre, Skane University Hospital, Malmo, Sweden</p> <p>Skouby, Sven; The Fertility Clinic, Department of Obstetrics and Gynaecology, Herlev University Hospital, Copenhagen, Denmark</p> <p>Mikkelsen, Anne Lis; The Fertility Clinic, Department of obstetrics and Gynaecology, Holbæk University Hospital, Holbæk, Denmark</p> <p>Andersen, Anders; The Fertility Clinic, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark</p> <p>Bergh, Christina; Department of Obstetrics and Gynaecology, Institute of Clinical Sciences, Sahlgrenska Academy, Gothenburg University, Reproductive Medicine, Sahlgrenska University Hospital, SE-413 45 Gothenburg, Sweden</p> <p>Humaidan, Peter; The Fertility Clinic, Skive Regional Hospital</p> <p>Pinborg, Anja; The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark</p>
Primary Subject Heading:	Reproductive medicine
Secondary Subject Heading:	Obstetrics and gynaecology

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Keywords:	ART, FET, Freeze-all, RCT, Ongoing pregnancy rate, OPR

SCHOLARONE™
Manuscripts

For peer review only

TITLE PAGE**PROTOCOL ARTICLE**

*Comparison of a “freeze all” strategy including GnRH agonist trigger versus a “fresh transfer” strategy including hCG trigger in assisted reproductive technology (ART)
– A study protocol for a randomised controlled trial*

Authors

Sacha Stormlund¹, Kristine Løssl¹, Anne Zedeler¹, Jeanette Bogstad¹, Lisbeth Prætorius¹,
Henriette Svarre Nielsen², Mona Bungum³, Sven O. Skouby⁴, Anne Lis Mikkelsen⁵, Anders Nyboe
Andersen², Christina Bergh⁶, Peter Humaidan⁷, Anja Pinborg¹

Author Affiliations:

¹The Fertility Clinic, Department of Obstetrics and Gynaecology, Hvidovre University Hospital, Hvidovre, Copenhagen, Denmark

²The Fertility Clinic, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark

³Reproductive Medicine Centre, Skane University Hospital, Malmö, Sweden

⁴ The Fertility Clinic, Department of Obstetrics and Gynaecology, Herlev University Hospital, Copenhagen, Denmark

⁵ The Fertility Clinic, Department of obstetrics and Gynaecology, Holbæk University Hospital, Holbæk, Denmark

⁶ Department of Obstetrics and Gynaecology, Institute of Clinical Sciences, Sahlgrenska Academy, Gothenburg University, Reproductive Medicine, Sahlgrenska University Hospital, SE-413 45 Gothenburg, Sweden

⁷ The Fertility Clinic, Skive Regional Hospital and Faculty of Health, Aarhus University, Aarhus, Denmark

Correspondence to: Sacha Stormlund; sacha.stormlund.01@regionh.dk

1
2
3 26 **ABSTRACT**
4
5

6 27 **Introduction** Pregnancy rates after frozen-thawed embryo transfer (FET) have improved in recent
7
8 28 years and are now approaching or even exceeding those obtained after fresh embryo transfer.

9
10 29 This is partly due to improved laboratory techniques, but may also be caused by a more
11
12 30 physiological hormonal and endometrial environment in FET cycles. Furthermore, the risk of
13
14 31 ovarian hyperstimulation syndrome (OHSS) is practically eliminated in segmentation cycles
15
16 32 followed by FET and the use of natural cycles in frozen-thawed embryo transfers may be beneficial
17
18 33 for the post-implantational conditions of foetal development. However, a freeze-all strategy is not
19
20 34 yet implemented as standard care due to limitations of large randomised trials showing a benefit of
21
22 35 such a strategy. Thus, there is a need to test the concept against standard care in a randomised
23
24 36 controlled design. This study aims to compare ongoing pregnancy and live birth rates between a
25
26 37 freeze-all strategy with GnRH agonist triggering versus hCG trigger and fresh embryo transfer in a
27
28 38 multicentre randomised controlled trial.

29 39 **Methods and analysis** Multicentre randomised, controlled, double-blinded trial of women
30
31 40 undergoing ART treatment including 424 normo-ovulatory women aged 18 to 39 from Denmark
32
33 41 and Sweden. Participants will be randomised (1:1) either A. GnRH agonist trigger and single
34
35 42 vitrified-warmed blastocyst transfer in a subsequent hCG triggered natural menstrual cycle or B.
36
37 43 hCG trigger and single blastocyst transfer in the fresh (stimulated) cycle. The primary endpoint is
38
39 44 to compare ongoing pregnancy rates per randomised patient in the two treatment groups after the
40
41 45 first single blastocyst transfer.

42 46 **Ethics and dissemination**

43 47 The study will be performed in accordance with the ethical principles in the Helsinki Declaration.
44
45 48 The study is approved by the Scientific Ethical Committees in Denmark and Sweden. The results
46
47 49 of the study will be publically disseminated.

48 50 **Trial registration numbers:** ClinicalTrials.gov identifier: NCT02746562; Ethical Approval,
49
50 51 Denmark: H-1600-1116, Ethical Approval, Sweden: Dnr. 2016/654
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

52 **Strengths and limitations of this study**

53 Strengths

- 54 • The design: A multicentre, randomised controlled double-blinded trial powered to identify
55 an increase in ongoing pregnancy rate in the freeze-all group compared to the conventional
56 fresh blastocyst transfer group
- 57 • The study includes normo-ovulatory women aged 18-39 years with a BMI < 35 thus results
58 can be extrapolated to the majority of the normo-ovulatory infertile population
- 59 • GnRH-agonist trigger in the freeze-all group adds a concept of an OHSS-free strategy

60 Limitations

- 61 • As both GnRH-agonist trigger and elective freeze-all are new treatment approaches, we will
62 not be able to distinguish the two effects from each other, but compare an OHSS-free
63 strategy to a conventional fresh transfer strategy
- 64 • The study is powered to detect a 12 % difference in ongoing pregnancy between the two
65 groups, thus smaller but yet clinically relevant differences may be overlooked

66 INTRODUCTION

67 The use of assisted reproductive technology (ART) is increasing and presently up to 5 % of birth
68 cohorts in certain countries are conceived by ART.¹ In recent years, pregnancy rates following
69 frozen embryo transfer (FET) have rapidly increased and may now be a viable and appropriate
70 alternative to the conventional fresh embryo transfer in ART. The main reason is the introduction of
71 vitrification, increasing post-thawing survival rates after blastocyst culture significantly as compared
72 to previous years.²⁻³ Implantation as well as clinical and ongoing pregnancy rates are
73 correspondingly improving in frozen cycles and approaching or even exceeding those associated
74 with fresh embryo transfer.⁴⁻⁶

75 A freeze-all strategy has been suggested as a way to further improve success rates in ART,
76 arguing that the use of the best embryo in frozen cycles instead of in fresh cycles may potentially
77 increase pregnancy rates and live birth rates.⁶⁻⁷ The rationale is that transfer of a frozen-thawed
78 embryo in a subsequent natural menstrual cycle has the advantage of an endometrium that has
79 not been exposed to the supraphysiological levels of estradiol and progesterone following
80 controlled ovarian stimulation (COS) in fresh cycles, which may negatively affect endometrial
81 receptivity.⁵⁻⁸ Elective FET (eFET) moreover has the benefit of essentially eliminating the risk of
82 developing late ovarian hyperstimulation syndrome (OHSS) associated with the pregnancy-related
83 rise in human chorionic gonadotropin (hCG) levels.⁹ If ovulation is induced with a GnRH agonist
84 instead of hCG and all embryos are frozen, even early OHSS is minimized making the overall
85 OHSS risk extremely low.¹⁰ Freezing and thawing of embryos additionally encourages an elective
86 single embryo transfer policy with cumulative pregnancy rates similar to those seen after double
87 embryo transfer.¹¹⁻¹²

88 Despite evidence suggesting that ART outcomes may be further improved with the adaptation of a
89 freeze-all strategy, the implementation remains a topic of ongoing debate and only one in five
90 transfers in Europe on average was performed with frozen-thawed embryos in 2012.¹ In a large
91 recent study, including 1508 patients with polycystic ovary syndrome comparing the freeze-all
92 strategy with conventional fresh embryo transfer, the authors found a significantly higher frequency
93 of live birth after the first frozen embryo transfer compared with fresh embryo transfer (49.3% vs.
94 42.0%).⁷ Correspondingly, in a meta-analysis including three trials accounting for 633 cycles in
95 women aged 27-33 years, eFET resulted in significantly higher clinical and ongoing pregnancy
96 rates compared with fresh embryo transfer.⁶ However, the included studies showed heterogeneity
97 and one of the included publications was later retracted due to serious methodological flaws. In
98 addition, the vast majority of the participants were high responders (496 out of 633) accounting for
99 a highly selected group of patients, mostly consisting of PCOS patients or patients with and

1
2
3 100 ovarian PCO like morphology.⁶ Moreover, previous studies were performed in China, US and
4 101 Japan making them less generalizable to a European ART setting. According to Clinicaltrials.gov
5 102 there are a few ongoing European RCT's on the freeze-all strategy, however none of these studies
6 103 investigate an almost complete OHSS-free strategy including GnRH-agonist trigger in the freeze-
7 104 all group.
8 105 OHSS is one of the most severe side effects of ART and is potentially life threatening. The present
9 106 protocol describes a randomised trial assessing a new ART treatment strategy, where OHSS can
10 107 be almost completely avoided. The results are very important as the majority of our patients could
11 108 avoid the OHSS risk by applying the "GnRH agonist and freeze-all" strategy, maybe even with a
12 109 higher chance of pregnancy. This concept has not been assessed before, and should relevantly be
13 110 considered when planning studies investigating the freeze-all strategy underlining the need for
14 111 large multicentre randomised controlled trials exploring the GnRH agonist and freeze-all strategy in
15 112 a broad population of ART patients. The present study will explore this approach in a bi-national
16 113 multicentre randomised controlled trial setting providing information on the prospect of a freeze-all
17 114 strategy.

