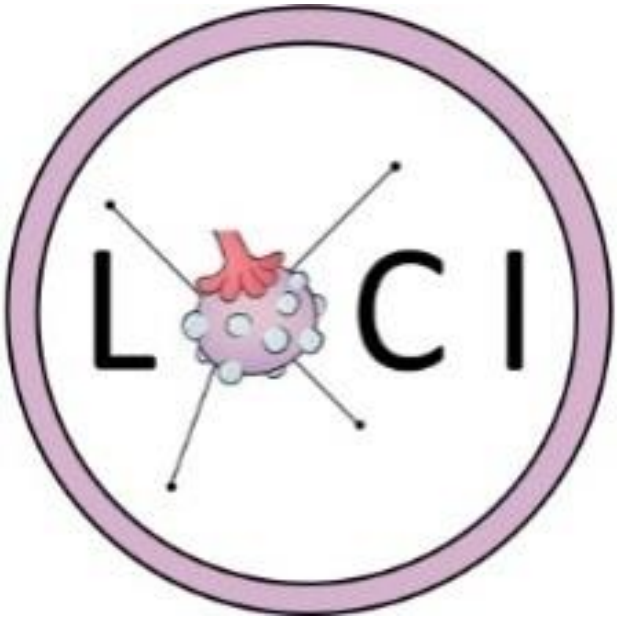

TRIAL PROTOCOL

The LOCI trial

Letrozole or Clomifene, with or without metformin, for ovulation induction in women with polycystic ovary syndrome: a 2x2 factorial design randomised trial

This protocol has regard for the HRA guidance and is compliant with SPIRIT

Version Number:	1.0
Version Date:	07-Nov-2019

Protocol development

Protocol Amendments				
The following amendments and/or administrative changes have been made to this protocol since the implementation of the first approved version.				
Amendment number	Date of amendment	Protocol version number	Type of amendment	Summary of amendment

Funding and Support in Kind	
Funder (s) (Names and contact details of all organisations providing funding and/or support in kind for this trial)	Financial and non-financial support given:
NIHR	Financial
Funding Scheme (if applicable)	HTA 17/116 Letrozole for improving fertility in women with polycystic ovary syndrome
Funder's reference number	17/116/01
Department of Health disclaimer The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health	

Protocol Sign Off

CI Signature Page

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the study publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

This protocol has been approved by:

Trial Name:	The LOCI trial
Protocol Version Number:	Version: __.__
Protocol Version Date:	__ __ / __ __ / __ __ __ __
CI Name:	Arri Coomarasamy
Trial Role:	Chief Investigator
Signature and date:	_____ __ __ / __ __ / __ __ __ __
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Signature and date:	_____ __ __ / __ __ / __ __ __ __

Sponsor statement:

Where the University of Birmingham takes on the sponsor role for protocol development oversight, the signing of the IRAS form by the sponsor will serve as confirmation of approval of this protocol.

Compliance statement:

This protocol describes the LOCI trial only. The protocol should not be used as a guide for the treatment of participants not taking part in the LOCI trial.

The study will be conducted in compliance with the approved protocol, UK Policy Framework for Health and Social Care Research 2017, the General Data Protection Regulations (GDPR) 2018, and the principals of Good Clinical Practice as defined by the European Good Clinical Practice (GCP) Directive. Every care has been taken in the drafting of this protocol, but future amendments may be necessary, which will receive the required approvals prior to implementation.

PI Signature Page

Reference Numbers	
Funder (NIHR HTA) Reference number	17/116/01
EudraCT number	2018-004641-16
Sponsor number	RG_18-272
ISRCTN reference number	TBC
IRAS reference number	257918

The undersigned confirm that the following protocol has been agreed and accepted and that the Principal Investigator agrees to conduct the trial in compliance with the approved protocol.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

This protocol has been approved by:

Trial Name:	The LOCI trial
Protocol Version Number:	Version: __.__
Protocol Version Date:	__ __ / __ __ / __ __ __ __
PI Name:	
Name of Site:	
Signature and date:	_____ __ __ / __ __ / __ __ __ __

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Trial website	https://www.birmingham.ac.uk/loci
Trial social media	Twitter: @LOCITrial

ABBREVIATIONS	
Abbreviation	**Term**
BCTU	Birmingham Clinical Trials Unit
BMI	Body Mass Index
CI	Chief Investigator
CRF	Case Report Form
DMEC	Data Monitoring and Ethics Committee
GnRH	Gonadotropin-Releasing Hormone
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GMP	Good Manufacturing Practice
GP	General Practitioner
hCG	Human Chorionic Gonadotropin
HDU	High Dependency Unit
ICF	Informed Consent Form
ISF	Investigator Site File
ITMS	Integrated Trial Management System
ITU	Intensive Therapy Unit
IUGR	Intrauterine Growth Restriction
IVF	In-vitro Fertilisation
MHRA	Medicines and Healthcare products Regulatory Agency
NHS	National Health Service
NICE	The National Institute for Health and Care Excellence
OCP	Oral Contraceptive Pill
OHSS	Ovarian Hyperstimulation Syndrome
PCOS	Polycystic Ovary Syndrome
PI	Principal Investigator
PIS	Participant Information Sheet
PSS	Personal Social Service
QALY	Quality-Adjusted Life Year

REC	Research Ethics Committee
RSI	Reference Safety Information
SmPC	Summary of Product Characteristics
TSC	Trial Steering Committee
UoB	University of Birmingham

TRIAL SUMMARY

Title Letrozole or Clomifene, with or without metformin, for ovulation induction in women with polycystic ovary syndrome (PCOS) (The LOCI trial)

Objectives To compare the effectiveness of letrozole versus clomifene, metformin versus placebo and letrozole plus metformin versus clomifene plus metformin, in woman with anovulatory PCOS and infertility on live birth rate (≥ 34 weeks of gestation).

Trial Design A 2x2 factorial randomised, double-blind, placebo-controlled multi-centre study, with health economic evaluation and a six month internal pilot

Setting Fertility clinics at secondary and tertiary level hospitals across the UK

Participant Population and Sample Size Women with anovulatory polycystic ovary syndrome (PCOS) and infertility. The sample size will be 2100 women, allowing greater than 99% power (at p=0.05) to detect a 10% difference in the letrozole vs clomifene comparison and the same power to detect differences in the metformin vs placebo comparison. The 10% absolute increase was identified in our survey as clinically minimally important. The sample size will ensure 90% power (p=0.05 and 1050 participants) to answer the question of letrozole plus metformin vs clomifene plus metformin.

Eligibility criteria – Inclusion criteria: Women diagnosed with PCOS (according to Rotterdam criteria) and evidence of anovulation (anovulation is defined as irregular cycles lasting <21 or more than 35 days or less than 8 periods per year OR absence of raised serum progesterone greater than 20nmol/l 7 days prior to a period); Presentation with infertility or wishing to conceive; Male partner with normal sperm count (≥ 15 million) and progressive motility (≥ 32%) in the last 3 years; Willing and able to give informed consent.

Exclusion criteria: Age <18 or >43 years at randomisation; Body Mass Index > 35; Three or more previous ovulation induction treatments with either letrozole or clomifene; Currently on metformin treatment or inositol supplements for ovulation induction or for other indications; Women opting for alternative methods of ovulation induction or treatment (GnRH agonists and antagonists, gonadotropins), triggering ovulation with hCG, or performing intrauterine or intracervical insemination; Contraindications to letrozole, clomifene, metformin use and/or pregnancy; Has previously participated in the LOCI trial.

Interventions: Letrozole for 5 days starting on day 2 or 3 of the menstrual cycle, plus metformin or placebo daily. Initial letrozole dose will be 2.5mg daily and increased to a maximum dose of 7.5mg daily until ovulation is confirmed, for a maximum of 6 treatment cycles. The comparison will be clomifene for 5 days starting on day 2 or 3 of the menstrual cycle, plus metformin or placebo daily. Initial clomifene dose will be 50mg daily and increased to a maximum dose of 150mg in a similar way to letrozole, for a maximum of 6 treatment cycles. The maximum dose of metformin will be 1500mg daily and continued until 14 weeks of pregnancy or until the end of the 6 treatment cycles.

TRIAL SUMMARY

Outcomes:

Primary outcome: Live births at and beyond 34 completed weeks of gestation, as a proportion of all women randomised.

Key secondary outcomes: Pregnancy loss (defined as pregnancy loss before 24 weeks of gestation)**;** number of ovulation induction cycles to live birth.

