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Effect of clindamycin and a live biotherapeutic on the reproductive outcomes of IVF patients with abnormal vaginal microbiota: protocol for a double-blind, placebo-controlled multicentre trial

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ABSTRACT

Introduction Recent studies in in vitro fertilisation (IVF) patients have associated abnormal vaginal microbiota (AVM) with poor clinical pregnancy rates of 6%–9% per embryo transfer. The biological plausibility for this finding is hypothesised to be ascending infection to the endometrium which in turn hampers embryo implantation. New molecular based diagnosis may offer advantages compared to microscopical diagnosis of AVM which has huge inter-study variability ranging from 4 to 38%; however, the important question is whether screening and treatment of AVM would improve reproductive outcomes in IVF patients. Herein, we describe a protocol for an ongoing double-blind, placebo-controlled multicentre trial of IVF patients diagnosed with AVM and randomised in three parallel groups 1:1:1.

Methods and analysis This is a drug intervention study where IVF patients will be screened for AVM, using a qPCR assay targeting *Atopobium vaginae* and *Gardnerella vaginalis*. If positive, patients will be randomised to one of the three study arms. The first arm consists of clindamycin 300 mg ×2 daily for 7 days followed by vaginal *Lactobacillus crispatus* CTV-05 until clinical pregnancy scan week 7–9. The second arm consists of clindamycin and placebo *L. crispatus* CTV-05, whereas patients in the third arm will be treated with placebo/placebo. We used a superiority design to estimate that active treatment in both arms will increase the primary outcome, clinical pregnancy rate per embryo transfer, from 20% to 40%. A potential difference between the two active arms was considered exploratory. With a power of 80% and an alpha at 5%, the sample size is estimated to be 333 patients randomised. A pre-planned interim analysis is scheduled at 167 patients randomised.

Ethics and dissemination All patients have to give informed consent. Dissemination of results is ensured in clinical trial agreements whether they be positive or not. Ethics committee, Central Denmark Region approved this protocol.

Strengths and limitations of this study

- Molecular-based diagnosis of abnormal vaginal microbiota was validated in pilot studies.
- The first randomised controlled trial in IVF patients diagnosed with abnormal vaginal microbiota investigating treatment effect on reproductive outcome of clindamycin and live *Lactobacillus* treatment.
- The *Lactobacillus crispatus* CTV-05 treatment is an investigational live biotherapeutic product regulated by the US FDA.
- ICH-GCP monitored trial.
- Inclusion criteria are relatively broad.

Trial registration number ICH-GCP monitored trial, EudraCT 2016-002385-31; Pre-results.

INTRODUCTION

Bacterial vaginosis (BV) is a common vaginal dysbiosis in women of reproductive age with a prevalence of 29% (95% CI 27% to 31%) as reported in a US population–based survey, n=3739.[1] It is well known that there is a higher BV rate among African Americans compared with Caucasian women.[1] However, this finding could be affected by the fact that asymptomatic African Americans seem to have a more diverse physiological vaginal microbiota as compared with Caucasians.[2 3] Other risk factors include vaginal douching and number of lifetime sex partners.[1] In the in vitro fertilisation (IVF) population, a recent meta-analysis (n=2980) reported that the prevalence of BV exhibited huge inter-study heterogeneity ranging from 4% to 38%.[4] In this study, BV was clearly associated

with tubal factor infertility, but not endometriosis. The most recent studies using a molecular-based analysis to determine an abnormal vaginal microbiota observed a prevalence of 17% and 28%, respectively.[5 6] It is known that despite diagnosed with BV by the gold standard Nugent method,[7] more than 80% of BV positives remain asymptomatic.[1] Hence, the important question is whether the many asymptomatic BV cases should be screened and treated. Clinical guidelines recommend screening and treatment for BV in patients undergoing gynaecological surgery or invasive diagnostic procedures with vaginal access to minimise infection.[8] However, most clinical guidelines do not support screening and treatment for asymptomatic BV to optimise reproductive outcome—a topic which has been thoroughly investigated in obstetric populations for preterm birth prevention.[9 10] Today, a new frontier is emerging with optimised molecular-based diagnosis of bacterial dysbiosis and new treatment possibilities including well-studied and well-characterised probiotics that have been designated 'live biotherapeutic products' by FDA.[11 12]