115 **Objectives**

116 *Primary objective*

117 The primary objective of the study is to investigate if the ongoing pregnancy rate per randomised
118 patient after the first potential single blastocyst transfer is superior in a freeze-all and transfer later-
119 strategy compared to the conventional hCG trigger and fresh transfer strategy.

120 Ongoing pregnancy rate is defined as an intrauterine pregnancy with a foetal heart beat at
121 transvaginal ultrasound in gestational week 7-8.

122 Ongoing pregnancy rate per first blastocyst transfer is also considered as a primary aim of the
123 study addressing possible differences in endometrial receptivity between the two groups.

124 *Secondary objectives*

125 Secondary objectives include:

- 126 1. To assess cumulative live birth rates after *one complete treatment cycle* including
127 consecutive single blastocyst transfers of all embryos deriving from that oocyte retrieval
128 (fresh and frozen) in the two study groups
- 129 2. To assess the transfer cancellation rate in the two study groups
- 130 3. To assess the prevalence of OHSS in the two study groups
- 131 4. To compare neonatal outcomes (preterm birth, low birth weight, SGA (small-for-gestational
132 age), LGA (large-for-gestational age) and perinatal mortality) and the incidence of
133 preeclampsia in the two study groups

- 1
2
3 134 5. To measure time-to-pregnancy from the date of start of COS to the date of the first ongoing
4 pregnancy in the two study groups
5 135
6 136 6. To assess quality of life for both female and male partners during the two treatment
7 protocols
8 137
9 138 7. To assess physical well-being by way of questionnaires and VAS scores regarding pain
10 and discomfort at four and 16 days after oocyte retrieval in the two study groups
11 139
12
13

14 140 **METHODS AND ANALYSIS**

17 141 **Study design**

18 142 The study is designed as a multicentre randomised, controlled double-blinded trial with seven
19 fertility clinics in Denmark and Sweden participating. All seven clinics are part of a University
20 Hospital setting and perform standardized treatments according to the public health care system in
21 Denmark and Sweden. Patient enrolment started in May 2016 and the last patients are expected to
22 be included in the study in May 2018 with the primary outcome measure, ongoing pregnancy rate,
23 being known for these patients approximately four months later for the patients allocated to the
24 freeze-all group.
25
26
27
28
29
30

31 149 **Study population/Participants and recruitment**

32 150 The study participants will consist of women and their partners initiating ART treatment at one of
33 the seven participating public clinics in Denmark and Sweden. Before initiating treatment patients
34 will attend an information meeting, where they will be informed about the standard ART
35 procedures, treatment regimens as well as ongoing clinical studies at the treatment sites. Those
36 patients not able to participate in the information meeting will instead be informed by a doctor at an
37 outpatient clinic consultation. Recruitment will be carried out by the doctors and study nurses at the
38 fertility clinics. Prior to the initiation of treatment, patient files will be browsed by investigators at the
39 clinics to assess if the patient fulfills the immediate inclusion criteria. Screening, including
40 ultrasound examination of the uterus and ovaries is done on menstrual cycle day two or three
41 securing that all inclusion criteria are met. Patients fulfilling the study criteria will start COS using a
42 GnRH antagonist co-treatment in accordance with the standard routines of the trial site.
43
44
45
46
47
48
49

50 161 **Eligibility criteria**

51
52 162 To participate in the study, women will be required to meet the following inclusion criteria: Female
53 age 18 to 39 years; eligibility to initiate the first, second or third ART cycle with oocyte aspiration
54 (IVF or ICSI); AMH level > 6.28 pmol/L (Roche Elecsys assay) corresponding to the AMH
55 threshold level used in the Bologna criteria to characterize poor responders; regular menstrual
56
57
58
59
60

166 cycle \geq 24 days and \leq 35 days; body mass index 18–35 kg/m²; preservation of both ovaries and
 167 capability of signing informed consent. For specific exclusion criteria see Table 1.

Table 1. Specific exclusion criteria

Endometriosis stage III to IV
Ovarian cysts with a diameter > 30 mm at day of start of stimulation
Submucosal fibroids
Women with severe co-morbidity (IDDM (insulin dependent diabetes mellitus), NIDDM (non-insulin dependent diabetes mellitus), gastrointestinal, cardiovascular, pulmonary, liver or kidney disease)
Dysregulated thyroid disease
Non-Danish or English speaking
Contraindications or allergies to use of gonadotropins or GnRH antagonists
TESA (testicular sperm aspiration)
OD (oocyte donation)
Previous inclusion in the study

168 **Randomisation and blinding**

169 Patients who meet the inclusion criteria are randomised 1:1 to one of the two treatment groups: A.
 170 Freeze-all including GnRH agonist trigger, blastocyst vitrification and subsequent FET in an hCG
 171 triggered natural cycle or B. Traditional hCG trigger and fresh blastocyst transfer.
 172 The randomisation is carried out by a study nurse or a non-treating doctor using a computerised
 173 randomisation program that runs a minimization algorithm, initially seeded using a random block
 174 sequence for the first subjects. The minimization algorithm is balancing the following variables:
 175 Female age (mean, and frequency of age \geq 37 years), previously performed cycles (frequency of
 176 0/1/2 cycles), nulliparous (frequency of yes/no), fertilisation method (frequency of IVF/ICSI),
 177 smoking (frequency of yes/no), AMH (\leq 12 pmol/L, 13-28 pmol/L, >28 pmol/L) and mean BMI. It
 178 selects with high (but less than 1.0) probability the treatment arm that provides the optimal balance
 179 between the arms. It also enforces predefined maximum allowed differences in number of subjects
 180 in each treatment arm at each study site (fertility clinic) and within the whole study.
 181 Furthermore, the starting dose of FSH is entered into the randomisation program before
 182 randomisation is performed to make sure that the FSH dose is decided upon before randomisation.
 183 Both the treating consultants and patients are blinded to the randomisation results during the
 184 controlled ovarian stimulation until the day when ovulation trigger is planned.

1
2
3
4
5 185 **Treatment arms and interventions**

6 186 The short GnRH antagonist protocol and blastocyst culture is applied in both treatment arms. The
7 187 starting dose and type of gonadotropin is decided by the doctor on stimulation day one (cycle day
8 188 two or three) and entered into the randomisation program prior to randomisation. Individualized
9 189 gonadotropin dosing based on AMH, age, weight, previous COH cycles are applied. Recombinant
10 190 follicular stimulating hormone (rFSH) or human menopausal gonadotropin (hMG) can be used
11 191 according to the preference of the site, but the daily dose cannot exceed 300 IU. The gonadotropin
12 192 stimulation will be performed according to the routine in the clinics and can be changed during the
13 193 treatment according to the ovarian response to stimulation evaluated through ultrasound
14 194 examination. GnRH antagonist co-treatment is initiated at a daily dose of 0.25 mg on stimulation
15 195 day five or six according to the general standards in each clinic and is continued throughout the
16 196 rest of the gonadotropin stimulation period.

17 197 Ultrasound examination is performed on cycle day two or three (baseline), stimulation day six or
18 198 seven and subsequently every second to third day until ovulation trigger is decided according to
19 199 the hCG/GnRH agonist trigger criterion: as soon as three follicles are ≥ 17 mm or one day later. At
20 200 baseline a comprehensive ultrasound examination will estimate endometrial thickness, ovarian
21 201 volume as well as number and size of antral follicles divided into the following three subclasses: 2-
22 202 4 mm, 5-7 mm and 8-10 mm. On the day of ovulation trigger endometrial thickness and
23 203 morphology as well as follicular development with number and size of follicles > 10 mm are
24 204 registered.

25 205 When ovulation trigger is decided, the result of the randomisation is disclosed to both doctors and
26 206 patients and ovulation and oocyte maturation is triggered with a GnRH agonist trigger injection (0.5
27 207 mg Buserelin) in the freeze-all group or a single injection of 250 μg of hCG in the fresh embryo
28 208 transfer group. If > 18 follicles with a diameter > 11 mm are observed in the fresh embryo transfer
29 209 group GnRH agonist triggering with Buserelin and vitrification of all embryos will be performed to
30 210 avoid severe OHSS. All fertilised oocytes are cultured to the blastocyst stage and the embryos are
31 211 scored and ranked according to standardised criteria ascribed to this study. The ranking will assure
32 212 that the blastocyst with the highest implantation potential is transferred first in both groups. In the
33 213 fresh transfer group, single blastocyst transfer is performed on day five after oocyte retrieval if a
34 214 good quality blastocyst has developed. Surplus good quality blastocysts will be vitrified on day five
35 215 or six. Luteal phase support is administered as vaginal progesterone according to the clinics
36 216 standard procedures from day two after oocyte retrieval until the day of hCG test; thus luteal
37 217 support is not extended into early pregnancy. In the freeze-all group all blastocysts of good quality
38 218 are vitrified on day five or six depending on when the blastocyst stage is reached. The blastocyst
39 219 with the highest rank is marked and will be the first one used in a subsequent hCG triggered

40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 220 modified natural cycle FET. There should be *at least one completed menstrual cycle in between*
4 221 *the stimulation and the embryo transfer*. In FET cycles a single injection of 250 µg hCG is
5 222 administered, when the leading follicle is ≥ 17 mm. Blastocyst transfer is performed six or seven
6 223 days after the hCG injection. No luteal phase support is given.