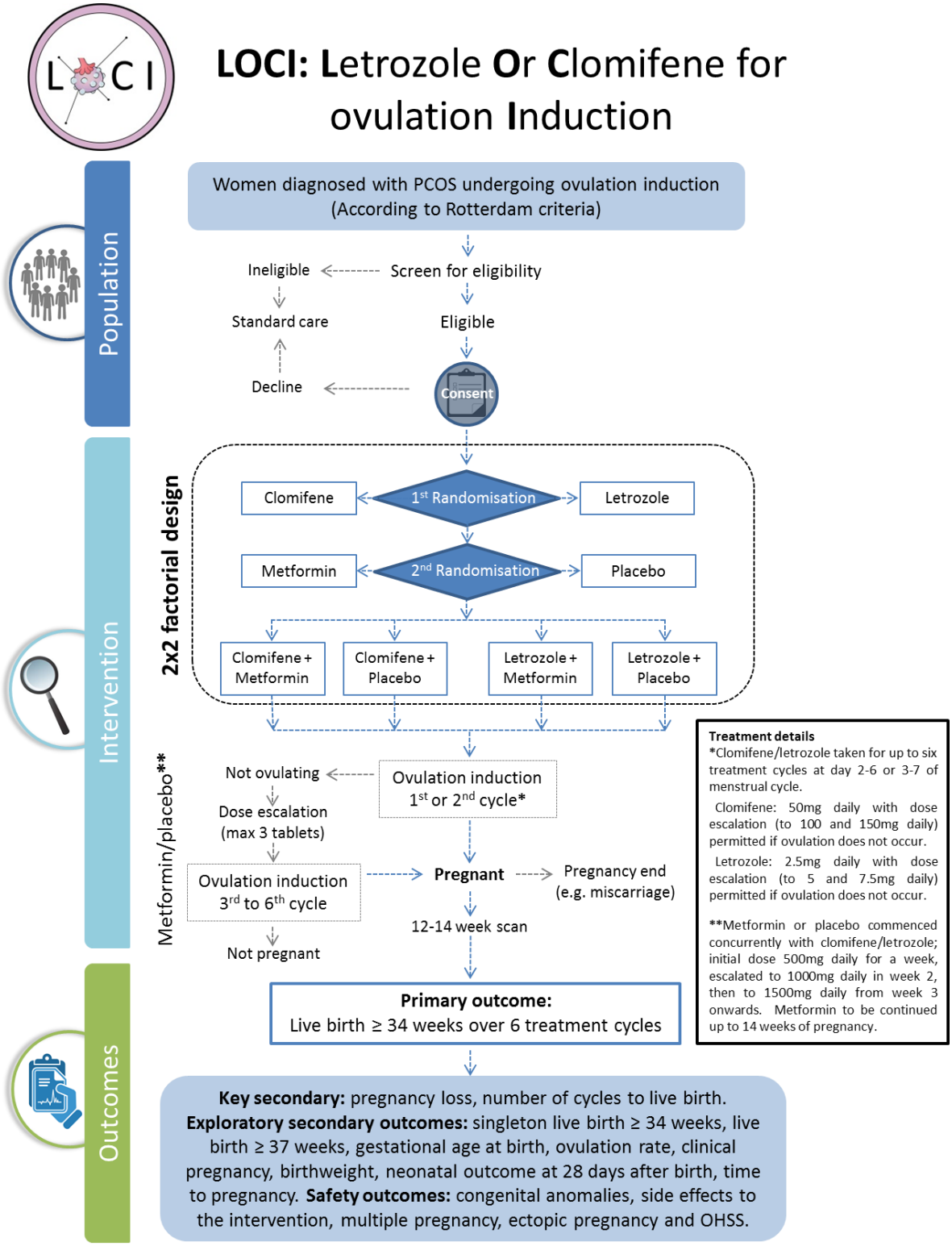
Exploratory secondary outcomes: Ovulation rate, time from randomisation to pregnancy, number of ovulation induction cycles required for pregnancy, ongoing pregnancy at 12 weeks (range 11 to 14 weeks) of gestation, termination, stillbirth, molar pregnancy, pregnancy of unknown location, twin live births, gestational age at live birth. ***Where live birth ≥24 weeks:*** time from conception to delivery (gestational age), gestational age <28/<32/<37 weeks, singleton live births at and beyond 34 completed weeks of gestation, live births at and beyond 37 completed weeks of gestation, mode of birth (unassisted vaginal, instrumental vaginal, elective caesarean section, emergency caesarean section, vaginal breech birth, other), birth weight, APGAR score<7 out of 10 (at 1, 5 and 10 minutes), pregnancy-induced hypertension, pre-eclampsia, obstetric cholestasis, cervical cerclage, preterm (<37 weeks) pre-labour rupture of membranes, gestational diabetes (other complications will be tabulated but not formally analysed), chorioamnionitis, intrauterine growth restriction (IUGR), macrosomia (other complications will be tabulated but not formally analysed), haemorrhage (other complications will be tabulated but not formally analysed), maternal outcomes: admission to high dependency unit (HDU), admission to intensive therapy unit (ITU), (other complications will be tabulated but not formally analysed)

Neonatal outcomes: discharge to hospital, early infection, retinopathy of prematurity, necrotising enterocolitis, intraventricular haemorrhage, respiratory distress syndrome, ventilation or oxygen support (other complications will be tabulated but not formally analysed), survival at 28 days of neonatal life.

Safety outcomes: Neonatal congenital or chromosomal abnormalities, maternal adverse events (tabulated but not formally analysed), multiple pregnancies, ectopic pregnancies, ovarian hyperstimulation syndrome (OHSS), serious adverse events.

Heath economic evaluation: Hospital resource use and EQ-5D-5L questionnaire.

Trial Schema



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1. BACKGROUND AND RATIONALE

1.1. Background

Infertility affects one in six couples, with 25% of infertility being due to anovulation (not releasing eggs from the ovaries).[1] Polycystic ovary syndrome (PCOS) is also very common; (approximately 10% of women of reproductive age in the UK have this condition) and it is responsible for 85% of anovulation.[2] Invasive treatments, such as an operation called ovarian diathermy or In-vitro fertilisation (IVF), may overcome anovulation from PCOS, but are associated with significant risks and costs.[3] A successful oral tablet treatment would reduce the need for invasive, risky and costly fertility treatments for women with PCOS, and may improve patient experience.

1.2. Trial Rationale

1.2.1. Justification for participant population

The trial will include women wishing to conceive and diagnosed with PCOS using the Rotterdam criteria and have evidence of anovulation (anovulation is defined as irregular cycles lasting <21 or more than 35 days or less than 8 periods per year OR absence of raised serum progesterone greater than 20nmol/l 7 days prior to a period). These criteria are in line with current clinical practice described in the latest European Society of Human Reproduction and Embryology (ESHRE) guidelines.[4] Women will be aged between 18 and 43 years of age at randomisation and have a Body Mass Index (BMI) < 35. These upper age and BMI limits are chosen because the probability of a successful pregnancy with natural conception beyond this limits decreases significantly. The male partner should have a normal sperm count (≥ 15 million) and progressive motility (≥ 32%) that has been tested in the last 3 years; this is necessary to rule out male factor infertility.

1.2.2. Choice of intervention

The current standard is the oral tablet clomifene.[3,5] A new oral tablet option is letrozole.[6] There is evidence that addition of another oral drug called metformin may enhance the effects of clomifene or letrozole.[3,7] We have carried out a number of systematic reviews and meta-analyses to fully examine the underpinning evidence of the three drugs for managing anovulation (please see ***Box 1***).

Box 1. Systematic review of ovulation drugs: methods

Systematic review methods:
Databases: MEDLINE, EMBASE, CCTR, CDSR, DARE.
Search period: From respective database inception to April 2018.
Search terms (MESH): (letrozole OR clomi* OR metformin) AND pregnancy (restricted to randomised trials).
Review Outcome: Clinical pregnancy and live birth.

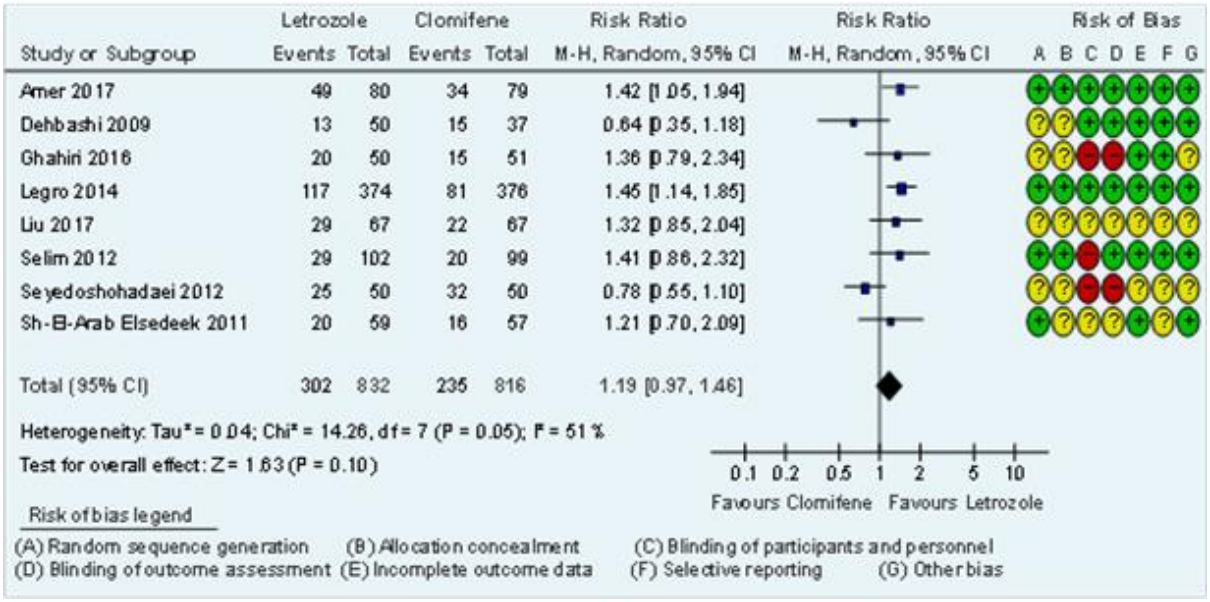
Systematic review findings
Our search yielded a total of 1,687 citations. After review of titles and abstracts 1,598 citations were judged to be not relevant and were therefore excluded. Full manuscripts of the remaining 103 citations were retrieved. We excluded 19 studies where ovulation induction was given as 2nd line treatment to women with PCOS and confirmed clomifene resistance. We also excluded 22 studies where ovulation was triggered using hCG as this is not standard UK practice. In total, we included 89 randomised trials in the review where a direct comparison was made between the various drug combinations or had pregnancy data on at least one of the drugs.

Clomifene: Current guidance in the UK is to use clomifene with or without metformin for a maximum of 6

treatment cycles before more complex and invasive second-line interventions are considered.[3] Clomifene is licensed and has been used for decades for this indication, but is associated with unpleasant side effects such as mood changes and hot flushes. It is also linked to approximately 10-fold higher risk of multiple pregnancies compared with natural conception.[6] The biggest risks of multiple pregnancies are prematurity and low birth weight, which often necessitate hospitalisation in the early neonatal period, and are linked to a significant risk of neonatal death and longer term health and cognitive effects.[8] For example, twins are at least six times more likely than singletons to suffer cerebral palsy.[8]

Letrozole: Letrozole has a different mechanism of action, and appears to be associated with fewer side effects and multiple pregnancies compared with clomifene.[5] The use of letrozole for women with PCOS was examined by the Cochrane Collaboration[5] and considered by NICE in 2016 with the conclusion that "*in women with PCOS, letrozole appears to be associated with a higher live birth rate, lower rates of multiple pregnancy and lower incidence of OHSS than clomifene. However, because of the low quality of the evidence base, no impact on NICE CG156 is expected*".[3] Our updated systematic review included 3 additional studies comparing letrozole to clomifene. The meta-analysis indicated a trend favouring letrozole, but the result was not statistically significant (clinical pregnancy: RR 1.19; 95% CI 0.97 to 1.46; ***Figure 1***). Furthermore, most of the trials were small and of variable quality (see risk of bias grading in ***Figure 1***), precluding us from drawing any firm inferences.