Haahr *et al* reported the advantages of a molecular-based diagnosis of vaginal dysbiosis in IVF patients.[5] The main advantages were (1) a more objective diagnosis as microscopists had significant inter-rater variability with the prior gold standard, Nugent score; (2) dichotomisation of the Nugent intermediate group which was difficult to interpret clinically; and (3) the establishment of quantitative thresholds using key vaginal bacteria to detect IVF patients at risk of a poor reproductive outcome. Hence, a new terminology termed abnormal vaginal microbiota (AVM) was proposed for IVF patients.[5 13] AVM was significantly associated with poor clinical pregnancy rates as compared with normal vaginal microbiota patients, 9% (2/22) versus 44% (27/62).[5] Later, these findings were corroborated by Koedooder *et al*[6] who found clinical pregnancy rates of 6% (2/34) versus 41% (65/158) in patients with unfavourable and favourable vaginal microbiota, respectively.

In the field of reproductive medicine, there have been two different approaches to investigate the potential influence of the genital tract microbiota on IVF outcomes: either (1) to directly investigate the endometrial microbiota by transcervical swabs/suctions[14–16] or (2) to investigate the vaginal microbiota as a proxy for the endometrial microbiota.[5 6 17] The bacterial load in the uterus as compared with the vagina is very low,[18] and for this reason the studies on endometrial microbiota have been criticised for reporting contamination from the transcervical sampling approach—and not a genuine endometrial microbiota.[19] Nevertheless, endometrial samples from women undergoing hysterectomy provide evidence for a genuine endometrial microbiota[18 20] that seems to be highly influenced by the vaginal microbiota,[20] especially in the case of BV where the odds of having endometrial colonisation, including *Gardnerella vaginalis* biofilm infection, was significant as compared to normal vaginal microbiota patients: OR 5.7 (95% CI 1.8 to 18.3, p=0.002).[21] Several groups are developing or further optimising molecular-based approaches to diagnose IVF women at risk of poor reproductive outcomes caused by genital tract dysbiosis. However, only one study validated a molecular diagnostic approach in IVF women against the gold standard for vaginal dysbiosis—Nugent score of Gram-stained vaginal smears.[5] Two other studies applied arbitrary cut-offs for *Lactobacillus* dominance in the vaginal microbiota.[6 17] Subsequently, these studies were criticised for insufficient methods,[22 23] including the application of arbitrary thresholds based on relative abundances which does not sufficiently take into account differences in the total abundance.[22–24]

The recommended first-line treatments for BV are antibiotic therapy with either metronidazole or clindamycin as reported by the 2015 CDC (Center for Disease Prevention and Control) Sexually Transmitted Disease Guideline and the 2018 European IUSTI/WHO (International Union against Sexually Transmitted Infections) guideline. Clindamycin was reported to effectively eradicate BV-related bacteria in the endometrium of patients with endometritis,[25] while it was also proven to enter the endometrial tissue in high concentrations if administered orally.[26] In contrast, metronidazole was less effective against *Gardnerella vaginalis* both in vivo[27] and in vitro.[25]

Finally, a recent systematic review and meta-analysis reported that the use of additional probiotic treatment alongside standard treatment of BV could improve BV cure rates, risk ratio (RR) 1.28 (95% CI 1.05 to 1.56).[28] However, due to primarily poor study quality,[29] there is currently no consensus on which vaginal *Lactobacillus* product, if any, should be recommended.[30]

The pioneering work by Ravel and colleagues established that the vaginal microbiota consisted of four *Lactobacillus*-dominated community state types (CSTs) using taxonomic stratification at the species level, with each CST dominated by a different vaginal *Lactobacillus* species or a diverse CST not dominated by *Lactobacillus*.[2] Although such stratification was based on hierarchical clustering and relative abundance, in contrast to absolute abundance, these CSTs have been adopted by the majority of researchers in the vaginal microbiome field. Consistently, publications have reported the *Lactobacillus crispatus* CST to be associated with optimal genital health and reproductive outcomes.[13 17 31–33] Moreover, abundant in vitro evidence point towards a beneficial production of both D and L lactic acid isomers by *L. crispatus* that not all other common vaginal lactobacilli produce.[34 35] At the time of planning the present study, only one *L. crispatus* product, LACTIN-V, existed as an investigational live biotherapeutic product regulated by FDA—at that time in phase II development.[11] Recently, adjuvant LACTIN-V after vaginal metronidazole was reported to lower BV recurrence rates in a phase IIb trial, RR 0.66 (95% CI 0.44 to 0.87; P=0.01).[36]