7
8 224 A plasma hCG test is performed 11 days after blastocyst transfer. Ongoing clinical pregnancy is
9 225 defined as foetal heart beat at gestational age 7-8 confirmed by transvaginal ultrasound 3 to 4
10 226 weeks after a positive plasma-hCG test. For overview of study design see figure 1.

14 227 **Data collection and management**

15
16 228 Treatment related data is collected at 1) Baseline (cycle day two or three), 2) Day of ovulation
17 229 trigger and 3) five days after oocyte retrieval. Data on blastocysts are collected at culture day
18 230 five/six. Follow-up data on all pregnancies resulting from blastocysts transferred according to the
19 231 study protocol will be followed from study inclusion and one year onwards. Data is transferred to
20 232 an online eCRF system called MediCase with an underlying Microsoft SQL server database
21 233 located in a guarded underground facility in Sweden. Data is backed up daily (one back-up to
22 234 another computer in the same physical location as the server, and a second back-up to a
23 235 physically separate location, also in Sweden). MediCase has a complete audit trail and is designed
24 236 to only contain de-identified data and is entirely based on anonymous subject ID numbers used in
25 237 the trial.

32 238 **Sample collection**

33
34 239 Blood samples will be collected three times during the treatment process: 1) Baseline (cycle day
35 240 two or three), 2) Day of ovulation trigger and 3) 16 days after oocyte retrieval (day of pregnancy
36 241 test in the fresh embryo transfer group). For overview of samples see Table 2. Furthermore one
37 242 serum, plasma and fullblood sample are drawn at baseline and on the day of triggering and stored
38 243 according to a trial specific laboratory manual in a project-specific biobank as back-up for analysis
39 244 of endocrine and immunological factors of relevance for pregnancy. The frozen samples will be
40 245 kept anonymised in the biobank with only the patient specific project ID number and collection date
41 246 marked on the sample. The samples will be store in the participating fertility clinics and destroyed 5
42 247 years after the end of the study period if not analysed.

43
44
45
46
47
48
49
50 248 Further blood samples will be collected during the luteal phase for a smaller subgroup of 30
51 249 patients in each treatment group as part of a luteal phase subgroup analysis of differences in
52 250 hormone levels in the two groups. The following blood samples will be collected at 1) Day of
53 251 ovulation induction and 2) Day of ovulation trigger, day of ovulation trigger +7, +11, +14, +16 and
54 252 +19: Estradiol, Inhibin-A, OH-Progesterone, Progesterone, LH and hCG.

Table 2. Blood sample collection

Baseline (cycle day 2 or 3)	AMH FSH LH Estradiol Progesterone TSH TPO-antibodies Vitamin D CRP suPAR*
Day of ovulation induction	FSH LH Estradiol Progesterone CRP suPAR*
16 days after oocyte retrieval	CRP suPAR* hCG**

253 * Soluble urokinase-type plasminogen activator receptor, only measured at Hvidovre Hospital

254 ** only fresh embryo transfer group

255 Questionnaires

256 Women as well as male partners will be asked to fill in quality of life validated questionnaires twice
 257 during the treatment process: 1) Four days after oocyte retrieval and 2) 16 days after oocyte
 258 retrieval. The questionnaires consist of standardized questions specially developed to explore
 259 emotional aspects as well as quality-of life related aspects of the treatment process. The women
 260 will at the same time be asked to fill in questionnaires regarding physical discomfort including a
 261 VAS score of physical pain in relation to the treatment.

262 Statistics

263 *Sample size calculation*

264 The trial is designed as a superiority study. Sample size calculation indicates that 424 participants
 265 (n = 212 in each arm) are required to have a 80 % chance of detecting, at a significance level at
 266 0.05, an increase in the primary outcome measure (ongoing pregnancy rate per randomised after
 267 first potential blastocyst transfer) from 30% in the control group (fresh embryo transfer) to 43 % in
 268 the experimental group (freeze-all).

1
2
3 269 *Outcome measurements (primary and secondary)*
4

5 270 The primary endpoint is the ongoing pregnancy rate per randomised patient after the transfer of
6 271 the first potential blastocyst. Ongoing pregnancy is defined as a pregnancy with a positive foetal
7
8 272 heart beat at gestational week 7-8.

9 273 Other endpoints explored in the study contribute to the assessment of other relevant aspects of the
10 274 freeze-all strategy including ongoing pregnancy rates per transfer, per started stimulation and per
11 275 oocyte pick-up (percentage of participants with an ultrasound confirmation of foetal heart beat at
12 276 gestational age 7-8) as well as live birth rate and cumulative live birth rates (percentage of
13 277 participants with 1 live born neonate after 1 year of follow-up). The study furthermore aims to
14 278 document the prevalence of OHSS assessed by the number of patients admitted to hospital under
15 279 this diagnosis and the number of patients having ascites puncture. In addition, it is planned to
16 280 evaluate pregnancy related complications as well as neonatal outcomes in both groups. For
17 281 complete overview of all secondary endpoint measures see Table 3.
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 3. Secondary endpoints

◆ Ongoing pregnancy rate per start of <i>per started ovarian stimulation and per oocyte retrieval</i>
◆ Live birth rate after the first blastocyst transfer calculated <i>per randomized patient, per started ovarian stimulation, per oocyte retrieval and per transfer</i>
◆ Cumulative live birth rate after one stimulated cycle with oocyte retrieval
◆ Cumulative live birth rate after use of all frozen blastocyst or after at least 1 year of follow-up
◆ Number of cycles with no embryo transfer
◆ Time-to-pregnancy (from start of ovarian stimulation to positive hCG)
◆ Time-to-delivery
◆ Cancelled embryo transfers
◆ Ovarian hyperstimulation syndrome (OHSS)
◆ Preterm birth
◆ Low birth weight
◆ Small-for-gestational age (SGA)
◆ Large-for-gestational age (LGA)
◆ Perinatal mortality
◆ Preeclampsia
◆ Placental rupture
◆ Positive hCG 11 days post embryo transfer
◆ Miscarriage, biochemical pregnancies, ectopic pregnancies
◆ Quality of life for female and male partner
◆ Cost-effectiveness
Other outcome measurements
◆ Number of good blastocysts
◆ Number of fertilized oocytes
◆ Number of high quality embryos day 2
◆ Number of grade 1 blastocysts
◆ Number of frozen blastocyst
◆ Paraclinical data: Endocrine, genetic and immunological parameters

1
2
3 282 *Statistical analyses*

4
5 283 Analyses of cumulative pregnancy rates and live birth rates after one oocyte retrieval including
6
7 284 fresh and all frozen embryo transfer cycles will be compared by Cox-regression analyses.
8
9 285 Comparisons between treatment groups will be performed primarily according to the intention-to-
10
11 286 treat (ITT) principle but per-protocol analyses will also be done. Continuous data will be compared
12
13 287 by students *t*-test or Mann-Whitney U test and Kruskal-Wallis test as appropriate. Proportions will
14
15 288 be compared with chi-square test. Predictive factors for ongoing pregnancy in the two treatment
16
17 289 groups will be tested with multivariate logistic regression analyses. A p-value of < 0.5 will be
18
19 290 considered as statistically significant.

20
21 291 *Patients in fresh embryo transfer group with GnRH agonist triggering*

22
23 292 Patients allocated to the fresh transfer group who end up receiving GnRH agonist trigger and
24
25 293 vitrification of all blastocysts due to risk of OHSS (> 18 follicles with a diameter > 11 mm on trigger
26
27 294 day) will still be analysed as part of the fresh transfer group according to the intention-to-treat
28
29 295 principle. Their first blastocyst transfer will derive from their first FET cycle and ongoing
30
31 296 pregnancies from these first transfers will be included in the numerator together with ongoing
32
33 297 pregnancies derived from the majority of patients with first blastocyst transfer in the fresh cycle.
34
35 298 The denominator will be all randomised patients.

36
37 299 **ETHICS, SAFETY AND DISSEMINATION**

38
39 300 The study has been approved by the Danish regional committee on Health Research Ethics of the
40
41 301 Capital Region and the Swedish national council on medical ethics.
42
43 302 Following oral and written information outlining the rationale, trial design, aims and treatment
44
45 303 procedures written informed consent will be obtained from women and male partners prior to the
46
47 304 enrolment in the study.

48
49 305 The participants are stimulated using individualised doses of gonadotropin stimulation in
50
51 306 accordance with the clinical practice at each site. In all clinics serum AMH is considered when the
52
53 307 FSH dose is determined. All medicine used in the study is part of standard ART care.

54
55 308 The overall safety of the patients is high in both treatment groups. The risk of OHSS is expected to
56
57 309 be similar to the standard clinical protocol in the fresh embryo transfer group and lower in the
58
59 310 freeze-all group in which GnRH agonist is used for ovulation trigger. In women in the fresh embryo
60
311 transfer group with a risk of OHSS development (more than 18 follicles with a diameter over 11

312 mm), GnRH agonist will be used for trigger instead of hCG and all blastocysts will be vitrified and
313 the transfer postponed.