Figure 1. Meta-analysis of studies of letrozole versus clomifene for ovulation induction (Outcome: Clinical pregnancy)

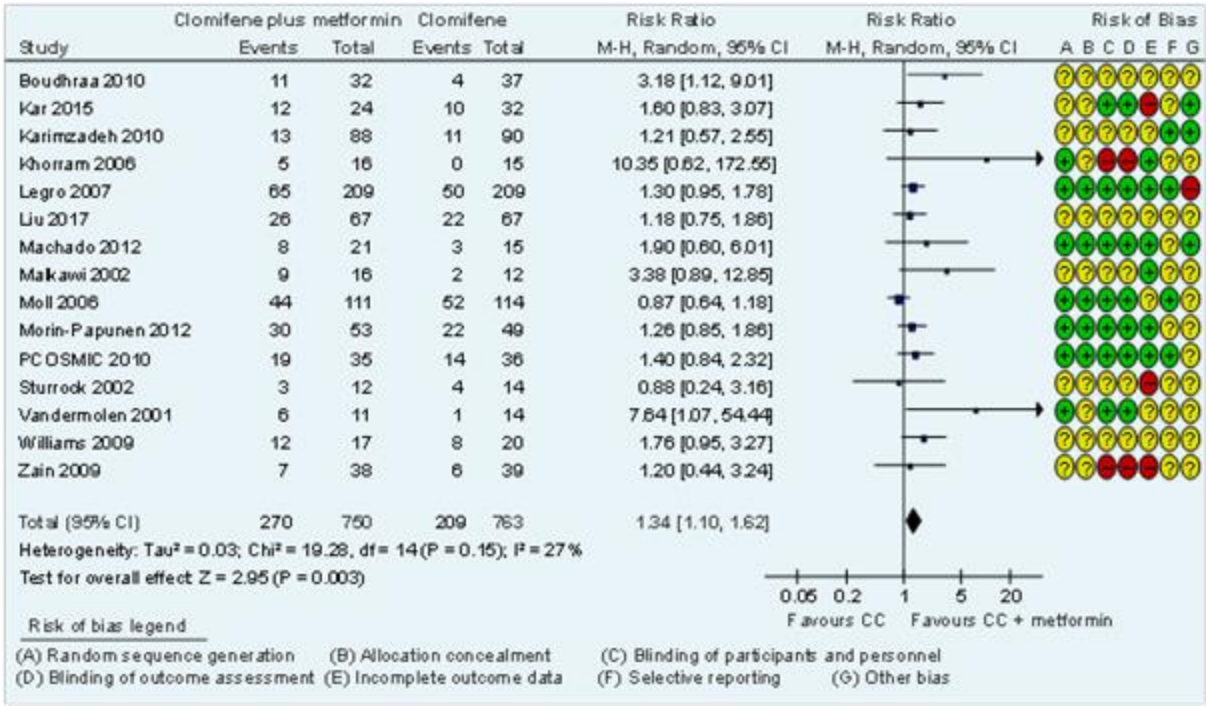


Metformin: Metformin has been used off-license for ovulation induction for over 20 years. While earlier, small, single-centre studies suggested a beneficial effect on reproductive outcomes, larger randomised studies have failed to demonstrate a significant benefit, either as a single agent or in combination with clomifene.[7,9,10] In a large Dutch multicentre trial, 228 women with PCOS were randomly allocated to receive clomifene plus metformin or clomifene plus placebo.[9] There was no difference in the rates of ongoing pregnancy (40% for clomifene plus metformin vs 46% for clomifene plus placebo, RR 0.87, 95% CI 0.6 to 1.2), but more women who received metformin discontinued the therapy because of gastrointestinal side effects (16% vs 5%; risk difference 11%,

95% CI 5% to 16%).[9] Another large placebo-controlled trial enrolled 676 anovulatory women with PCOS and randomised them to (i) metformin, (ii) clomifene, or (iii) metformin plus clomifene. The conception rates were 12% (25/208), 29.7% (62/209) and 38.3% (80/209), respectively. The live birth rates were 7.2% (15/208), 22.5% (47/209) and 26.8% (56/209), respectively.[10] In this study, the live birth rate did not improve significantly by the combination of metformin plus clomifene despite the higher conception rates with the combination therapy.[10]

Our updated systematic review of clomifene plus metformin versus clomifene alone found an increase in clinical pregnancy rate with clomifene plus metformin (RR 1.34; 95% CI 1.10 to 1.62; ***Figure 2***). However, the quality of the trials was again variable (please see risk of bias grading in ***Figure 2***, resulting in weak inferences.

Figure 2. Meta-analysis of studies of clomifene plus metformin versus clomifene alone for ovulation induction (Outcome: Clinical pregnancy)

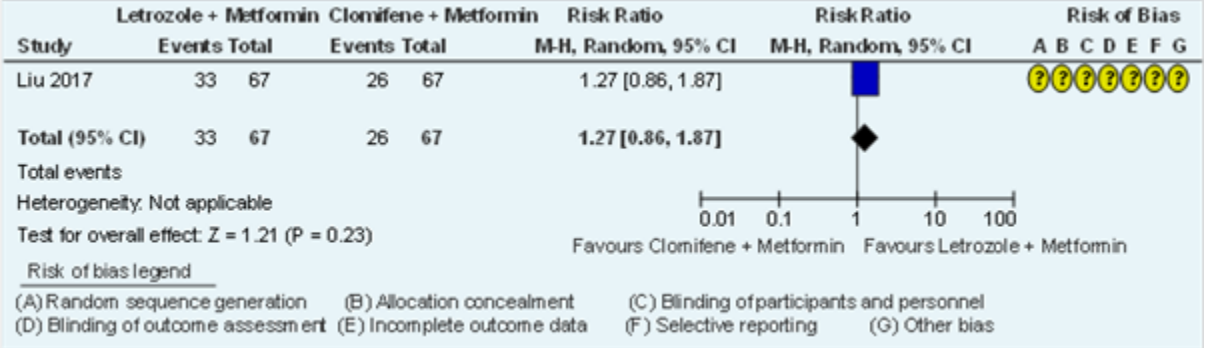


A Cochrane review on the same comparison came to a similar conclusion noting that metformin may be more beneficial than placebo for improving live births, but the evidence did not reach statistical significance and was of low quality (OR 1.21, 95% CI 0.92 to 1.59, 9 studies, 1079 women, I^2 = 20%, *low certainty*).[7] Women taking metformin alone or with combined therapy suffered significantly more gastrointestinal side effects (OR 3.97, 95% CI 2.59 to 6.08, 3 studies, 591 women, I^2 = 47%, moderate quality evidence) compared to women receiving clomifene alone.[7]

Use of metformin with letrozole is uncommon, both in clinical practice and in clinical trials; there is only one single-centre randomised trial from China in which women with PCOS were randomised to letrozole plus metformin (n=67) or letrozole alone (n=67) in the context of a 4-arm trial (***Figure 3***).[11] There were no significant differences in the pregnancy outcomes, but the size of the study limits the

power to detect meaningful differences.[11] A recent network meta-analysis of all ovulation induction agents, published in the BMJ by three of the co-investigators (Siladitya Bhattacharya, Madelon van Wely, Ben Mol) found that either letrozole or clomifene plus metformin could be considered as first-line treatment, but evidence was generally of poor quality and there was instability in sensitivity analyses.[12] Our proposed trial will investigate with sufficient power the most common comparisons for ovulation induction and will inform future iterations of national and international guidelines.

Figure 3. A summary of letrozole plus metformin versus clomifene plus metformin for ovulation induction (Outcome: Clinical pregnancy)



1.2.3. Justification for design

We conducted a UK-wide clinician survey, with representatives from most of the established fertility clinics in the UK (104 responses). Clomifene alone is the most commonly used first line treatment for ovulation induction (69/104; 66.3%, ***Figure 4***). Letrozole alone is used rarely as a first line treatment (3/104; 2.9%, ***Figure 4***). Clomifene plus metformin use is common (32/104, 30.8%), but none of the participants reported using the combination of letrozole plus metformin as a first line treatment. The most common second line treatment is clomifene plus metformin (39/104; 37.5%, ***Figure 5***). Letrozole is also frequently used as a second line treatment (23/104; 22.1%, ***Figure 5***), as well as letrozole plus metformin (8/104; 7.7%, ***Figure 5***). The survey highlighted the uncertainty among clinicians on the added value of metformin to either clomifene or letrozole. The reasons given for not using metformin were the lack of evidence for its added value and concerns regarding side effects. The 2x2 factorial design of this study will not only supply a definitive answer to the research of whether letrozole or clomifene is the best treatment for ovulation induction in women suffering from PCOS, but also allow us to explore the effects of metformin. Finally, we explored whether care providers will be willing to invite patients to participate in the LOCI study. The vast majority (103/104, 99%) agreed to offer the trial to patients under their care.

Figure 4. Current first line treatments for ovulation induction.

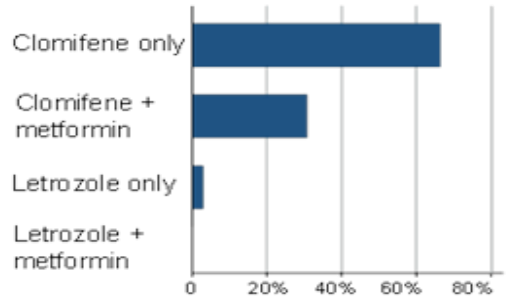
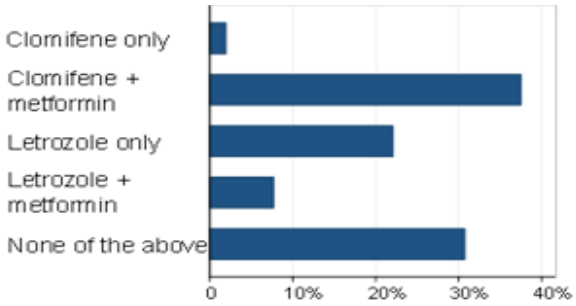


Figure 5. Current second line treatments for ovulation induction.