Taking into consideration the aforementioned evidence, the research question of the present study is: does antibiotic alone or in combination with live

biotherapeutic treatment of an abnormal vaginal microbiota improve the reproductive outcomes of IVF patients? The intervention is clindamycin either alone or in combination with LACTIN-V, a live biotherapeutic product containing *L. crispatus* CTV-05.[11] The study is designed as a double-blind, placebo-controlled multicentre trial of three parallel groups randomised 1:1:1. Randomisation is by computer-generated code and allocation concealment is performed by the pharmacy who will send out medication to the participating clinics with identical appearance and randomisation numbers. The randomisation code is with the pharmacy and can only be opened in case of emergency by the principal investigators or as planned by the sponsor-investigator. The benefit of the intervention would potentially lead to increased pregnancy rates and, for those suffering from symptomatic BV, also relief of BV symptoms. In contrast, the expected adverse reactions of concern are especially gastrointestinal symptoms caused by clindamycin, whereas LACTIN-V might cause increased vaginal discharge but is otherwise not expected to cause adverse reactions as based on prior studies.[11 37]

METHODS AND ANALYSIS

Setting and eligibility criteria

The present trial will be conducted at four University-affiliated clinics and one private fertility clinic in Denmark. The list of study sites is available with EudraCT clinical trial identifier: 2016-002385-31, first registration day 2016-07-11. The current version of the protocol is 9, 2020-02-07. Patients are enrolled in a cohort study (ClinicalTrials.gov NCT03420859) from which we will recruit patients for the randomised trial (EudraCT: 2016-002385-31). Eligibility criteria are described in table 1.

In brief, IVF patients attending their first, second or third IVF stimulation cycle or embryo transfer therefrom will be approached for informed consent by the study nurse or treating physician. Patients are told about the project in a private room with the right to have an assessor, allowing time to reflect whether they will participate. They are handed out written information material with a link to the study website with full information about the project (www.reproflor.dk). The vaginal swab can be taken by the treating physician or the patient herself after careful instruction. In this case, patients are instructed to place the swab at least 8 cm into the vaginal cavity for 10 s and rotate. This is to ensure that the vaginal bacteria in the fornix or in its close proximity will be caught by the flocked swab. Subsequently, the vaginal swab will be sent to a central laboratory at Statens Serum Institut, Copenhagen to be analysed for AVM within 7 days as previously reported.[5] If AVM positive, patients are asked to provide informed consent that they are willing to participate in the randomised controlled trial. Patients should ideally be randomised on the first day of ovarian stimulation with exogenous gonadotropins, allowing a minimum of 12 days of study medication to be acceptable for inclusion in the study. If elective frozen embryo transfer (FET) is planned, patients should be randomised during the first days of the FET cycle allowing for at least 12 days of study medication. If patients enter the trial and have less than 12 days of study medication despite the aforementioned inclusion criteria (eg, when hormonal stimulation is shortened due to an unexpected ovarian response), it is considered a protocol violation and they will be excluded from the per-protocol analysis, not from intention-to-treat (ITT) analysis.

Interventions

Active treatment 1

Oral clindamycin 300 mg two times per day for 7 days followed by LACTIN-V (Osel) until completion of the clinical pregnancy scan at week 7–9. LACTIN-V containing *L. crispatus* CTV-05 (2×10^9 CFU/dose, 200 mg, delivered with pre-filled, single-use vaginal applicators) regimen is once daily from the clindamycin stop day and for 7 consecutive days, thereafter twice weekly as explained in table 2.