314 No financial incentive exists for the participants as all couples are reimbursed for their first three
315 ART treatments in the public health care system in the Nordic countries.

316 The results of the study will be publically disseminated in peer-reviewed scientific journals and
317 presented at relevant international scientific meetings such as ESHRE (European Society of
318 Human Reproduction and Embryology) and ASRM (American Society for Reproductive Medicine).
319 In addition results will be published in popular science journals and other media.

320 **DISCUSSION**

321 The increasing interest in possible benefits of a freeze-all strategy and the limitations of existing
322 randomised controlled trials comparing this strategy with conventional fresh embryo transfer
323 underline the need for additional studies. The few previous RCT's have demonstrated significantly
324 increased pregnancy- and delivery rates with freeze-all, however these studies were performed in
325 highly selected patient populations with poor generalizability.⁶⁻⁷ Further, the treatment strategy
326 combining GnRH agonist trigger and freeze-all minimizing the risk of severe OHSS development
327 has not yet been investigated in a RCT setting. As GnRH agonist trigger does not hamper the yield
328 of mature oocytes¹² and reduces the risk of OHSS to an absolutely minimum, it seems rational to
329 include GnRH agonist trigger in the freeze-all concept. Evidently, we are unable to distinguish
330 between the effect of the GnRH-agonist trigger and the effect of elective freeze-all, when both are
331 included in the freeze-all treatment arm. The present study therefore compares an 'OHSS-free'
332 freeze-all strategy including GnRH agonist trigger with a fresh transfer strategy with hCG trigger. In
333 both treatment arms individualized gonadotropin dosing is used with the possibility of conversion to
334 GnRH agonist trigger and segmentation in case of risk of OHSS development in the fresh embryo
335 transfer group. Individualized gonadotropin dosing based on female age and weight, antral follicle
336 count, AMH and results of previous COH cycles is applied, as this is the standard treatment
337 approach used routinely in all of the participating clinics. The AMH cut-off value at 6.28 pmol/L
338 (Roche Elecsys assay) corresponding to the Bologna criteria for poor ovarian response was
339 chosen to have a reasonable chance of the patient ending up with at least one usable blastocyst
340 after aspiration. It could be argued that an open randomisation, rather than a double-blinded study
341 design, would allow a better exploration of the concept as higher gonadotropin doses and more
342 oocytes could be safely aimed for in the freeze-all group. However, as this is the first RCT of a
343 freeze-all strategy including GnRH agonist trigger, a double-blinded design was chosen to

1
2
3 344 minimize differences between the two treatment arms and gonadotropin dosing is decided upon
4 345 independently of allocation to treatment group, as this is done prior to randomisation. In addition,
5 346 even though a strategy combining GnRH agonist trigger and freeze-all is near OHSS free,
6 347 increasing gonadotropin dosing would nonetheless add a potential risk of early OHSS in the
7 348 patients.

8 349 The primary endpoint of this study is to investigate ongoing pregnancy rates per randomised
9 350 patient after the first potential blastocyst transfer. Cumulative rates are additionally planned to be
10 351 calculated, but as the number of aspirated oocytes is expected to be the same in both treatment
11 352 groups due to gonadotropin dosing being decided upon independently of allocated treatment
12 353 group, the effect of the freeze all strategy on the results of the first transfer may be diluted with the
13 354 inclusion of additional FET's.

14 355 The strengths of this study include the design as a multicenter randomised controlled double-
15 356 blinded trial as well as preregistration and publication of the study protocol for more transparency.

16 357 The investigation of several outcome measures related to different aspects of success parameters,
17 358 including quality of life may furthermore add important information as regards the future potential of
18 359 the freeze-all strategy in assisted reproduction.
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

FIGURE LEGEND Figure 1:

Figure 1. Flowchart of the Freeze-all study design

For peer review only

1
2
3 **Contributor statement** ANA, AP and SS participated in the conception, design, and writing of the
4 study protocol. KL, HSN and CB contributed to the revision and editing of the study protocol. AP,
5 SS, KL, JB, LP, ANA, HSN, CB, PH, MB, ALM and SOS will be involved in the recruitment of
6 patients and the acquisition of data. SS wrote the first draft of this manuscript. AZ was involved in
7 developing the laboratory criteria for the study. SS, AP, ANA, PH, CB, KL, JB, LP, HSN, MB, ALM
8 and SOS will AP, ANA, PH, CB, KL, AZ, JB, LP, HSN, MB, ALM and SOS were all involved in
9 critical revision of the manuscript. All authors approved the final version of the manuscript to be
10 submitted.
11

12 **Competing interests** None declared

13 **Funding** The study is part of and fully funded by the Repronion Collaborative study, co-financed
14 by the European Union, Interreg, V ÖKS.

15 **Data sharing statement** This manuscript is a study protocol. Data from the final study will be
16 shared according to the coming ICJME guidelines.
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

REFERENCES

1. Calhaz-Jorge C, de Geyter C, Kupka MS, et al. Assisted reproductive technology in Europe, 2012: results generated from European registers by ESHRE. *Human reproduction* 2016;31(8):1638-52.
2. Loutradi KE, Kolibianakis EM, Venetis CA, et al. Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. *Fertility and sterility* 2008;90(1):186-93.
3. Cobo A, de los Santos MJ, Castello D, et al. Outcomes of vitrified early cleavage-stage and blastocyst stage embryos in a cryopreservation program: evaluation of 3,150 warming cycles. *Fertility and sterility* 2012;98(5):1138-46 e1.
4. Zhu D, Zhang J, Cao S, et al. Vitrified-warmed blastocyst transfer cycles yield higher pregnancy and implantation rates compared with fresh blastocyst transfer cycles--time for a new embryo transfer strategy? *Fertility and sterility* 2011;95(5):1691-5.
5. Shapiro BS, Daneshmand ST, Garner FC, et al. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfers in high responders. *Fertility and sterility* 2011;96(2):516-8.
6. Roque M, Lattes K, Serra S, et al. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. *Fertility and sterility* 2013;99(1):156-62.
7. Chen ZJ, Shi Y, Sun Y, et al. Fresh versus Frozen Embryos for Infertility in the Polycystic Ovary Syndrome. *The New England journal of medicine* 2016;375(6):523-33.
8. Kansal Kalra S, Ratcliffe SJ, Milman L, et al. Perinatal morbidity after in vitro fertilization is lower with frozen embryo transfer. *Fertility and sterility* 2011;95(2):548-53.
9. Devroey P, Polyzos NP, Blockeel C. An OHSS-Free Clinic by segmentation of IVF treatment. *Human reproduction* 2011;26(10):2593-7.
10. Youssef MA, Van der Veen F, Al-Inany HG, et al. Gonadotropin-releasing hormone agonist versus HCG for oocyte triggering in antagonist-assisted reproductive technology. *The Cochrane Database of systematic review* 2014;31;(10):CD008046.
11. Pinborg A. To transfer fresh or thawed embryos? *Seminars in reproductive medicine* 2012;30(3):230-5.
12. Engmann L, Benadiva C, Humaidan P. GnRH agonist trigger for the induction of oocyte maturation in GnRH antagonist IVF cycles: a SWOT analysis. *Reproductive BioMedicine Online* 2016 Mar;32(3):27485

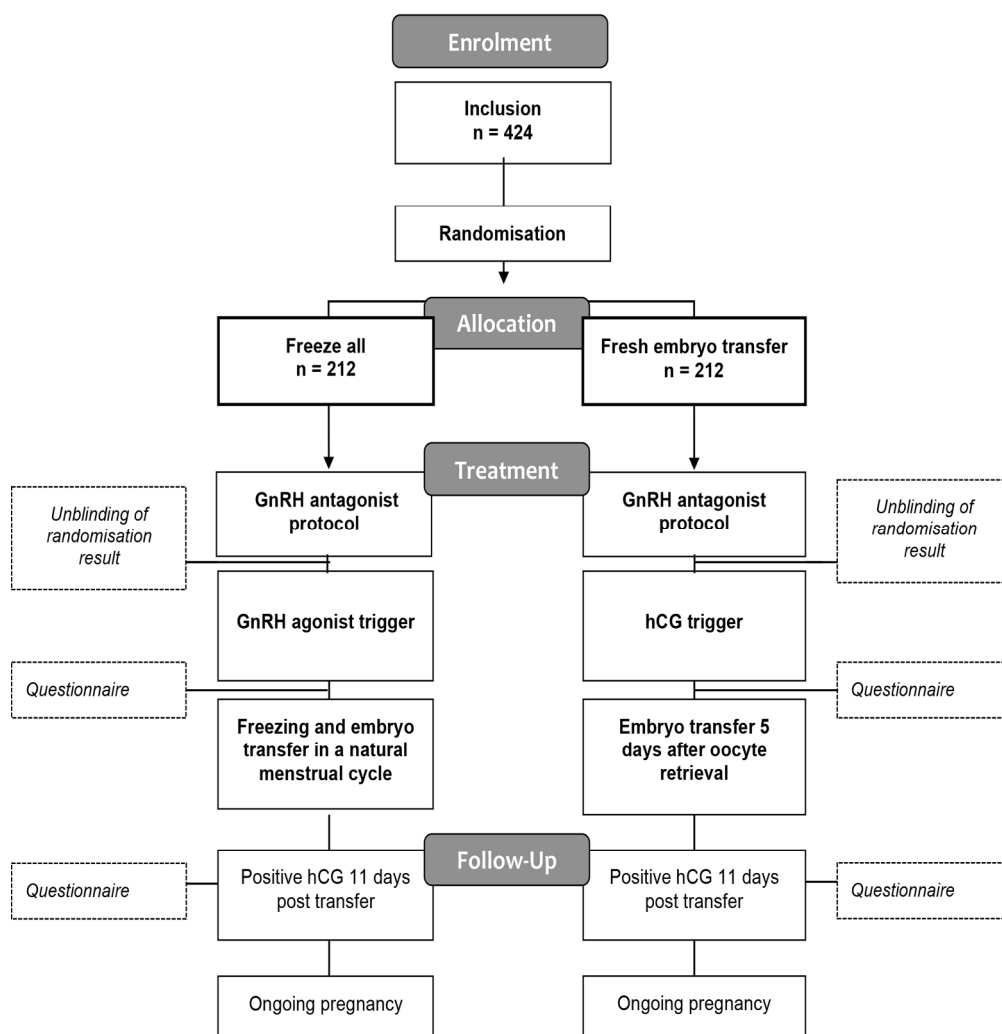


Figure 1. Flowchart of the Freeze-all study design

173x186mm (300 x 300 DPI)