We also conducted an online UK patient survey (n=82 participants) in partnership with Fertility Network UK. All patients answered further research to decide on the safest and most effective medical therapy for women with PCOS and infertility is needed, and 97.6% (80/82) answered that the LOCI trial aiming to investigate the safety and effectiveness of letrozole versus clomifene and the added value of metformin is required (***Figure 6***). Reassuringly, 85.4% (70/82) stated they would agree to take part if offered the study (***Figure 6***). Many patients shared their experiences of ovulation induction, and highlighted their preference for oral therapy with the least amount of side-effects.

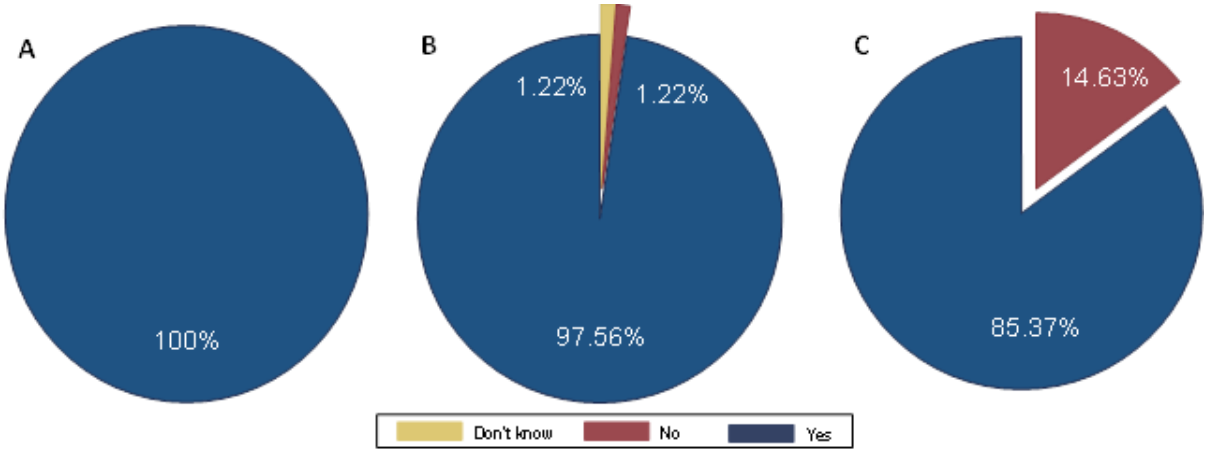


Figure 6. Patient opinion on (A) whether they think further research is needed for women with PCOS and infertility, (B) whether the LOCI trial is needed and (C) if they would be willing to take part?

Given this weight of evidence, discussions elicited a unanimous preference for carrying out a 2x2 factorial randomised, double-blind, placebo-controlled multi-centre superiority trial.
studying the commissioning brief as a primary question with sufficient power, as well as the added value of metformin in a 2x2 factorial design:

Population: Women with anovulatory PCOS and infertility wishing to conceive.

Intervention: Letrozole 2.5-7.5mg daily for 5 days starting on day 2 or 3 of the menstrual cycle for 6 treatment cycles, plus metformin at a maximal dose of 1500mg or placebo daily up to 14 weeks of pregnancy or until end of the 6 treatment cycles.

Comparison: Clomifene 50-150mg daily for 5 days starting on day 2 or 3 of the menstrual cycle for 6 treatment cycles, plus metformin 1500mg or placebo daily up to 14 weeks of pregnancy or until end of the 6 treatment cycles.

Primary Outcome: Live birth ≥ 34 weeks of gestation.

2. AIMS AND OBJECTIVES

2.1. Trial Objectives

2.1.1. Aims and Objectives

Aim

To investigate the clinical and cost-effectiveness of letrozole versus clomifene with or without metformin for ovulation induction in women with PCOS and infertility.

2.1.1 Primary Objective

To test the hypothesis that in women with PCOS and infertility, letrozole plus metformin versus clomifene plus metformin increases the live birth rate (≥ 34 weeks of gestation) by at least 10%.

2.1.2. Secondary Objectives

- To compare the paired drug effectiveness in women with anovulatory PCOS and infertility on live birth rate (≥ 34 weeks of gestation).

Objectives

Letrozole vs clomifene

To compare the effectiveness of letrozole vs clomifene in women with anovulatory PCOS and infertility on live birth rate (≥ 34 weeks of gestation).

Metformin vs Placebo

To compare the effectiveness of metformin vs placebo in women with anovulatory PCOS and infertility undergoing ovulation induction on live birth rate (≥ 34 weeks of gestation).

Letrozole plus metformin vs clomifene plus metformin

To compare the effectiveness of letrozole plus metformin vs clomifene plus metformin in women with anovulatory PCOS and infertility on live birth rate (≥ 34 weeks of gestation).

- Collect key secondary data on:

    (i) Pregnancy loss (defined as pregnancy loss before 24 weeks of gestation)
    (ii) Number of ovulation induction cycles to live birth

- Collect exploratory secondary data on:

    (i) Treatment outcomes
    (ii) Pregnancy end outcomes
    (iii) Where live birth ≥24 weeks: gestational characteristics
    (iv) Antenatal Outcomes
    (v) Intrapartum Outcomes
    (vi) Post-partum outcomes
    (vii) Maternal outcomes
    (viii) Neonatal outcomes
    (ix) Survival at 28 days of neonatal life
    (x) Heath economic evaluation: Hospital resource use and EQ-5D-5L questionnaire

- Collect Safety outcomes data
    (i) Neonatal congenital or chromosomal abnormalities
    (ii) Maternal adverse events
    (iii) Multiple pregnancies
    (iv) Ectopic pregnancies
    (v) Ovarian hyperstimulation syndrome (OHSS)
    (vi) Serious adverse events

2.1.3. Economic Aims and Objectives

To assess the cost-effectiveness of letrozole and the added value of metformin in the management of ovulation induction for women with PCOS and infertility based on an outcome of cost per additional live birth at ≥ 34 weeks of gestation from an NHS and personal social service (PSS) perspective. A secondary analysis reporting results in terms of cost per quality-adjusted life year (QALY) will also be carried out.

3. TRIAL DESIGN AND SETTING

3.1. Trial Design

A 2x2 factorial randomised, double-blind, placebo-controlled multi-centre superiority trial of investigational medicinal products, with health economic evaluation and a six month internal pilot to ensure ability to recruit and randomise.

3.2. Trial Setting

Approximately 45 gynaecology departments and/or fertility centres in the United Kingdom.

3.3. Identification of participants

Potential participants will be identified and approached by clinic doctors, nurses, research nurses and research midwives, after having received appropriate training relating to the trial. Recruitment will take place in gynaecology clinics and fertility clinics located across the United Kingdom.

The participant eligibility pathway to recruitment and randomisation is illustrated by the trial flowchart. They will be advised that participation in the study is entirely voluntary with the option of withdrawing from the study at any stage, and that participation or non-participation will not affect their usual care. Potential participants will be provided with a Study Participant Information Sheet (PIS) and given time to consider their involvement.

Eligible women will be given the opportunity to decide if they wish to participate, or if they need more time to consider their decision, or if they do not wish to participate. In all three scenarios, the decision of the woman will be respected. If a woman needs more time to consider her potential involvement, she will be asked to call the research nurse or midwife when she has decided. If an undecided woman does not call within 1 week, then the research nurse or midwife will contact her. If an initially undecided woman decides to participate later, the research nurse or midwife will arrange a mutually convenient opportunity for the woman to be consented, providing she still meets the eligibility criteria.

Women who give consent will proceed to randomisation if they are eligible to participate in the trial. Consent will be recorded on the approved consent form, which must be retained in the site file with a copy given to the participant and a copy sent to the LOCI Trial Office.

3.4. Assessment of Risk

All clinical trials can be considered to involve an element of risk and, in accordance with Birmingham Clinical Trials Unit (BCTU) operating procedures this trial has been risk assessed, to clarify any risks relating uniquely to this trial. This risk assessment concluded that the risk of participating to this trial is no higher than the risk of standard medical care and is there a Type A trial in accordance with risk-adapted approach to CTIMPs.

4. ELIGIBILITY

4.1. Inclusion Criteria

- Women diagnosed with PCOS (according to Rotterdam criteria)[13] and evidence of anovulation (anovulation is defined as irregular cycles lasting <21 or more than 35 days or less than 8 periods per year OR absence of raised serum progesterone greater than 20nmol/l 7 days prior to a period);
- Presentation with infertility or wishing to conceive;

- Male partner with normal sperm count (≥ 15 million) and progressive motility (≥ 32%) in the last 3 years;
- Willing and able to give informed consent.

4.2. Exclusion Criteria

- Age ≤18 or ≥43 years at randomisation;
- Body Mass Index ≥35;
- Three or more previous ovulation induction treatments with either letrozole or clomifene;
- Currently on metformin treatment or inositol supplements for ovulation induction or for other indications
- Women opting for alternative methods of ovulation induction or treatment (GnRH agonists and antagonists, gonadotropins), triggering ovulation with hCG, or performing intrauterine or intracervical insemination;
- Contraindications to letrozole, clomifene, metformin use and/or pregnancy (see section 7.3 for full details on contraindications).
- Woman has previously participated in the LOCI trial.

4.3. Co-enrolment

Co-enrolment may be permissible, but in all instances the recruiting centre should contact the LOCI Trials Office prior to offering the other trial.

5. CONSENT

It will be the responsibility of the Investigator to obtain written informed consent for each participant prior to performing any trial related procedures. A research nurse, research midwife or clinician is able to take consent providing that local practice allows this and responsibility has been delegated by the Principal Investigator as captured on the Site Signature and Delegation Log.

A Participant Information Sheet (PIS) will be provided to facilitate this process. Investigators, or delegates, will ensure that they adequately explain the aim, trial intervention, anticipated benefits and potential hazards of taking part in the trial to the participant. They will also stress that participation is voluntary and that the participant is free to refuse to take part and may withdraw from the trial at any time. The participant will be given an appropriate amount of time to read the PIS and to discuss their participation with others outside of the site research team. The participant will be given the opportunity to ask questions. If the participant expresses an interest in participating in the trial they will be asked to sign and date the latest version of the Informed Consent Form (ICF). The participant must give explicit consent for the regulatory authorities, members of the research team and/or representatives of the sponsor to be given direct access to the participant's medical records.