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Abnormal vaginal microbiota as described previously. The screening swab should be repeated if more than 3 months old at randomisation day	HIV, hepatitis B or C positivity
First, second or third IVF stimulation cycle or embryo transfer therefrom	HPV CIN 2 or higher
BMI <35	Known or suspected hypersensitivity to clindamycin
Informed consent	Former or current inflammatory bowel disease
18–42 years old	Severe concomitant disease, including diabetes
A maximum of 2 embryos to be transferred	Artificial heart valve
	Intrauterine malformations with operation indication as determined by treating physician (polyps, septum, fibroma)

BMI, body mass index; CIN, Cervical Intraepithelial Neoplasia; HIV, Human Immunodeficiency Virus; HPV, Human Papilloma Virus.

Table 2 Study medication scheme

	Clindamycin 'Alternova'	LACTIN-V
Dose	300 mg	200 mg/2×10^9 CFU/applicator
Dose schedule	Two times per day minimum 6 hours interval. Maximum 14 tablets	Before sleeping Maximum 21 applicators
Allocation	Patients start medication at least 12 days prior to embryo transfer in a fresh or a frozen cycle	Patients start medication at least 12 days prior to embryo transfer in a fresh or a frozen cycle
Route of administration	Oral	Vaginal/topical
Treatment period	7 days	Once per day in 7 days followed by administration twice weekly until clinical pregnancy scan or confirmed not pregnant. In the event of negative hCG test (not pregnant), patients are, however, allowed to continue LACTIN-V treatment until all applicators have been used*
Follow-up period in the present RCT	Clinical pregnancy scan 7–9 weeks later	Clinical pregnancy scan 7–9 weeks later
Medication permitted	All other than the below mentioned	All other than the below mentioned
Medication not permitted	Other antibiotics (unless medically indicated), probiotics, neuromuscular blocking drugs, immunosuppressive medication. Investigational drug preparations other than the study product	Antibiotics (unless medically indicated), other probiotics and investigational drug preparations other than the study product

*Patients not pregnant are informed to contact the department in case of any LACTIN-V–related side effect.
CFU, colony-forming unit; RCT, randomised controlled trial.

Active treatment 2

Oral clindamycin 300 mg two times per day for 7 days followed by LACTIN-V placebo (Osel) until completion of the clinical pregnancy scan at week 7–9. The LACTIN-V placebo regimen is once daily from the clindamycin stop day and for 7 consecutive days, thereafter twice weekly as explained in table 2.

Inactive treatment (placebo)

Matching clindamycin placebo two times per day for 7 days followed by LACTIN-V placebo (Osel) until completion of the clinical pregnancy scan at week 7–9. LACTIN-V placebo regimen is once daily from clindamycin stop day and for 7 consecutive days, thereafter twice weekly as explained in table 2.

If there are embryos to transfer (approximately 90% of patients), then LACTIN-V/placebo treatment is continued twice weekly until clinical pregnancy scan, however, with a maximum of 21 applicators per patient. If the patient has no embryos to transfer or is confirmed not pregnant (negative hCG test), then LACTIN-V treatment can be stopped by the patient, although at least 7 days of LACTIN-V administration need to be administered. An overview of the study medication and allocation can be seen in table 2. Patients are not allowed to take other antibiotics (unless medically indicated), probiotics, neuromuscular blocking drugs, immunosuppressive medication or investigational drug preparations other than the study product. Placebo clindamycin consists of encapsulated mannitolum. The placebo LACTIN-V formulation contains the same inactive ingredients as LACTIN-V, without *Lactobacillus crispatus* CTV-05.

Labelling and packaging

Labelling and packaging of the medication are performed by Glostrup Pharmacy, Denmark in accordance with ICH-GCP guideline and EU GMP Annex 13. Patients are informed that it is important not to have penile-vaginal intercourse within 12 hours after LACTIN-V application. Patient compliance will be measured by tablet counting of the medication packs (clindamycin). Patients who are not pregnant (negative hCG test) and who decided to continue LACTIN-V treatment are informed to contact the respective clinics in case of adverse events and these will be captured in the electronic case report form (eCRF). If patients decide to end study product treatment, they are informed to contact the clinics and to deliver the unused LACTIN-V to the clinic at which point they would be asked about any adverse events. Study personnel will verify in the eCRF what patients decided to do with remaining LACTIN-V applicators after a negative hCG test or no embryos for transfer.