February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

A multicentre randomized controlled trial of a “Freeze all and transfer later”
versus a conventional “Fresh Embryo Transfer” strategy for assisted
reproductive technology (ART) in women with a regular menstrual cycle

- A Multicentre Randomized Controlled Trial of patients undergoing IVF/ICSI

Multicenter studium med otte deltagende fertilitetsklinikker i Danmark og Sverige

Projektansvarlig læge

Anja Pinborg, professor, overlæge, dr.med.

Fertility Clinic, Gynecologic/Obstetric Department, Hvidovre Hospital

Kettegaard Allé 30, DK – Hvidovre 2650

Telephone: +45 38 62 26 56

e-mail: anja.bisgaard.pinborg.01@regionh.dk

AutorisationsID: 00QKV

Deltagende Afdelinger:

Hvidovre Hospital

Sacha Stormlund (læge 1.februar 2016, kommende PhD stud)

Kristine Løssl, overlæge, PhD,

Anne Zedeler, laboratorieleder, Ph.D.

Jeanette Wulff Bogstad, lægelig klinikleder, overlæge

Lisbeth Prætorius, overlæge

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

Herlev Hospital

Sven Skouby, professor, dr. med.

Marie Louise Grøndahl, laboratorieleder, Ph.D

Fertility Clinic, Herlev Hospital, Copenhagen University Hospital

Rigshospitalet

Henriette Svarre Nielsen, overlæge, dr.med.

Anne Loft, overlæge

Anders Nyboe Andersen, professor, overlæge, dr.med.

Fertility Clinic, Rigshospitalet, Copenhagen University Hospital

Holbæk Hospital

Anne Lis Mikkelsen, klinikleder, overlæge, dr.med.

Fertility Clinic, Region Sjælland, Holbæk Hospital

Malmö University Hospital

Aleksander Giwercman, forskningsleder, professor, overlæge, dr.med.

Fertility Centre, Malmö University Hospital, Lunds Universitet

Sahlgrenska University Hospital

Christina Bergh

Department of Obstetrics and Gynaecology, Institute of Clinical Sciences, Sahlgrenska Academy

Gothenburg University, Reproductive Medicine

Sahlgrenska University Hospital, SE-413 45 Gothenburg, Sweden

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

Fertility Clinic Skive

Peter Humaidan, professor, overlæge, dr. med

The Fertility Clinic, Skive Regional Hospital and Faculty of Health, Aarhus University, Aarhus, Denmark

Storklinik,

Nina la Cour Freisleben, speciallæge, phd

Vivaneo Copenhagen, Storklinik, Store Kongesgade 40 G, 1264-Copenhagen K, Denmark

Statistical advisor

Julie Forman, Cand. Scient, Associate Professor

Section of Biostatistics

University of Copenhagen

1. BACKGROUND

In recent years improved cryopreservation techniques have made frozen embryo transfer (FET) a viable and promising alternative to fresh embryo transfer in assisted reproduction (ART). The optimization of cryopreservation techniques from slow-freezing to vitrification and prolonged embryo culture from cleavage to blastocyst state encourages the use of FET as the embryo survival rate following freezing and thawing is now significantly higher reaching 95-97% (Loutradi et al., 2008). Success rates including implantation as well as clinical and on-going pregnancy rates in FET are also significantly improving and approaching or even exceeding those associated with fresh embryo transfer (Kupka et al., 2014; Roque et al., 2013; Shapiro et al., 2011; Zhu et al., 2011). This is partly due the improved laboratory techniques, but may also be due to the endometrial environment in the FET cycles, which mirrors the natural cycle. In the stimulated cycle supraphysiological levels of estradiol and progesterone are present and may cause impaired endometrial receptivity (Shapiro et al., 2011). Furthermore, obstetric and perinatal outcomes after cryopreservation of embryos have been investigated and follow-up data from children born after FET have shown lower perinatal morbidity compared with fresh

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 embryo transfer (Kansal et al., 2011), but FET may also give rise to more large-for-gestational
5 age babies (Pinborg et al., 2014). In addition, a recent systematic review and meta-analysis on
6 data from 11 observational studies has shown better perinatal outcomes including lower
7 perinatal mortality in singleton pregnancies following frozen-thawed embryo transfer
8 compared with pregnancies after fresh embryo transfer (Maheshwari et al., 2012). Moreover,
9 FET has the benefits of minimizing the risk of ovarian hyperstimulation syndrome (OHSS),
10 which is the most severe side effect of ART and potentially life threatening. Finally, improved
11 cryopreservation techniques favour an elective single embryo transfer (eSET) policy minimizing
12 multiple pregnancies after ART (Pinborg, 2012).
13
14
15
16
17
18
19

20
21 Despite the noticeable advantages of embryo cryopreservation, fresh embryo transfer has
22 persistently been the conventional in vitro fertilisation (IVF) procedure as only one in five
23 transfers were made using frozen-thawed embryos in Denmark in 2013
24 (www.fertilitetsselskab.dk). This favour of a fresh embryo transfer strategy is however
25 reflected in other European countries including Finland, Sweden and Iceland where
26 approximately every third ART child is born after FET (Kupka et al., 2014). Some evidence
27 suggests that IVF outcomes can be further improved with the adaptation of a 'freeze-all' or
28 elective frozen embryo transfer (eFET) strategy with replacement of thawed embryos in
29 natural cycles (Evans et al., 2014; Devroey et al., 2011; Maheshwari et al., 2013; Roque et
30 al., 2013).
31
32
33
34
35
36
37
38
39

40 In a recent meta-analysis including three trials accounting for 633 cycles in women aged 27–33
41 years (Roque et al., 2013), FET resulted in significantly higher ongoing pregnancy rates (RR
42 1.32, 95% CI 1.10–1.59) and clinical pregnancy rates (RR 1.31, 95% CI 1.10–1.56). The studies
43 showed heterogeneity and only 137 of the participants were normal responders, while the rest
44 was high responders. Moreover one study included only cleavage stage embryo transfer while
45 the other two included blastocyst transfers only. The studies were performed in Japan and in
46 the US, while no European RCT has been published yet. Further, one of the included papers
47 (Aflatoonian et al., 2010) was later retracted based on findings of serious methodological flaws
48 in the study. This accentuates the need for a large multicentre, randomized controlled trial to
49
50
51
52
53
54
55
56
57
58
59
60

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 evaluate the prospect and clinical consequences of a “freeze all embryos and transfer later”
5
6 policy compared with conventional fresh embryo transfer.
7

8
9 The aim of this multicentre randomized controlled trial is to compare a “freeze-all” embryo
10 strategy with a conventional single fresh embryo transfer strategy in women 18 to 40 years of
11 age undergoing their first to third IVF/ICSI cycle women with regard to treatment outcomes,
12 risks for mother and child, quality of life and cost-effectiveness aspects of the two treatment
13 modalities in a short GnRH antagonist protocol with blastocyst transfer and vitrification as the
14 freezing method.
15
16
17
18
19
20
21
22
23

24 **2. STUDY AIMS**

- 25
26 1. The primary aim is to compare ongoing pregnancy rates per randomized patient and ongoing
27 pregnancy rates per transfer in the “freeze-all” versus “fresh embryo transfer” group.
28
29
- 30
31 2. To assess live birth rates per randomized patient and per transfer in the “freeze-all” versus
32 “fresh embryo transfer” group
33
34
- 35
36 3. To assess cumulative live birth rates after one stimulated cycle with oocyte retrieval in the two
37 study arms.
38
39
- 40
41 4. To compare perinatal outcomes (preterm birth, low birth weight, small-for-gestational age,
42 large-for-gestational-age, preeclampsia and perinatal mortality) in the two groups.
43
44
- 45
46 5. To measure time to pregnancy from start of ovarian stimulation and quality of life in both
47 females and males in the two groups.
48
49
- 50
51 6. To explore VAS scores regarding pain and discomfort at the day of embryo transfer and 11 days
52 post transfer in the two study arms
53
54
- 55
56 7. To assess female physical well-being during the two treatment modalities and to assess
57 quality of life for both female and male partners during the two treatment protocols.
58
59
60