The Investigator, or delegate, will then sign and date the ICF. A copy of the ICF will be given to the participant, a copy will be filed in the medical notes, and the original placed in the Investigator Site File (ISF). Once the participant is entered into the trial, the participant's trial number will be entered on the ICF maintained in the ISF. In addition, if the participant has given explicit consent a copy of the signed ICF will be sent to the BCTU trials team for review.

Details of the informed consent discussions will be recorded in the participant's medical notes. This will include date of discussion, the name of the trial, summary of discussion, version number of the PIS given to participant and version number of ICF signed and date consent received. Where consent is obtained on the same day that the trial related assessments are due to start, a note should be made in the medical notes as to what time the patient was approached about taking part in the trial, the time consent was obtained and what time the procedures started.

At each visit the participant's willingness to continue in the trial will be ascertained and documented in the medical notes. Throughout the trial the participant will have the opportunity to ask questions about the trial. Any new information that may be relevant to the participant's continued participation will be provided. Where new information becomes available which may affect the participants' decision to continue, participants will be given time to consider and if happy to continue will be re-consented. Re-consent will be documented in the medical notes. The participant's right to withdraw from the trial will remain.

Electronic copies of the PIS and ICF will be available from the Trials Office and for UK trials will be printed or photocopied onto the headed paper of the local institution. Details of all participants approached about the trial will be recorded on the Participant Screening/Enrolment Log and with the participant's prior consent, their General Practitioner (GP) will also be informed that they are taking part in the trial.

6. ENROLMENT AND RANDOMISATION

6.1. Enrolment and Screening

The medical records of potential participants will be screened for eligibility by clinic doctors, nurses, research nurses and research midwives, after having received appropriate training relating to the trial. Clinic doctors will confirm eligibility for the trial.

6.2 Randomisation

6.2.1. Randomisation Methodology

Participants will be randomised on-line via a secure internet facility at the level of the individual in a 1:1 ratio to either letrozole or clomifene and at the same time randomised to metformin or placebo.

A minimisation algorithm will be used within the online randomisation system to ensure balance in the treatment allocations (both randomisations will occur simultaneously effectively resulting in four allocation groups) over the following variables:

- maternal age (<35 and ≥35 years);
- body mass index (<30 and ≥30);
- any previous pregnancy (yes and no)
- previous exposure to either clomifene or letrozole;
- any periods in the preceding 6 months (yes and no)
- randomising centre

A ‘random element’ will be included in the minimisation algorithm, so that each participant has a probability (unspecified here), of being randomised to the opposite treatment that they would have otherwise received.

Full details of the randomisation specification will be stored in a confidential document at BCTU.

6.2.2. Blinding

This trial will be double-blinded, with over-encapsulation of the letrozole and clomifene and a matched placebo controlled IMP for metformin. Dose escalations for letrozole and clomifene will be identical in terms of the number of tablets the patient has to take in each cycle (e.g. month one = 1 tablet, month two = 2 tablets, month three = 3 tablets) therefore, the blinding will be maintained throughout the trial.

6.2.3. Blinded Personnel

Participants, investigators, research midwives/nurses, laboratory outcome assessors and other attending clinicians will remain blind to the trial treatment allocation throughout the duration of the trial.

6.2.4. Allocation Concealment

Given the randomisation methodology described above, allocation concealment will be maintained throughout the trial.

6.2.5. Unblinding

Investigators will have access to unblinding in case of a medical emergency via the online code-break system or by contacting the LOCI Trial office.

Should any Serious Adverse Event occur, the management and care of the participant will be initiated as though the woman is taking metformin and either letrozole or clomifene. Cases that are considered serious, unexpected and possibly, probably or definitely related (please refer to section 9.6) will be unblinded only at the Trial Office by the LOCI Trial Manager (or other nominated individual), for reporting purposes. The attending clinician and local PI will not be made aware of the actual trial treatment unless it is deemed clinically necessary by the local clinical team.

In all other circumstances, investigators and research midwives/nurses will remain blind to treatment allocation whilst the participant remains in the trial. However, if a participant is withdrawn from the trial and only if the treatment allocation is required for the continued medical management of the withdrawn participant, care providers should attempt to contact the LOCI Trial Office in the first instance or use the online LOCI code-break system. This online service will be available 24 hours a day, seven days a week and will facilitate rapid unblinding. If they wish, participants may enquire and find out about their treatment allocation after the trial has ended by contacting the hospital or trial office directly.

6.2.6. Randomisation Process

Immediately after all eligibility criteria have been confirmed, consent has been obtained and baseline prognostic factors gathered, the participant can be randomised into the trial. Participants will be randomised into the trial by a secure online randomisation system which is available via the MedSciNet Clinical Trial Framework.

Each participating centre and each authorised member of the research team will be provided with a unique log-in username and password for this purpose. Online randomisation will be available 24 hours a day, seven days a week apart from short periods of scheduled maintenance. As a back-up, authorised members of the research team will be able to call the toll-free randomisation service (0800 953 0274). Telephone randomisations will be available Monday to Friday, between 09:00 and 17:00, except for bank holidays and University of Birmingham closed days.

Randomisation Notepads (a sample document is available separately) will be provided to investigators and may be used to collate the necessary information prior to randomisation. All the questions and data items on the Randomisation Notepad must be answered before a trial number and pack number may be given. If some data items are missing, randomisation will be suspended but may be resumed once the information is available. Only when all the eligibility criteria and baseline data items have been provided, will the trial and pack numbers be given and a confirmatory email sent to the randomising investigator, the local PI and the research midwife/nurse.

The trial number will be linked to a treatment pack number that will be available in the local hospital pharmacy, and the pharmacy will also receive notification of the randomisation by email.

6.2.7. Randomisation Records

Following randomisation, a confirmatory e-mail will be sent to the randomiser, local research nurse, local PI, local pharmacist and the trial office (loci@trials.bham.ac.uk).

Investigators will keep their own study file log which links participants with their allocated trial number in the **LOCI Participant Recruitment and Identification Log**. The Investigator must maintain this document, which is **not** for submission to the Trials Office. The Investigator will also keep and maintain the **LOCI Participant Screening/Enrolment Log** which will be entered into the trial database and kept in the Investigator Site File (ISF), and will be available to the Trials Office at all times. The **LOCI Participant Recruitment and Identification Log** and **LOCI Participant Screening/Enrolment Log** should be held in strict confidence.

6.3. Informing Other Parties

If the participant has agreed, the participant's GP should be notified that they are in LOCI trial, using the LOCI **GP Letter.**

7. TRIAL TREATMENT / INTERVENTION

7.1. Patient pathway

Randomisation into the trial will be considered the first visit (Visit 1) in the trial. Subsequent visits will depend on whether the participant has become pregnant. At this first visit, baseline medical details will be collected by staff prior to ovulation induction (OI) lasting up to six months. Participants will also be given a treatment diary to complete during the trial.

Should pregnancy be confirmed, participants will be advised to contact the trial team and arrange for Visit 2. At six months, if the participant has not contacted the research team, a member of the site team will call the participant to determine whether they have become pregnant. If a pregnancy is confirmed, Visit 2 may be booked at this point. Visit 2 is only for participants who have become pregnant. They will be asked to return any unused OI drugs and collect additional Metformin/placebo supply to last until 14 weeks gestation.

For participants who have not become pregnant by the end of the six months, the compliance assessment and end of study forms should be completed. Participants may return unused drugs at their next routine fertility clinic appointment.

Please see Figure 7 for an illustration of the participant pathway.

7.2. Intervention(s) and Schedule

Planned IMP interventions: Letrozole oral tablet 2.5-7.5mg daily or clomifene 50-150mg daily for 5 days of each menstrual cycle for up to 6 treatment cycles, with concomitant randomisation to an escalating dose of metformin to 1500mg or placebo daily. Letrozole, clomifene, metformin and placebo will be provided as over-encapsulated tablets in numbered treatment packages. The Metformin/placebo will be provided at the same time as letrozole/clomifene.

Dose: The ideal dose of letrozole and clomifene for ovulation induction is not known. The choice of 2.5mg for letrozole and allowing for 2 dose escalations up to 7.5mg was made after a) careful review of the existing literature, b) a survey of UK health professionals who use letrozole for this indication, and c) reviewing the safety profile of the drug in a previous large randomised trial involving letrozole.[14] Summary of Product Characteristics and the British National Formulary[15] suggest a starting dose of 2.5mg. The choice of clomifene 50mg with 2 dose escalations up to 150mg was based on NICE recommendations.[3] Our systematic review of literature and UK-wide survey of health professionals supported these regimens. The choice of metformin dose was based on our systematic review of literature and the UK-wide survey of health professionals. However, when clinicians feel the participant should be started on a higher dose because of previous cycle experience or preference, this will be allowed for a maximum of 3 tablets daily.

Route: All drugs are recommended for oral use.

Regimen: Letrozole and clomifene will be given for 5 days starting on day 2 or 3 of the menstrual cycle or following the start of withdrawal bleeding for up to 6 treatment cycles (Table 1). This regimen was the most commonly used in our survey of UK health professionals. The dose of metformin will be increased gradually from 500mg daily for the 1st week, 500mg twice daily for the 2nd week, and 500mg thrice daily from the 3rd week, and continued until the end of treatment or up to 14 weeks of pregnancy (Table 2). The gradual increase of metformin was suggested by our national investigator group to minimise the side-effects of metformin. Metformin will be continued up to 14 weeks of pregnancy as this was the most commonly used regimen in our national survey of UK health professionals. However, when clinicians feel the participant should be started on a higher dose because of previous experience or preference, this will be allowed for a maximum of 3 tablets daily.

	Month 1	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**
Letrozole	2.5mg (1 tablet per day)	5mg (2 tablets per day)	7.5mg (3 tablets per day)	7.5mg (3 tablets per day)	7.5mg (3 tablets per day)	7.5mg (3 tablets per day)
Clomifene	50mg (1 tablet per day)	100mg (2 tablets per day)	150mg (3 tablets per day)	150mg (3 tablets per day)	150mg (3 tablets per day)	150mg (3 tablets per day)

Table 1. Suggested dosing regimen for letrozole/clomifene. If clinically indicated, participants can be started on a higher dose from month 1, this will be permitted.