Patients can withdraw their informed consent at any given time and without any reason according to Danish law. If available, the reason for discontinuation has to be stated in the eCRF. Moreover, in case of protocol deviations, this also has to be stated in the eCRF and the principal investigator should decide whether trial medication can continue or not. Furthermore, trial medication is stopped should the patient develop hypersensitivity,

Table 3 Study timeline

	Enrolment	Allocation				
Timepoint	**Maximum 3 months prior to allocation day**	**Minimum 12 days prior to embryo transfer**	**After 7 days clindamycin/placebo treatment**	**Embryo transfer**	**Pregnancy scan week 7-9**	**Gestational weeks 22, 37 and after birth**
Enrolment for screening	X					
Eligibility screen	X					
Informed consent	X					
Vaginal swab	X	X	X	X	X	X
Enrolment for RCT, Intervention allocation		X				
Clindamycin/placebo intervention		◆———	———◆			
LACTIN-V/placebo intervention			◆———	———	———◆	
IVF treatment		◆———	———	———	———◆	
Adverse event questionnaire*				X	X	

*In case of no embryos to transfer or deferred embryo transfer, we sought the questionnaire from patients at oocyte pick-up or when we knew there was no embryo to transfer.
IVF, in vitro fertilisation; RCT, randomised controlled trial.

allergy or severe diarrhoea that a primary investigator suspects may be trial medication related. Vaginal swabs will be taken alongside treatment to monitor the vaginal microbiota and its response to treatment (see table 3 and online supplemental appendix 1). Specifically, vaginal swabs will be taken on the day of randomisation immediately before study medication, after clindamycin treatment, on the day of embryo transfer and again on the day of clinical pregnancy scan. In a substudy, a total of 20 patients will be asked to deliver vaginal samples for each day they take medication and the swab should be taken immediately before the medication on that specific day.

Outcomes

The primary outcome is the clinical pregnancy rate per first embryo transfer defined as ultrasound proven fetal heartbeat in gestational weeks 7–9. The secondary outcomes are the live birth rate per embryo transfer, biochemical pregnancy rate (hCG positive at 9–11 days after embryo transfer according to local laboratory standards), implantation rate, early miscarriage, late miscarriage, preterm birth rates, birth weight and adverse effects of the medication through a safety analysis. The effect of treatment on the vaginal microbiota of the mother throughout study participation and potential pregnancy will be determined using quantitative PCR (qPCR) and next-generation sequencing methods. The colonisation of the *L. crispatus* CTV-05 strain will also be investigated using qPCR. It is pre-planned that reproductive outcome analysis will lead to a first publication by itself, whereas the more laborious sequencing results will arrive in a later publication. Later, we plan to investigate cumulative live birth results of subsequent transfer of spare frozen-thawed embryos of patients attending the study in a fresh cycle.

Sample size

In 2014, the average clinical pregnancy rate per embryo transfer in our fertility clinic was approximately 40% for an IVF cycle. In our pilot study,[5] the adjusted OR between the AVM group and the normal group was 0.06 (95% CI 0.01 to 0.47) for clinical pregnancy per embryo transfer. Taken together, we estimated a superiority design where women in each AVM arm and treated with active medication will have at least a 40% chance for clinical pregnancy per embryo transfer as compared with the placebo arm which was estimated to have a maximum of 20% chance of clinical pregnancy/transfer. By two-sample proportion test with a power of 80% and an alpha at 5%, the aim was to randomise 92 patients in each group. A potential difference between the two active arms was considered exploratory and consequently this was not part of the power calculation, but we decided to include the same number of patients in the active/active arm to investigate a potential added benefit of live biotherapeutic treatment.

An interim analysis will be performed, and to adjust for this, we add 10% to the 92 randomised patients as suggested in Wittes .[38] Approximately 10% of couples will have no embryos for transfer; we adjusted for this by adding another 10% to each randomised group, that is, 19+92=111 (see figure 1). Considering an estimated 20% AVM rate, a total of 1850 IVF patients will be screened to randomise 333 patients (three arms). It was estimated that inclusion will be distributed according to the size of