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

3. ENDPOINTS

Primary endpoints

- Ongoing pregnancy rate *per randomized patient*
(pregnancy with positive fetal heart beat in gestational week 7-8)
- Ongoing pregnancy rate *per transfer* of the first blastocyst
(pregnancy with positive fetal heart beat in gestational week 7-8)
- Ongoing pregnancy rate *per oocyte pick-up*
(pregnancy with positive fetal heart beat in gestational week 7-8)
- Ongoing pregnancy rate *per start of ovarian stimulation*
(pregnancy with positive fetal heart beat in gestational week 7-8)

Secondary endpoints

- Live birth rates calculated per randomized patient, per started ovarian stimulation, per oocyte pick-up and per transfer
- Cumulative live birth rate after one stimulated cycle with oocyte retrieval
- Cumulative live birth rate after use of all frozen blastocysts or after at least 1 year of follow-up.
- Number of cycles with no embryo transfer
- Time-to-pregnancy (from start of ovarian stimulation to positive hCG)
- Time-to-delivery
- Cancelled embryo transfers
- OHSS
- Preterm birth
- Low birth weight
- Small-for-gestational age (SGA)
- Large-for-gestational age (LGA)
- Perinatal mortality

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

- Preeclampsia
- Placental rupture
- Positive hCG 11 days or according to the local routine post embryo transfer
- Miscarriage, biochemical pregnancies, ectopic pregnancies
- Quality of life for female and male partner
- Cost-effectiveness

Other outcomes

- Number of good quality blastocyst
- Number of fertilized oocytes
- Number of high quality embryos day 2 (defined by the study laboratory manual)
- Number of grade 1 blastocysts (defined by the study laboratory manual)
- Number of frozen blastocysts
- Para-clinical data: Endocrine, genetic and immunological parameters influencing pregnancy

4. STUDY POPULATION

Inclusion criteria

- Women > 6.28 pmol/L with the Roche Elecsys assay* (AMH > 1.1 ng/ml ~ 7.85 pmol/L old assay).
This is according to the Bologna criteria for POR; AMH < 0.5–1.1 ng/ml (3.57-7,85 pmol/l (old assay) ~ 2,86 – 6,28 pmol/l Elecsys)(Ferraretti et al., 2011)
- Female age 18 year to less than 40 years
- 1.-3. IVF/ICSI cycle with oocyte aspiration
- Regular menstrual cycle ≥ 24 days and ≤ 35 days
- BMI ≥ 18 or < 35 kg/m²
- Two ovaries
- Can and will sign the informed content

Exclusion criteria

- Women who do not fulfil the inclusion criteria

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

- Endometriosis stage III to IV
- Ovarian cysts with diameter > 30 mm at day of start of stimulation
- Submucosal fibroids
- Women with severe co-morbidity (i.e Insulin Dependent Diabetes Mellitus (IDDM), Non-Insulin Dependent Diabetes Mellitus (NIDDM), gastrointestinal, cardio-vascular, pulmonary, liver or kidney disease)
- Dysregulation of thyroid disease
- Not Danish, Swedish or English speaking women
- Contraindications or allergies to use of gonadotrophins or GnRH antagonists
- TESA (testicular sperm aspiration)
- OD (oocyte donation)
- Previous inclusion in the study

5. METHODS

Inclusion of patients

- All couples or single/lesbian women starting IVF/ICSI treatment participate in a standard information meeting arranged by the clinic. During this 2 hour meeting patients and their partners are informed about the normal IVF/ICSI procedures, treatments and research in the clinic as well as this study. If patients are not attending the information meeting, they will be informed about the study at their first outpatient visit at the clinic.
- Few patients do not participate in the information meeting and they will have an appointment at the outpatient clinic in the Fertility clinic, where they will receive information about the IVF/ICSI treatment and be informed about this study.
- Patient files are browsed by one of the investigators, who decide if the patient is eligible. After the information meeting all patients receives a phone call from a doctor/study nurse, where they are informed about the treatment plan. If the inclusion criteria are fulfilled, the couples will receive oral information about the study and asked if they are interested in participating in the study, if so, the written patient information is sent to the couples by

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 email. If the couples are interested, a visit is planned on menstrual cycle day 2-4. The
5 couple is informed that they can bring an assessor to the oral information visit. The written
6 information is send by email, which leaves possibility of reading and reflection.
7
8
9

10 11 12 **Informed consent**

13
14
15 At the fertility clinic the patient will be seen by one of the investigators. Patients will be informed
16 about the aim of the project and risks in accordance with the guidelines from the Scientific Ethical
17 Committee. In case of questions, these will be answered. If the patients need more time for
18 reflection, a new visit will be arranged. After signing the informed consent, the patient will be
19 screened.
20
21
22
23
24

25 **Screening – cycle day 2-4**

- 26
27
- 28 • Medical and gynaecological history inclusive reproductive history including menstrual
29 cycle length, smoking (yes/no), years of infertility
 - 30 • Transvaginal ultrasound examination including ovarian volume, antral follicle count (AFC),
31 and endometrial thickness and morphology and exclusion of pathology
 - 32 • Height and weight
 - 33 • Blood samples: AMH, FSH, LH, estradiol, progesterone, TSH, TPO antibodies, vitamin D,
34 CRP and suPAR* (*only done at Hvidovre Hospital)
 - 35 • One full blood, one plasma and one serum sample is cryopreserved as back-up and for
36 analysis of endocrine and immunological factors of relevance for pregnancy
- 37
38
39
40
41
42
43
44

45 Screening should be performed no later than 3 months before randomization.
46
47
48

49 **Randomization**

50
51
52 When the patient has signed the informed consent, has been screened and it is confirmed that the
53 inclusion criteria are meet, the patient is randomized to one of the two arms:
54
55

- 56
57 I. hCG arm with traditional hCG triggering and fresh blastocyst transfer
58
59
60

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 II. GnRH agonist triggering arm with blastocyst cryopreservation and subsequent
5 transfer in a natural cycle.
6
7
8
9

10 Computerized randomization is performed according to the 1) Trial site and to 2) Female age ≤ 37
11 years or >37 years. The gonadotrophin stimulation dose is decided upon before randomization and
12 entered into the database before randomization. The doctor and patient are blinded to the
13 randomization until the day of hCG or GnRH agonist triggering.
14
15
16
17
18
19

20 **Blood samples**
21

22 Blood samples are collected at
23

- 24
25
26
 - Baseline before the first gonadotrophin injection (cycle day 2-4): AMH, FSH, LH,
27 estradiol, progesterone, TSH, TPO-antibodies, vitamin D CRP and suPAR* (*only
28 done at Hvidovre Hospital)
 - Day of trigger-injection: FSH, LH, estradiol, progesterone, CRP and suPAR* (*only
29 done at Hvidovre Hospital)
 - Day 16 after oocyte pick-up: hCG (only in the fresh embryo transfer group), CRP and
30 suPAR* (*only done at Hvidovre Hospital)

31
32
33
34
35
36
37
38
39

40 At baseline and at day of trigger an extra full blood, plasma and serum sample is collected and
41 stored according to a trail-specific laboratory manual. This will be stored in the freezer as back-up
42 and for analysis of endocrine and immunological factors of relevance for this study.
43
44
45

46 *Subgroup analyses in the luteal phase*
47

48 During the lutealphase with embryo transfer of the stimulated fresh cycle and non-stimulated FER
49 cycle blood samples are taken on day of hCG injection, hCG injection day+7, +11, +14, +16 and +19
50 for patients included at Hvidovre Hospital until 30 patient in each arm has been achieved. The
51 following blood samples are collected; Estradiol, Inhibin-A, OH-progesterone, Progesterone, LH
52 and hCG.
53
54
55
56
57
58
59
60

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 All blood samples are confidential. The frozen samples are anonymous, so no person identifiable
5 date is left on the sample. Only the patient project ID number and the collection date identifies
6 the sample. The study will be approved by the Danish Data Protection Agency and the Scientific
7 Ethical Committee of the Capital Region, Region Zealand and the Region Skåne in Sweden. The
8 blood samples will be stored in the participating fertility clinics and if not used then five years after
9 end of the study, at December 1st, 2023 blood samples will be definitively destroyed.
10
11
12
13
14

15 16 17 18 **Gonadotropin stimulation treatment**

19
20 The dose of gonadotrophin is decided and entered into the computer programme before
21 randomization. The doctor and patient are blinded to the randomization until the day of hCG or
22 GnRH agonist triggering. The study nurse is not blinded.
23
24
25

26
27 The ovarian stimulation with recombinant follicular stimulating hormone (rFSH) or human
28 menopausal gonadotrophin (hMG) can start immediately after randomization in a short GnRH
29 antagonist protocol. The gonadotrophin stimulation is performed according to the general
30 standards in each of the clinics and can be altered according to the ovarian response. The GnRH
31 antagonist is initiated at a daily dose of 0.25 mg at stimulation day 5 or 6 according to the clinical
32 standards and continued throughout the rest of the gonadotropin stimulation period. The
33 gonadotrophin dose cannot exceed a daily dose of 300 IU. Both groups are treated according to
34 the short GnRH-antagonist protocol, where a higher dose of gonadotropin than 300 IU has been
35 shown to be of no added value for further follicular growth. The maximum stimulation period is 20
36 days.
37
38
39
40
41
42
43

44
45 The medication for the study is bought by the patients themselves according to general
46 prescription rules.
47
48
49

50 51 52 **Ultrasound**

53
54
55 Ultrasound examination is performed on cycle day 2-3 (Stim1), Stim 5-8 and thereafter every 2-3
56 days until ovulation trigger is decided. At the start of stimulation comprehensive sonography is
57
58
59
60

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

performed with details on each ovary, including ovarian volume, number of antral follicle in the following subclasses: 2-4mm, 5-7mm and 8-10mm.