	Week 1	**Week 2**	**Weeks 3-46***
Metformin	500mg (1 tablet per day)	1000mg (2 tablets per day)	1500mg (3 tablets per day)

Table 2. Suggested dosing regimen for metformin. If clinically indicated, participants can be started on a higher dose from week 1, this will be permitted. *Maximum number of weeks if participant takes 6 months to get pregnant, then remains pregnant up to 12 weeks of gestation.

7.3. Drug Interaction or Contraindications

The following drugs and contraindications will be in place for this trial and women will be excluded if:

- Pregnant or breastfeeding women
- Liver disease or a history of liver dysfunction
- Hormone-dependent tumours
- Abnormal uterine bleeding of undetermined origin and in the presence of an ovarian cyst, except polycystic ovary, since further enlargement of the cyst may occur
- Acute conditions with the potential of altering renal function, including:
    - Dehydration
    - Severe infection

- Shock
- Intravascular administration of iodinated contrast agents
- Acute or chronic disease which may cause tissue hypoxia such as:
  - Cardiac or respiratory failure
  - Recent myocardial infarction
  - Shock
  - Hepatic insufficiency
  - Acute alcohol intoxication
  - Alcoholism
  - Lactation
  - Any type of acute metabolic acidosis (such as lactic acidosis, diabetic ketoacidosis) and severe renal failure (GFR <30 mL/min)

- Other medications known to affect reproductive function or metabolism, including:
  - Oral contraceptive pills (OCPs)
  - GnRH agonists and antagonists
  - Anti-androgens
  - Gonadotropins
  - Anti-obesity drugs
  - Somatostatin
  - Diazoxide, ACE inhibitors
  - Calcium channel blockers

We will allow a 2-month washout period for subjects who desire to participate and discontinue exclusionary medications (most commonly OCPs), and a period of observation or treatment for correctable conditions

All drugs are contraindicated for patients with hypersensitivity to letrozole, clomifene and metformin or to any of their excipients.

Concomitant therapy will be at the discretion of the care-providing clinicians, and all concomitant treatments and medications relevant to ovulation induction (e.g. inositol) will be documented in the electronic data capture system.

7.4. Treatment Modification

If any patient reports serious adverse effects from any of the trial drugs, or wishes to discontinue for any reason they will be allowed to and the reasons carefully logged. Clinicians may vary the dosage of any of the trial mediations in line with standard clinical practice. Both the dosage and the reasons for the change will be recorded.

7.5. Cessation of Treatment / Continuation after the Trial

A participant may be withdrawn from trial treatment if it becomes medically necessary in the opinion of the investigator(s) or clinician(s) providing patient care. In the event of such premature treatment cessation, LOCI study personnel will make every effort to obtain and record information about the reasons for discontinuation and any adverse events, and to follow up all safety and efficacy outcomes as appropriate.

A participant may voluntarily decide to cease taking the LOCI trial treatments at any time. If a participant does not return for a scheduled visit, attempts will be made to contact her and (where possible) to review compliance and adverse events. If a woman decides after randomisation that she does not wish to continue her pregnancy, she may withdraw herself from the trial. We will aim to document the reason(s) for self-withdrawal.

Clear distinction will be made between withdrawals from trial treatments whilst allowing further follow-up, and any participants who refuse any follow-up. If a participant explicitly withdraws consent to any further data recording then this decision will be respected and recorded on the electronic data capture system. All communications surrounding the withdrawal will be noted in the participant's records and no further data will be collected for the participant.

7.6. Treatment Supply and Storage

7.6.1. Treatment Supplies, Packaging and Labelling

Investigational medicinal products will be procured, assembled, packed and quality assured by MODEPHARMA Ltd, a global clinical services company we have used for previous clinical trials. MODEPHARMA Ltd holds a Manufacturer Licence for tablets and capsules under the Good Manufacturing Practice requirements (GMP; EU Directive 2003/94/EC) and in compliance with Good Clinical Practice (GCP; Clinical Trials Directive 2001/20/EC) Annex 13 requirements. MODEPHARMA Ltd does not hold the Marketing Authorisation for the trial IMPs.

7.6.2. Drug Storage

The study drugs will be stored and dispensed from the pharmacies of participating hospitals. All pharmacies will comply with the relevant guidelines and regulations including the Duthie report of 1988 and the Royal Pharmaceutical Society Practice Guidance on Pharmacy Services for Clinical Trials, 2005. Clinical trial medication will be dispensed against an appropriate prescription form that carries the title the LOCI Trial, the EudraCT number, investigator and sponsor name, instructions for use and a unique trial number according to GCP Annex 13 requirements. Detailed dispensing records will be kept by each dispensing pharmacy. The SmPC for clomifene and metformin state that they should be stored below 25^{o}C. Shipments from MODEPHARMA Ltd will be temperature-monitored. Participants will be advised to keep their trial treatment packs away from extremes of temperature.

7.7. Accountability and Compliance Procedures

7.7.1 Compliance

The dispensing of the LOCI trial drugs will be recorded in the pharmacy drug accountability log. The Trial Manager will periodically request the trial drug chart to verify that the dispensing system is being followed. Any deviations from the protocol schedule should be logged locally and both the PI and Trial Office informed.

Participants will be asked to return completed, partially used and unused treatment packs to the trial centres at their routine follow-up hospital appointments (e.g. fertility clinic appointments, 12 week booking scan). The research nurse at each local centre will receive the empty/partially used/unused treatment packs, and record the information for each trial participant, in the database. To monitor compliance, women who fail to return the treatment packs, whether empty or not, will be contacted by telephone or email by the research nurse for advice and support. The research nurse will ask the participant for an honest assessment of how many of the trial drugs were taken and record this information.

7.7.2. Accountability

At randomisation, the trial treatment number will be provided and this reference will correspond to a trial treatment pack available in the local hospital pharmacy. The pharmacist will receive notification of the name and trial number of the randomised woman and will prepare the trial treatment pack for dispensing. The trial treatment packs will cover six treatment cycles for clomifene or letrozole and seven calendar months for metformin or placebo. A separate pack will contain 14 weeks supply of metformin or placebo to cover the time from conception up until 14 weeks of gestation for women that conceive while on the trial.

The local pharmacist should keep accurate records of trial drugs dispensed using a pharmacy log provided by the LOCI Trial Office. Trial drugs must be kept in the packaging supplied and under no circumstances used for other participants or non-participants.

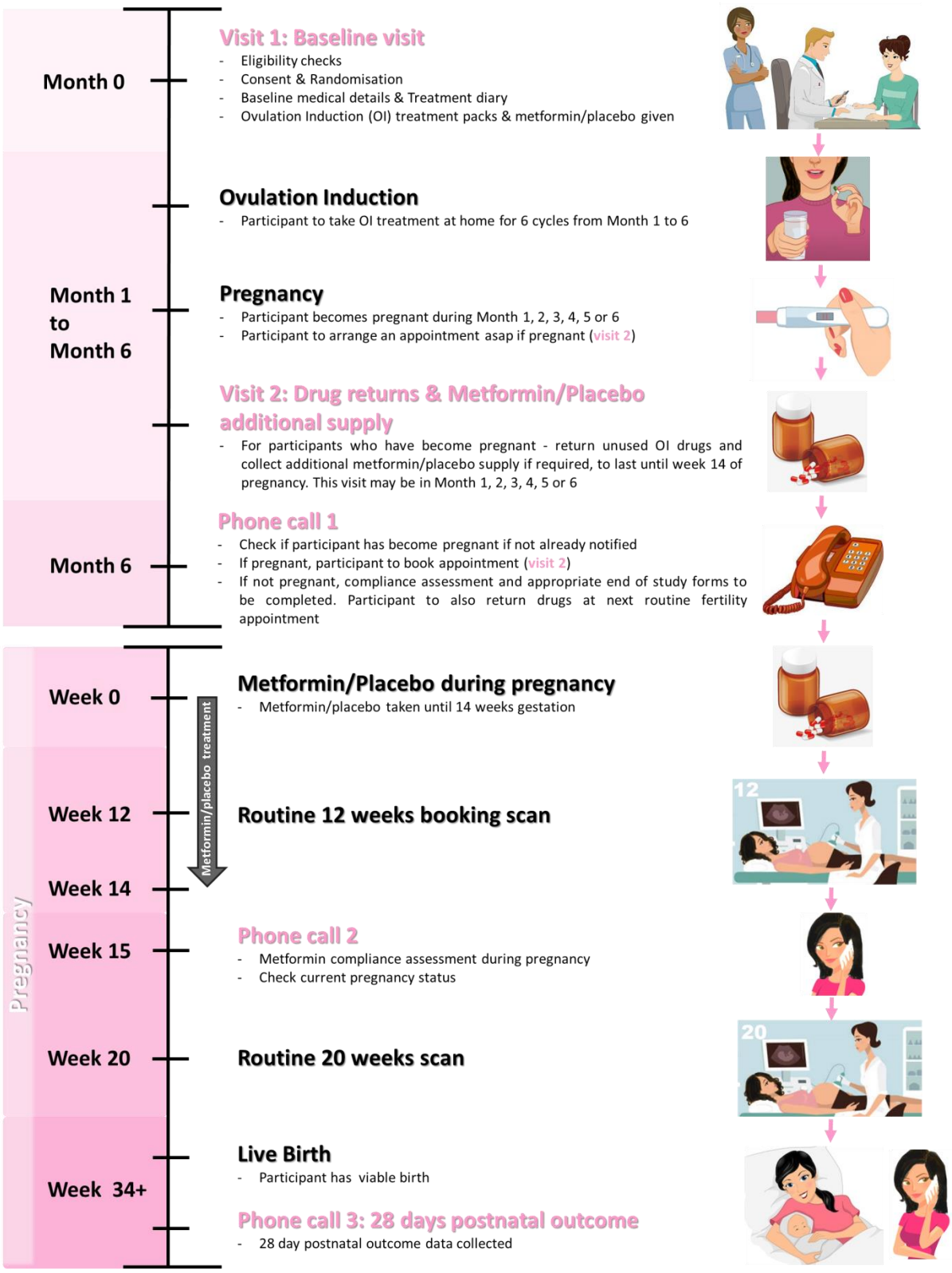


Figure 7. Patient pathway.

8. OUTCOME MEASURES AND STUDY PROCEDURES

8.1. Trial Outcomes

8.1.1. Internal pilot outcome

1. Open a minimum of 7 recruiting hospitals within six months of the first recruiting hospital being activated.
2. Recruit a minimum of 72 patients within six months of the first recruiting hospital being activated.