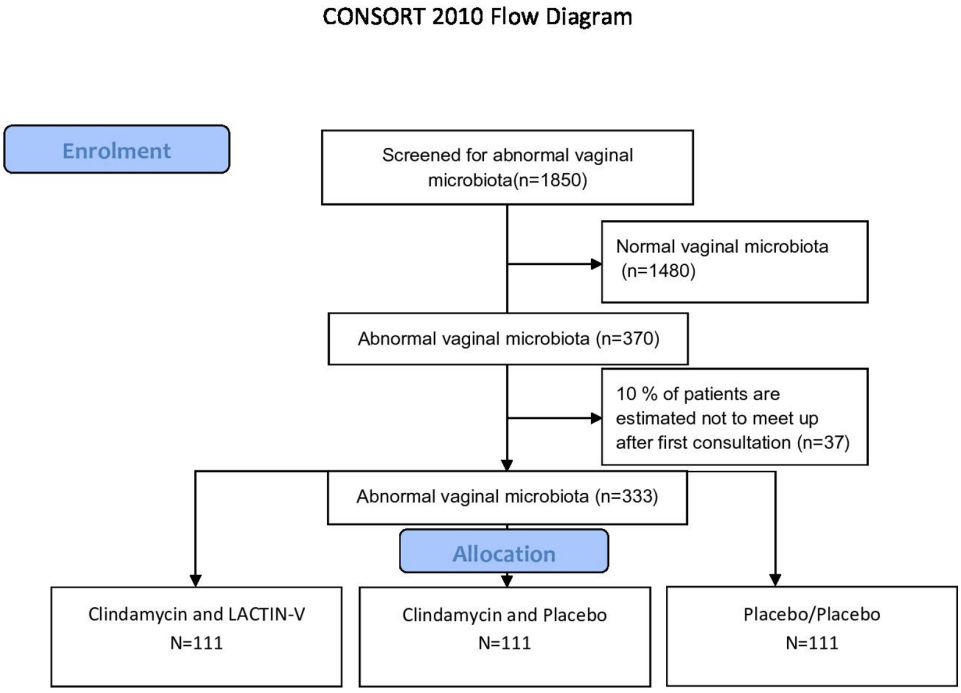

Figure 1 Study flowchart. We add 20% more patients to the powered sample size of 92 randomised patients to adjust for couples who have no embryos for transfer and to adjust for the interim analysis, that is, 19+92=111. Considering an estimated 20% abnormal vaginal microbiota rate, a total of 1850 in vitro fertilisation patients will be screened to randomise 333 patients (three arms).

the centres. Furthermore, we make the following assumptions: (1) very limited loss to follow-up, (2) near full compliance to study medication and (3) homogeneity in the treatment effect.

Allocation

Randomisation is performed by Glostrup pharmacy by a computer-generated code (www.randomization.com). The medication packs labelled with the randomisation number are received at the IVF centres from the pharmacy in blocks of 15, five of each of the three treatments, to secure equal distribution of treatment arms at the centres. The medication has identical appearance and only the randomisation number differ, hence both patients and study personnel are blinded for the intervention. A block of 15 medication packs will be sent from the pharmacy from start of study and new blocks can be requested when five medication packs are left. The 15 medication packs are mixed and appear identical to both personnel and patients. The randomisation number is continuous and unique for each patient, starting from 1 to 333 and the number is prelabelled from the pharmacy before distribution to the clinics. The last three medication packs from 331 to 333 is also one block.

The randomisation list is secured by the pharmacy throughout the trial, and only the sponsor has the authority to unblind the trial. However, in case of medical emergency, the principal investigator can call the pharmacy to unblind. Each participant's medication package is labelled with a randomisation number that is linked to their study ID number in the eCRF. Although both patients and clinicians will be blinded to allocation, they may suspect active medication in case of 'signature' side effects. This small risk of bias seems to be unavoidable. However, to investigate such an effect, patients are asked if they believed that they received active or inactive medication.

Data collection methods

Study data are collected and managed using REDCap (Research Electronic Data Capture) electronic data capture tools hosted at Aarhus University, Denmark.[39 40] REDCap is a secure, web-based software platform designed to support data capture for research studies, providing (1) an intuitive interface for validated data capture, (2) audit trails for tracking data manipulation and export procedures, (3) automated export procedures for seamless data downloads to common statistical packages, and (4) procedures for data integration and interoperability with external sources. All data collectors of the study have to be trained in good clinical practice (GCP) procedures and as minimum to have passed the course provided by the Danish GCP institution. All inclusion and exclusion criteria as well as outcome data will be monitored by external GCP monitors to ensure optimal data quality. Data collection forms and other data entry–related information can be requested from the corresponding author.