The following parameters are measured on the day of ovulation trigger or the day before:

Follicular development with size and number of follicles >10 mm, endometrial thickness and echogenicity and uterine pathology.

Ovulation induction

As soon as three follicles of ≥ 17 mm are observed or one day after a single injection of 250 μ g of human chorionic gonadotrophin (hCG) is administered in the “fresh transfer“-arm, while GnRH agonist triggering with GnRH agonist Buserelin 0.5 mg is administered in the “freeze-all” arm.

Triggering of ovulation with a GnRH agonist Buserelin 0.5 mg is allowed in the fresh embryo transfer arm in case of risk of severe OHSS after the following the criteria: In the fresh embryo arm: If > 18 follicles with a diameter > 11 mm are observed on the day of triggering, GnRH agonist triggering should be used and all blastocysts frozen (Papanikolaou et al., 2011). Coasting / surfing cannot be used.

Oocyte retrieval

Oocyte retrieval is performed 36 ± 2 hours after hCG or GnRH agonist administration.

IVF/ICSI

Oocytes are fertilised by either IVF or ICSI and embryos are cultured individually according to the normal procedure in the clinics.

Embryo transfer

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 **I. “Fresh embryo transfer” group**
5

6 Single blastocyst transfer is always performed on day five after oocyte pick-up if a blastocyst is
7 developed. Surplus good quality blastocyst are vitrified on day five or six.
8

9
10 *Luteal phase support* is administered as vaginal progesterone (vaginal gel (Crinone) 90 mg/dose x 1
11 daily or vaginal tablets 100 mg x 3 daily (Lutinus)) according to the standard procedure in each of
12 the individual clinics from day 2 after oocyte retrieval and to confirmation of pregnancy or
13 negative hCG 11-15 days post transfer. In case of a positive pregnancy test an ultrasound scan is
14 performed three to four weeks later to confirm an intrauterine pregnancy with a live foetus.
15
16

17
18 Triggerring of ovulation with a GnRH agonist Buserelin 0.5 mg is allowed in the fresh embryo
19 transfer arm in case of risk of severe OHSS, following the criteria: If > 18 follicles with a diameter >
20 11 mm are observed, GnRH agonist triggering should be used and all blastocysts frozen
21 (Papanikolaou et al., 2011). Coasting / surfing cannot be used.
22
23
24
25
26
27
28
29
30

31 **II. “Freeze all and transfer later” group**
32

33 For patients in this group all embryos of a good quality are vitrified at the blastocyst stage day 5 in
34 the stimulated cycle. Criteria for freezing of blastocyst are according to the criteria in the specific
35 clinic. The “best” embryo (i.e. of the highest quality is selected after predefined strict criteria
36 according to the specific trial laboratory manual) is marked and is the first one to be warmed after
37 at least one menstrual cycle that is considered as a wash out period.
38
39
40
41
42

43 In the menstrual cycle with blastocyst transfer, an hCG injection of 6500 units is given when the
44 leading follicle is ≥ 17 mm. Embryo transfer is performed 6-7 days after hCG injection. No luteal
45 phase support is needed.
46
47
48
49
50

51
52 **Pregnancy test**
53
54
55
56
57
58
59
60

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 A serum beta-hCG test is performed 11 days after blastocyst transfer or according to local routine.
5
6 Clinical pregnancy is confirmed by transvaginal ultrasound 3 to 4 weeks after a positive serum-
7
8 hCG.
9

10 11 12 **Follow-up both groups**

13
14
15 A follow-up of all pregnancies will be performed within three months after delivery or termination
16
17 of pregnancy on predefined information sheets.
18

19
20 Information on background data, pregnancies and deliveries are returned to Hvidovre Fertility
21
22 clinic on predefined case report forms (CRF) including pregnancy and delivery information sheets.
23
24 All pregnancies resulting from blastocyst retrieved and thawed according to this study protocol
25
26 will be followed from study inclusion (Stim day 1) and one year onwards.
27

28
29 All data are anonymized by encryption in the database with no personal identifiable data.

30
31 We will retrieve data from the patient clinical files and clinical databases with information
32
33 regarding previous diseases, hospital admissions, former and current fertility treatment,
34
35 pregnancy and delivery data on pregnancies related to this study. Both females and males will be
36
37 informed about this in the patient information. This collected information will be used to
38
39 characterize the populations and to minimize risk of bias.

40
41 We will also gain information regarding the coming child and the female and partner will sign a
42
43 separate informed consent in Denmark and in Sweden a single consent form for the couple
44
45 regarding this.

46 **VAS-score and physical discomfort questionnaire**

47
48 Women in both arms will be requested to fill-in their level of pain and discomfort on a VAS-score
49
50 scale and a physical discomfort questionnaire as well as a quality of life questionnaire at the day of
51
52 oocyte pick-up +4 and the day of oocyte pick up + 16.
53
54

55 56 57 **Visits**

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 Every visit is registered on a standardized stimulation scheme made specifically for this study. The
5 schemes are normally used as standards in the clinics.
6
7
8
9

10 **Criteria for withdrawal**

11
12
13 A patient can be withdrawn from the study at any time, if the patient wishes to do so or if there is
14 a medical indication decided by the investigator. The patient participation in the study can be
15 interrupted, if one of the following criteria is present:
16
17

- 18 • The patients general condition contraindicates participation
- 19 • Protocol violation, which the investigator assess to have influence on the treatment
- 20 • Safety

21
22
23
24
25
26 Patients will be carefully monitored from stimulation start, at Stim6 and thereafter every 2-3 day
27 in the clinic. The treatment will be monitored by transvaginal ultrasound of the ovaries. After each
28 visit the patients will receive thorough information on the drug dosage and administration. This
29 will follow the normal procedure in the clinic. If a patient is taking the wrong dosage, it will be
30 documented on the stimulation scheme. This is not dangerous to the patient as the treatment is
31 monitored by ultrasound scans hence a risk of OHSS will be discovered there.
32
33
34
35
36

37 In case of risk of OHSS in the “fresh-embryo-transfer”-arm, the ovulation trigger will not be
38 induced by hCG but with Buserelin 0.5 mg. The further treatment of this patient will be handled
39 according to the routine of the clinic.
40
41
42

43 In case of OHSS the patient is monitored at the clinic until recovery. Overall the safety of the
44 patients is high in both the fresh embryo transfer and the freeze all group as the gonadotrophin
45 stimulation corresponds to the normal program for patients at risk of OHSS. Furthermore patients
46 with irregular cycles i.e. as part of polycystic ovarian syndrome, who in general have a higher risk
47 of OHSS, are not included in this study.
48
49
50
51
52

53 **6. STATISTICS AND SAMPLE SIZE**

54
55
56
57
58
59
60

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

Superiority study

In all 424 (n= 212 in each arm) patients are required to have an 80% chance of detecting, as significant at the 5% level, an increase in the primary outcome measure ongoing pregnancy rate per randomised patient and per transfer from 30% in the control group to 43% in the experimental group. A difference of 15% was found in a randomized controlled trial by Shapiro et al, 2011 between the “freeze-all” arm and the fresh transfer group.

The statistical analyses will be performed by investigator together with statistical experts at Hvidovre Hospital and associate professor Julie Forman, Department of Biostatistics, Faculty of Health and Medical Sciences, University of Copenhagen.

A Statistical Analysis Plan (SAP) will be presented before closing of the database and before any statistical analyses are performed.

7. STUDY MEDICATION

All medicine used in this study is normally used as standard care for the patients in the short GnRH antagonist protocol. Patients will have prescriptions on all the medicine and will take all the medicine at home as is the routine in the clinics.

Dosage and administration

Treatment dose at the first day and during the ovarian stimulation is planned by the investigator and the patient is further instructed by a nurse, so that the patient is confident in self administration at home according to the normal clinical routine.

Side effects

Most side effects are mild and related to the medication during the stimulation. Unwanted OHSS is a risk in all IVF treatment but is considered low in this project as a standard IVF/ICSI protocol is used with individualized dosing. Further, in the freeze-all group the risk of OHSS is expected to be lower than in the standard care group as all blastocyst transfers are postponed to cycles without ovarian stimulation.

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

8. DATA SECURITY AND ETHICAL ASPECTS

Data security

One full blood and one serum sample at baseline and at the day of ovulation induction will be collected and stored according to the trial specific laboratory manual on all women included in this trial for future analyses of endocrine, immunological and protein markers. All data will be collected in a single database including all project subjects with an identification code thus data on each subject will be anonymous, when entered into the database.

Ethical aspects

The study will be performed according to the Danish Law and ethical principles in the Helsinki Declaration. This covers that study subjects receive both oral and written information and the opportunity of time for reflection and that they can discuss their participation with a third person.

The participants will be given a individualized dose of gonadotropin according to their serum AMH level, which is standard for patients at all five Fertility Clinics in Denmark and Sweden. The risk of OHSS will be similar to the standard clinical protocol and lower in the “freeze-all” group.

With a “freeze-all embryo and transfer later” protocol in ART, the risk of OHSS in women undergoing IVF/ICSI will be minimized and the embryo development will benefit from an endometrium less influenced by supra-physiological levels of estradiol and progesterone in the fresh embryo transfer cycle. This may also be beneficial for the children born after the treatments.