8.1.2. Primary Outcome

1. Live births at and beyond 34 completed weeks of gestation, as a proportion of all women randomised.

8.1.3. Secondary Outcomes

8.1.3.1. Key secondary outcomes

1. Pregnancy loss (defined as pregnancy loss before 24 weeks of gestation)
2. Number of ovulation induction cycles to live birth

8.1.3.2. Exploratory secondary outcomes

1. Treatment outcomes: Ovulation rate, time from randomisation to pregnancy, number of ovulation induction cycles required for pregnancy.
2. Pregnancy end outcomes: Ongoing pregnancy at 12 weeks (range 11 to 14 weeks) of gestation, termination, stillbirth, molar pregnancy, pregnancy of unknown location, twin live births, gestational age at live birth.
3. Where live birth ≥24 weeks: time from conception to delivery (gestational age), gestational age <28/<32/<37 weeks, singleton live births at and beyond 34 completed weeks of gestation, live births at and beyond 37 completed weeks of gestation, mode of birth (unassisted vaginal, instrumental vaginal, elective caesarean section, emergency caesarean section, vaginal breech birth, other), birth weight, APGAR score <7 out of 10 (at 1, 5 and 10 minutes).
4. Antenatal outcomes: antepartum haemorrhage, pregnancy-induced hypertension, pre-eclampsia, obstetric cholestasis, preterm (<37 weeks) pre-labour rupture of membranes, gestational diabetes (other complications will be tabulated but not formally analysed).
5. Intrapartum outcomes: chorioamnionitis, intrauterine growth restriction (IUGR), macrosomia (other complications will be tabulated but not formally analysed).
6. Post-partum outcomes: haemorrhage (other complications will be tabulated but not formally analysed).
7. Maternal outcomes: admission to high dependency unit (HDU), admission to intensive therapy unit (ITU), (other complications will be tabulated but not formally analysed).
8. Neonatal outcomes: discharge to hospital, early infection, retinopathy of prematurity, necrotising enterocolitis, intraventricular haemorrhage, respiratory distress syndrome, ventilation or oxygen support (other complications will be tabulated but not formally analysed).

9. Survival at 28 days of neonatal life.
10. Heath economic evaluation: Hospital resource use and EQ-5D-5L questionnaire.

8.1.3.3. Safety outcomes

1. Neonatal congenital or chromosomal abnormalities.
2. Maternal adverse events (tabulated but not formally analysed).
3. Multiple pregnancies.
4. Ectopic pregnancies.
5. Ovarian hyperstimulation syndrome (OHSS).
6. Serious adverse events.

8.2. Schedule of Assessments

			From randomisation							
Visit	**Screening before clinic**	**Baseline clinic**	**Month 1**	**Month 2**	**Month 3**	**Month 4**	**Month 5**	**Month 6**	**Month 4-9***	**Month 2-46****
Eligibility check	x	x								
Valid informed consent		x								
Relevant medical history taken	x	x								
Concomitant medication		x	x	x	x	x	x	x	x	x
Randomisation		x								
Routine blood tests	x	x								
D21 progesterone concentration blood test (where applicable)			x	x	x	x	x	x		
Dispensing of IMP		x								
Ultrasound monitoring of ovulation (optional, depending on local practice)			x							
Drug returns (participant visit)								x†		
Phone call to participant								x	x[#]	
EQ-5D-5L questionnaires		x							x	x
11-14 week ultrasound scan (if participant becomes pregnant)									x	
Final outcomes (conception failure; pregnancy, antenatal, intrapartum, post-partum, Day 28 neonatal and maternal outcomes)										x

Table 3. Schedule of assessments. *Exact month determined by when the participant becomes pregnant (e.g. if they become pregnant in month 1, subsequent assessments will be made in month 4). **Exact month determined by when participant becomes pregnant, and if they miscarry or have a successful live birth. †Pregnancy to be confirmed by month 6, visit 2 scheduled accordingly. [#]Second phone call to be made at week 15 of pregnancy.

Relevant trial data will be transcribed directly into the web-based database. Source data will comprise the research clinic notes, hospital notes, hand-held pregnancy notes and laboratory results.

Women will be encouraged to report pregnancies and their outcome, any adverse events and any additional visits to non-participating hospitals to the research midwife. Self-reports will be verified against clinical notes by the research team.

The trial pathway fits within the current standard care pathway for women presenting with anovulatory PCOS and infertility. Women would normally be referred to a gynaecology or fertility clinic and will be offered baseline investigations and ovulation induction treatment as part of standard care. Potential participants in the LOCI trial will be identified, approached and invited to participate in the trial by clinic doctors, research nurses and midwives in these clinics. Randomisation and prescribing of study medications will take place in the clinics. The schedule of assessments is detailed in Table 2.

8.3. Participant Withdrawal and Change of Status Within Trial

Informed consent is defined as the process of learning the key facts about a clinical trial before deciding whether or not to participate. It is a continuous and dynamic process and participants should be asked about their ongoing willingness to continue participation.

Participants should be aware at the beginning that they can freely withdraw (discontinue participation) from the trial at any time. A participant who withdraws from the trial does so completely (i.e. from trial treatment and all follow up).

A participant, who wishes to cease to participate *in a particular aspect of the trial*, will be considered as having changed their status within the trial.

The changes in status within trial are categorised in the following ways:

No trial intervention: The participant would no longer like to receive the trial intervention, but is willing to be followed up in accordance with the schedule of assessments and if applicable using any central UK NHS bodies for long-term outcomes (i.e. the participant has agreed that data can be collected and used in the trial analysis).

No trial related follow-up: The participant would no longer like to receive the trial intervention AND does not wish to attend trial visits in accordance with the schedule of assessments but is willing to be followed up at standard clinic visits and if applicable using any central UK NHS bodies for long-term outcomes (i.e. the participant has agreed that data can be collected at standard clinic visits and used in the trial analysis, including data collected as part of long-term outcomes).

No further data collection: The participant would no longer like to receive the trial intervention AND is not willing to be followed up in any way for the purposes of the trial AND does not wish for any further data to be collected (i.e. only data collected prior to the withdrawal can be used in the trial analysis). The details of either withdrawal or change of status within trial (date, reason and category of status change) should be clearly documented in the source data.

The details of either withdrawal or change of status within trial (date, reason and category of status change) should be clearly documented in the source documents. Patients subsequently found to be ineligible will still have their data analysed unless they explicitly withdraw consent.

9. ADVERSE EVENT REPORTING

9.1 Definitions

Adverse Event	AE	Any untoward medical occurrence in a participant or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.
Adverse Reaction	AR	All untoward and unintended responses to an IMP related to any dose administered.
Serious Adverse Event	SAE	Any untoward medical occurrence or effect that: • Results in death is life-threatening* • Requires hospitalisation or prolongation of existing hospitalisation • Results in persistent or significant disability or incapacity • Is a congenital anomaly/birth defect • Or is otherwise considered medically significant by the Investigator**
Serious Adverse Reaction	SAR	An Adverse Reaction which also meets the definition of a Serious Adverse Event
Unexpected Adverse Reaction	UAR	An AR, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator Brochure for an unapproved IMP or (compendium of) Summary of Product Characteristics (SPC) for a licensed product). When the outcome of an AR is not consistent with the applicable product information the AR should be considered unexpected.
Suspected Unexpected Serious Adverse Reaction	SUSAR	A SAR that is unexpected i.e. the nature, or severity of the event is not consistent with the applicable product information. A SUSAR should meet the definition of an AR, UAR and SAR.

9.2 Reporting Requirements

The collection and reporting of Adverse Events (AEs) will be in accordance with the UK Policy Framework for Health and Social Care (2017) and the requirements of the Health Research Authority (HRA). Definitions of different types of AEs are listed in the table of abbreviations and definitions. The Investigator should document all AEs experienced by the trial participant in the source data and assess the seriousness and causality (relatedness) with reference to the Reference Safety Information (RSI). The RSI is based on section 4.8 'Undesirable Effects' of the Summary of Product Characteristics (SmPC) located in the 'SmPC Reference Document'.

9.3 Adverse Events Requiring Reporting in the LOCI trial

The safety profile for this trial population and interventions are well established so although the severity and causality of all AEs should be recorded in the source data, a strategy of targeted recording of AEs will therefore not affect the safety of participants.

The recording of only the following subset of AEs via the Case Report Forms (CRFs), for the appropriate period, is consistent with aims of the trial:

- Adverse events that occur during the trial intervention treatment period (up to 14 weeks of pregnancy).

The assessment of severity of AEs and SAEs to the trial drug is a clinical decision based on all available information at the time. The following categories, as outlined in Table 3, will be used to define the severity of the AE/SAE.

Category	Definition
Grade 1	**Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.**
Grade 2	**Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.**
Grade 3	**Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care activities of daily living (ADL)**.**
Grade 4	**Life-threatening consequences; urgent intervention indicated.**
Grade 5	**Death related to AE.**

Table 4. Categorisation of severity for all events. * Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc. ** Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bed ridden.

The assessment of relationship of AEs and SAEs to the trial drug is a clinical decision based on all available information at the time.

9.4 Serious Adverse Advents (SAE) Reporting in the LOCI trial

9.4.1. Events not requiring reporting to the Sponsor/CTU on an SAE form

At whatever time they occur during an individual's participation, from randomisation to end of participant follow-up, the following are "protocol exempt" SAEs:

- Hospitalisation for other assisted conception treatment (e.g. IVF, IUI, ICSI, ovarian drilling)
- Hospitalisation for a non-ovulation induction or non-pregnancy related condition
- Hospitalisation for hyperemesis
- Hospitalisation for early pregnancy bleeding
- Hospitalisation for the management of pregnancy loss
- Hospitalisation for rest in pregnancy
- Hospitalisation for observation or monitoring of pregnancy
- Hospitalisation for maternal discomfort in pregnancy
- Hospitalisation for complications of pregnancy e.g. pre-eclampsia, urinary tract infection, pyelonephritis
- Hospitalisation for birth (including caesarean section)
- Prolonged hospitalisation for post-natal care
- Neonatal hospitalisation for sepsis
- Neonatal hospitalisation for prematurity

All events which meet the definition of serious must be recorded in the participant notes, including the causality and severity, throughout the participant's time on trial, including follow-up, but for trial purposes these events do not require reporting on the SAE Form.