Protocol deviations have to be stated in the eCRF. Loss to follow-up is unlikely for patients in IVF treatment who will be highly motivated to come to the clinic. The eCRF instruments have range checks and other data rules that have to be passed to ensure optimal data input. In case

of missing outcome data, we plan to use the framework proposed by White *et al*.[41]

Statistical methods

The total significance level of the study was set to be 5%. Based on the O'Brien-Fleming method, the total significance was split into 0.1% for the interim and 4.9% for the final analysis.[38] Therefore, a p value with 99.9% CI is calculated in the interim analysis to test the possible effect of one or both active treatment arms (combined or separately) on clinical pregnancy rate per embryo transfer (primary outcome) compared with placebo. A Wald χ^2 test for possible effect will be conducted comparing all three arms. Moreover, four analyses: (1) active/active versus active/placebo, (2) active/placebo versus placebo/placebo, (3) active/active versus placebo/placebo, and (4) average effect of active/active AND active/placebo versus placebo/placebo will be done as first a crude estimate and then secondly adjustment with confounders for double embryo transfer, quality of the embryo (cleavage/blastocyst), female age (continuous variable) and centre effect (public/private). If the trial is discontinued according to the criteria stated under the section 'Interim analysis', a full statistical analysis will be made as described next. First, a Wald χ^2 test for possible effect of active treatment on clinical pregnancy rate (primary outcome) will be made across all three groups. Moreover, pairwise comparisons for the aforementioned four tests will be made with ORs and RRs and 94.9% CIs calculated from logistic and linear regressions models, taking the aforementioned confounding factors into account. Analyses will be conducted at modified (m)ITT level defined as all randomised patients who have an embryo transfer following study treatment cycle, including also deferred/frozen embryo transfers due to for example, risk of ovarian hyperstimulation syndrome (OHSS). Patients are excluded from mITT analysis if they do not have embryos for transfer or in case embryo transfer is deferred to a later stage than actual study treatment. Per-protocol analysis will also be considered, that is, an analysis for patients having an embryo transfer as described previously and not violating the protocol as described herein. Sensitivity analyses will also be conducted including strict ITT analysis per randomised patient. The mITT analysis is considered the primary analysis under the assumption that study treatment is not affecting the probability of patients having an embryo transfer.

Interim analysis

An interim analysis as described earlier will be performed to evaluate the clinical pregnancy rate per embryo transfer when 167 patients have been randomised and completed the study for primary outcome evaluation. If study medication is affecting the clinical pregnancy rate statistically significant in either of the analyses, the trial will discontinue. Furthermore, the drop-out rate will be evaluated considering both the number of positive AVM declining to participate and the number of patients who drop out after randomisation. A drop-out rate above 20% will lead to discontinuation. External statisticians from Aarhus University, Denmark will conduct the interim analysis. Only a small study board, including sponsor and principal investigators, will know the result of the interim analysis. Sponsor-investigator makes the decision to continue or discontinue the trial. The study will continue in case there is no statistical difference in either of the tests, drop-out rate is acceptable, and the logistical requirements to finish the study can be met within reasonable time considering, for example, expiry of study medication and time to recruit all patients. The time to undertake the interim analysis and the decision to continue or discontinue is approximately 3 weeks.

Data monitoring

Investigator(s)/institution(s) will permit direct access to source data/documents for trial-related monitoring, audits, institutional review board/independent ethics committee (IRB/IEC) review and regulatory inspection(s). Primary investigators only have access to patients from their own centre in the eCRF. This study will be monitored by the Danish GCP units, primarily the GCP unit at Aarhus University and the GCP unit at Copenhagen University Hospital. Furthermore, this trial is open for audit and quality assurance by the Danish Medicines Agency as specified by Danish law.