The study is approved by the Scientific Ethical Committee in the Capital Region (H-1600-1116) and by the Scientific Ethical Committee in Region Skåne in Sweden (Dnr. 2016/654)

The study will be approved by the Data Protection Agencies in Denmark and Sweden.

9. TIME SCHEDULE AND PUBLICATION

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

1
2
3
4 Protocol will be send to the Scientific Ethical Committee in the Capital Region, Denmark in January
5
6 2016 and inclusion of patients will start as soon as the approval from SEC has been obtained. The
7
8 inclusion of patients will run from March 2016 to February 2018. Statistical analyses, writing and
9
10 preparing manuscripts will go on from February 2018 to January 2019.

11
12 The results of the study will be presented at national as well as international scientific congresses
13
14 and published in high impact international scientific journals in reproductive medicine such as
15
16 Human Reproduction or Fertility and Sterility. Further results of public interest will be reported in
17
18 the public press.

21 22 **10.FINANCING**

23
24
25 The project is initiated by Professor Anja Pinborg. This project is part of the Reprounion program,
26
27 which has been supported by the Interreg-program for Öresund-Kattegat-Skagerak from EU,
28
29 Capital Region of Denmark, Region Skåne and Ferring Pharmaceutical Company. The project has
30
31 been financed with 450.000 euro (3.375.000 dkk) by a grant from Interreg/EU.

32
33 Patients included in this study and the Scientific Ethical Committee will be informed if further
34
35 funding is obtained for this study. Funding will be transferred to a research account in the bank of
36
37 Hvidovre Hospital, Capital Region of Denmark.

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

11. REFERENCES

1. Aflatoonian et al. Can fresh embryo transfers be replaced by cryopreserved-thawed embryo transfers in assisted reproductive cycles? A randomized controlled trial. *J Assist Reprod Genet* 2010;27:357–63.
2. Devroey P, Polyzos NP, Blockeel C. An OHSS-Free Clinic by segmentation of IVF treatment. *Hum Reprod* 2011;26:2593-7.
3. Evans J, Hannan NJ, Edgell TA, Vollenhoven BJ, Lutjen PJ, Osianlis T, Salamonsen LA, Rombauts LJ. Fresh versus frozen embryo transfer: backing clinical decisions with scientific and clinical evidence. *Hum Reprod Update* 2014;20:808-21.
4. Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L; ESHRE working group on Poor Ovarian Response Definition. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. *Hum Reprod* 2011;26:1616-24.
5. Goswami M, Murdoch AP, Haimes E. To freeze or not to freeze embryos: clarity, confusion and conflict. *Hum Fertil (Camb)*. 2015;18:113-20.
6. Kansal Kalra S, Ratcliffe SJ, Milman L, Gracia CR, Coutifaris C, Barnhart KT. Perinatal morbidity after in vitro fertilization is lower with frozen embryo transfer. *Fertil Steril* 2011;95:548–553.
7. Kupka MS, Ferraretti AP, de Mouzon J, Erb K, D'Hooghe T, Castilla JA, Calhaz-Jorge C, De Geyter C, Goossens V; European IVF-Monitoring Consortium, for the European Society of Human Reproduction and Embryology. The European IVF-monitoring programme (EIM), for the European Society of Human Reproduction and Embryology (ESHRE). Assisted reproductive technology in Europe, 2010: results generated from European registers by ESHRE. *Hum Reprod* 2014;29:2099-113.
8. Loutradi et al. *Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis*. *Fertil Steril* 2008;90:186-93.
9. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and Perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in-vitro fertilisation treatment. A systematic review and meta-analysis. *Fertil Steril* 2012;98:368–377.
10. Maheshwari A and Bhattacharya S. Elective frozen embryo transfer cycles for all: ready for prime time? *Hum Reprod* 2013;28:6-9.

February 18th, 2017

Protokol_Freeze all and transfer later_V3_18022017

11. Papanikolaou EG, Humaidan P, Polyzos N, Kalantaridou S, Kol S, Benadiva C, Tournaye H, Tarlatzis B. New algorithm for OHSS prevention. *Reprod Biol Endocrinol* 2011;9:147.
12. Pinborg A. To transfer Fresh or Thawed embryos? *Semin Reprod Med* 2012;30:230-235.
13. Roque M, Lattes K, Serra S, Solà I, Geber S, Carreras R, Checa MA. Fresh embryo transfer versus frozen embryo transfer in in vitro fertilization cycles: a systematic review and meta-analysis. *Fertil steril* 2013; 99:156–62.
14. Roque M. Freeze-all policy: is it time for that? *J Assist Reprod Genet* 2015;32:171-6.
15. Roque M, Valle M, Guimarães F, Sampaio M, Geber S. Freeze-all policy: fresh vs. frozen-thawed embryo transfer. *Fertil Steril* 2015;103:1190-3.
16. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. *Fertil Steril* 2011;96:344–348.
17. Zhu D, Zhang J, Cao S, Zhang J, Heng BC, Huang M et al. Vitrified-warmed blastocyst transfer cycles yield higher pregnancy and implantation rates compared with fresh blastocyst transfer cycles – time for a new embryo transfer strategy?. *Fertil Steril* 2011;95:1691–95.



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

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			Page 1 of protocol
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Page 17 of protocol _____
	2b	All items from the World Health Organization Trial Registration Data Set	All accounted for see items below _____
Protocol version	3	Date and version identifier	Page 1 of protocol (header) _____
Funding	4	Sources and types of financial, material, and other support	Page 18 of protocol _____
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Page 1, 2, 3 of protocol _____

1			
2			
3		5b	Name and contact information for the trial sponsor
4			Page 1 of protocol
5			_____
6		5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and
7			interpretation of data; writing of the report; and the decision to submit the report for publication, including
8			whether they will have ultimate authority over any of these activities
9			_____
10		5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint
11			adjudication committee, data management team, and other individuals or groups overseeing the trial, if
12			applicable (see Item 21a for data monitoring committee)
13			_____
14			
15			
16			
17			
18			
19	Introduction		
20			
21	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant
22	rationale		studies (published and unpublished) examining benefits and harms for each intervention
23			Page 3, 4 5 of
24			protocol
25			_____
26		6b	Explanation for choice of comparators
27			Page 3, 4 5 of
28			protocol
29			_____
30	Objectives	7	Specific objectives or hypotheses
31			Page 5 of protocol
32			_____
33	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),
34			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)
35			Page 8-10 of
36			protocol
37			_____

37 **Methods: Participants, interventions, and outcomes**

1				
2				
3	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Page 8 List of study sites; page 1-3
4				
5				
6				
7				
8	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Page 7-8
9				
10				
11	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Page 11-13
12				
13				
14		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	Page 14-15
15				
16				
17		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Page 11 (visits)
18				
19				
20		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Not applicable
21				
22				
23				
24	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Page 5-7
25				
26				
27				
28				
29	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Page 5-13
30				
31				
32	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	Page 15
33				
34				
35	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 8
36				
37				
38				

Methods: Assignment of interventions (for controlled trials)

Allocation:

1				
2				
3	Sequence	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any	Page 9
4	generation		factors for stratification. To reduce predictability of a random sequence, details of any planned restriction	_____
5			(eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants	
6			or assign interventions	
7				
8	Allocation	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered,	Page 9
9	concealment		opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	_____
10	mechanism			
11				
12	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to	Page 9, 11
13			interventions	_____
14				
15	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome	Page 9
16			assessors, data analysts), and how	_____
17				
18		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's	No circumstances
19			allocated intervention during the trial	_____
20				
21				
22				
23	Methods: Data collection, management, and analysis			
24	Data collection	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related	Page 9, 10, 11 and
25	methods		processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of	14
26			study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known.	_____
27			Reference to where data collection forms can be found, if not in the protocol	
28				
29		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be	Page 13-14
30			collected for participants who discontinue or deviate from intervention protocols	_____
31				
32				
33				
34				
35				
36				
37				
38				
39				
40				
41				
42				
43				
44				
45				
46				
47				
48				
49				

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

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	Page 13,14, Page 16 Details data management can be obtained through contact to primary investigator (“projektansvarlig læge” of trial)
<hr/>			
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	Page 15 Details of planned statistical analysed can be obtained through contact to primary investigator (“projektansvarlig læge” of trial)
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Same as item 20a
	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)	Same as item 20a
<hr/>			
Methods: Monitoring			
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	No data monitoring committee used

1				
2				
3		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	No interim analyses will be performed
4				
5				
6				
7				
8	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	Not applicable
9				
10				
11	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Not available in protocol
12				
13				
14				
15				
16	Ethics and dissemination			
17				
18	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Page 17
19				
20				
21	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)	Not available in protocol
22				
23				
24				
25	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 8 of protocol
26				
27				
28		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Not applicable
29				
30				
31	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	Page 14, 16
32				
33				
34				
35	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Page 18 No competing interests exists
36				
37				
38				
39				
40				
41				
42				
43				
44				
45				
46				
47				
48				
49				

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

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	Not available in protocol
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	Not applicable
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	Not available in protocol
	31b	Authorship eligibility guidelines and any intended use of professional writers	Not available in protocol
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	No current plans
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Not available in protocol – Standard consent forms from the Danish ethical committee is used as well s participant information approved by the Ethical Committee
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	Page 10

1
2 *It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items.
3
4 Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons
5 [“Attribution-NonCommercial-NoDerivs 3.0 Unported”](#) license.
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

For peer review only