Adverse events and reactions

Adverse events and adverse reactions will be registered in a questionnaire handed out by study personnel to the patient on the day of embryo transfer and on the day of the clinical pregnancy scan. In case there are no embryos for transfer, patients will be approached to answer the questionnaire either by email or at oocyte retrieval day. Patients who undergo segmentation ('freeze-all') will use the same questionnaire on the oocyte retrieval day of the cycle where they have started study medication, corresponding to approximately 14 days of study medication. In the questionnaire, patients will also be asked to answer questions regarding gastrointestinal symptoms that might be related to the treatment with antibiotic clindamycin. Patients will be asked the same questionnaire concerning potential late occurring LACTIN-V–related side effects on the day of the clinical pregnancy scan. Moreover, patients are asked if they have symptoms at all study visits and if these symptoms are considered adverse reactions they are recorded in the eCRF, including an adverse reaction judgement from the treating physician.

Serious adverse events (SAEs)

At each centre, primary investigators will report SAEs to sponsor within 24 hours by email or phone. Sponsor ensures that all suspected unexpected serious adverse reactions (SUSARs) that are fatal or life-threatening are recorded and reported to the Danish Medicines Agency and the scientific Ethics Committee as soon as possible and no later than 7 days after the sponsor became aware

of such possible side effect. Within 8 days after a SUSAR has been reported, the sponsor must notify the Danish Medicines Agency and the Ethics Committee with all relevant information on the follow-up of any SUSAR that may occur. All other unexpected serious or suspected serious adverse reactions will be reported to the Danish Medicines Agency and the scientific Ethics Committee within 15 days after the sponsor become aware of these. An annual safety report regarding the trial participants will be performed, consisting of serious adverse event suspected to be related to the investigational drug will be submitted to Danish Medicines Agency and the Ethics Committee. At end of study, all adverse events and SAEs will be reported according to regulations in Denmark.

Ethics

Approvals from the Regional Scientific Ethical Committee, Central Denmark Region (M-2017-157-17), the Danish Data Protection Agency[1–16] and Danish Medicines Agency (2016-002385-31) were obtained prior to trial initiation 7 December 2017. Danish law will be complied with regarding the handling of personal information. Protocol amendments will be provided to the relevant parties, including the Regional Scientific Ethical Committees and Danish Medicines Agency. All protocol amendments have to be approved by the Danish Medicines Agency and the scientific ethical committee before taken into use. Logging of trial amendments is secured at both these institutions, the sponsor-investigator as well as updated at EudraCT. Patient confidentiality is ensured by data capture in REDCap. All patients are covered by a public insurance in Denmark.

Access to data

Only the sponsor-investigator has full access to the dataset. The interim analysis will be performed by external statisticians at the local university according to the pre-set plan explained previously. Principal investigators and statisticians may have access to data at the discretion of the sponsor-investigator. External parties can only gain access to trial data following establishment of a data handling agreement.

Dissemination

Positive, negative as well as inconclusive results will be published, aiming for high-impact journals with full data transparency. Dissemination of results is ensured in clinical trial agreements between the participating institutions and the sponsor's institution, Aarhus University, Denmark. The Vancouver guidelines for authorship will be followed.

Trial status

The first patient was screened 7 December 2017. By 7 September 2019, we had screened 533 patients and randomised 119 patients. Interim analysis is expected by March 2020. End of trial is expected to be summer 2021.

Patients and public involvement

Neither patients nor the public were directly involved in the planning of this trial.

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Competing interests PH, JSJ, NU, TP and TH are listed as inventors in an international patent application (PCT/US2018/040882), involving the therapeutic use of vaginal lactobacilli to improve IVF outcomes. TP is an employee of Osel, Inc. Not related to this trial, TH received honoraria for lectures from Ferring, IBSA, Besins and Merck. PH received unrestricted research grants from MSD, Merck and Ferring as well as honoraria for lectures from MSD, Merck, Gedeon-Richter, Theramex and IBSA. JSJ received speaker's fee from Hologic, BD, SpeeDx and Cepheid and serves on the scientific advisory board of Roche Molecular Systems, Abbott Molecular and Cepheid. NLCF received unrestricted research grant from Gedeon Richter and honoraria for lectures from Merck. HSN received unrestricted research grant from Ferring and honoraria for lectures from Merck, IBSA and Ferring.

Patient and public involvement Patients (other than those actually recruited/invited to participate) and/or the public were not directly involved in the design, or conduct of this research. Although it should be mentioned that lay persons are part of the Ethics Committee who approved this study. Reporting and dissimination to the public will be made on the study webpage www.reproflor.dk and in case the results have relevance to the public we plan to publish also in relevant media.

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