9.4.2 Events that require expedited reporting to the Sponsor on the SAE Form

All SAEs (except those listed in Sections 9.4.1) from the date of commencement of protocol defined treatment until the end of participant follow-up.

Therefore, for all SAEs the Investigator will do one of the following three procedures:

1. record protocol-exempt SAEs in the medical notes but such events do not require reporting to the sponsor
2. where the SAE does not require expedited (immediate) reporting it should be reported to the trials office as soon as reasonably possible after becoming aware of the event. This includes expected SAEs as defined in section 9.4.2 above
3. where the event requires expedited reporting (immediately and within 24hrs on the Investigator becoming aware of the event) to the trials office.

Note: when an SAE occurs at the same hospital at which the participant is receiving trial treatment or is being followed up for trial purposes, processes must be in place to make the trial team at the hospital aware of any SAEs, regardless which department first becomes aware of the event, in an expedited manner.

9.5 Reporting procedure

9.5.1. Reporting procedure for Serious Adverse Events by sites

On becoming aware that a participant has experienced an SAE, the Investigator or delegate(s) should report the SAE to their own Trust in accordance with local practice and to the BCTU trials office as per section 9.3, above.

To report an SAE to the BCTU trials office (where BCTU are delegated this function), the Investigator or delegate(s) must complete, date and sign the trial specific BCTU SAE form. The completed form together with any other relevant, appropriately anonymised, data should be faxed, or scanned, to the BCTU trials team using one of the numbers listed below as soon as possible for non-expedited SAEs and no later than 24 hours after first becoming aware of the event for expedited SAE.

To report an SAE, fax or email the SAE Form to:

Fax number: 0121 415 9136

bwh-tr.locitrial@nhs.net

On receipt of an SAE form, the BCTU trials team will allocate each SAE a unique reference number and return this via fax or email to the site as proof of receipt. If the site has not received confirmation of receipt of the SAE from the BCTU or if the SAE has not been assigned a unique SAE identification number within 1 working day, the site should contact the BCTU trials office. The site and the BCTU trials team should ensure that the SAE reference number is quoted on all correspondence and follow-up reports regarding the SAE and filed with the SAE in the Site File.

Where an SAE Form has been completed by someone other than the Investigator or delegate, initially, the original SAE form will need to be countersigned by the Investigator to confirm agreement with the causality and severity assessments.

9.5.2. Provision of follow-up information

Following reporting of an SAE for a participant, the participants should be followed up until resolution or stabilisation of the event. Follow-up information should be provided using the SAE reference number provided by the BCTU trials team. Once the SAE has been resolved, all critical follow-up information has been received and the paperwork is complete, the final version of the original SAE form completed at site must be returned to the BCTU trials office and a copy kept in the Site File.

9.6 Assessment of relatedness by the PI

When completing the SAE form, the PI will be asked to define the causality (relatedness) and the severity of the AE (as defined in Table 4). In defining the causality the PI must consider if any concomitant events or medications may have contributed to the event and, where this is so, these

events or medications should be reported on the SAE form. It is not necessary to report concomitant events or medications which do not contribute to the event.

Category	Definition	Causality
Definitely	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out	Related
Probably	There is evidence to suggest a causal relationship, and the influence of other factors is unlikely	
Possibly	There is some evidence to suggest a causal relationship, however, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant events or medication)	
Unlikely	There is little evidence to suggest there is a causal relationship; there is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant events or medication)	Unrelated
Not related	There is no evidence of any causal relationship	

Table 5. Definitions of serious adverse event causality

On receipt of an SAE Form the Trials Office will forward it, with the unique reference number, to the Chief Investigator (CI) or delegate(s) who will independently review the causality of the SAE. An SAE judged by the PI or CI or delegate(s) to have a reasonable causal relationship with the intervention will be regarded as a related SAE (SAR). The causality assessment given by the PI will not be downgraded by the CI or delegate(s). If the CI or delegate(s) disagrees with the PI's causality assessment, the opinion of both parties will be documented, and where the event requires further reporting, the opinion will be provided with the report.

9.7 Assessment of Expectedness by the CI

The CI or delegate(s) will also assess all related SAEs for expectedness with reference to the following criteria.

Category	Definition
Expected	An adverse event that is consistent with known information about the trial related procedures or that is clearly defined in the relevant safety information.
Unexpected	An adverse event that is not consistent with known information about the trial related procedures.

Table 6. Definitions of serious adverse event expectedness

The CI will undertake review of all SAEs and may request further information from the clinical team at site should be made available immediately upon request. The CI will not overrule the severity or causality assessment given by the site Investigator but may add additional comment on these. If the event is unexpected (i.e. is not defined in the approved version of the RSI, it will be classified as a Suspected Unexpected Serious Adverse Reaction (SUSAR).

9.8 Reporting SAEs to third parties

The independent Data Monitoring Committee (DMC) may review any SAEs at their meetings.

BCTU will report details of all SARs (including SUSARs) to the MHRA main REC and RGT (or external sponsor) annually from the date of the Clinical Trial Authorisation, in the form of a Development Safety Update Report (DSUR). Additionally, BCTU will report a minimal data set of all individual events categorised as a fatal or life threatening SUSAR to the Medicines and Healthcare products Regulatory Agency (MHRA), main REC and Research Governance Team (RGT) (or external sponsor) within 7 days. Detailed follow-up information will be provided within an additional 8 days.

All other events categorised as non-life threatening SUSARs will be reported within 15 days.

The main REC and RGT will be notified immediately if a significant safety issue is identified during the course of the trial.

Details of all SUSARs and any other safety issue which arises during the course of the trial will be reported to PIs. A copy of any such correspondence should be filed in the site file and TMF.

9.9 Urgent Safety Measures

If any urgent safety measures are taken, the BCTU shall immediately, and in any event no later than 3 days from the date the measures are taken, give written notice to the REC (and MHRA in the case of CTIMPs) of the measures taken and the circumstances giving rise to those measures.

9.10 Monitoring pregnancies for potential Serious Adverse Events

Since live birth is the primary outcome in the trial, congenital anomalies or birth defects will be routinely monitored and SAE data on congenital anomalies or birth defects will be collected.

10. DATA HANDLING AND RECORD KEEPING

10.1. Source Data

Source data is defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. In order to allow for the accurate reconstruction of the trial and clinical management of the subject, source data will be accessible and maintained. Illustrative examples of source data are provided in the Table 6 below:

Data	Source
Participant Reported Outcomes	The original participant-completed CRF is the source and will be kept with the participant's trial record at site, whilst copies will be provided to the Trials Office

Lab results	The original lab report (which may be electronic) is the source data and will be kept and maintained, in line with normal local practice. Information will be transcribed onto CRFs
Imaging	The source is the original imaging usually as an electronic file. Data may be supplied to the Trials Office as a password-protected, anonymised, copy of the electronic file, or as an interpretation of the imaging provided on a CRF. This will be transferred via fax or secure email, and stored on a secure computer server at the University of Birmingham. Where data is interpreted, the CRF onto which it is transcribed becomes the source. Copy of the CRF should be provided to the Trials Office.
Clinical event data	The original clinical annotation is the source data. This may be found on clinical correspondence, or electronic or paper participant records. Clinical events reported by the participant, either in or out of clinic (e.g. phone calls), must be documented in the source data.
Health Economics data	Often obtained by interview directly with the participant for transcription onto the CRF.
Recruitment	The original record of the randomisation is the source. It is held on MedSciNet servers as part of the randomisation and data entry system.
Drop out	Where a participant expresses a wish to withdraw, the conversation must be recorded in the source data.

Table 7. Source data definitions and examples

10.2. Case Report Form (CRF) Completion

A CRF is required and should be completed for each individual participant. For the LOCI trial this will be in the form of an eCRF. The data held on the completed original eCRFs are the sole property of the respective PIs whilst the data set as a whole is the property of the Sponsor and should not be made available in any form to third parties except for authorised representatives or appropriate regulatory authorities without written permission from the sponsor. Appropriate data sharing requests will be considered by the trial management group and the BCTU data sharing committee.

It will be the responsibility of the investigator to ensure the accuracy of all data entered in the eCRFs and confirm accordingly. The **LOCI Site Signature & Delegation Log** will identify all those personnel with responsibilities for data collection.

Data reported on each form will be consistent with the source data and any discrepancies will be explained. All missing and ambiguous data will be queried. Staff delegated to complete eCRFs will be trained to adhere to online completion of the eCRFs in the trial database from source data. Online data entry is achieved via unique passwords and usernames which must not be shared amongst the team. All time formats, where applicable, should be in accordance with the 24 hour clock. Rounding of numbers, where applicable, should be in the normal way (i.e. ≥*x*.5 is rounded up to the nearest whole number). Laboratory test data that is used to inform clinical decisions should always be supplied. If a test is repeated it is either to confirm or clarify a previous reading. Confirmatory tests should use the original test values. Protocol and GCP non-compliances should be added to a Protocol Deviation Log, held by the site, and reported to the Trials Office on discovery.

10.3. Participant completed Questionnaires

EQ-5D-5L questionnaires can be completed in clinic or by telephone. The questionnaire will be completed by the participant, and overseen by a member of the research team. At the time of completion, the member of the research team will check to make sure the questionnaire has been fully completed by the patient.

10.4. Data Management

Processes will be employed to facilitate the accuracy of the data included in the final report. These processes will be detailed in the trial specific Data Management Plan. Coding and validation will be agreed between the trial team and the trial database and will be signed off once the implementation of these has been assured. Data entry will be completed by site staff from source via the MedSciNet system. The data capture system will conduct automatic range checks for specific data values to ensure high levels of data quality. Queries on the trial data will be raised using the integrated data query system in the trial database, with the expectation that these queries will be completed by the site within 30 days of receipt. Overdue data queries will be requested on a monthly basis.

10.5. Data Security

The security of the System is governed by the policies of MedSciNet. MedSciNet's Data Protection Policy and the Conditions of Use of Computing and Network Facilities set out the security arrangements under which sensitive data should be processed and stored. All studies at the University of Birmingham have to be registered with the Data Protection Officer and data held in accordance with the General Data Protection Regulation (GDPR) 2018. The University will designate a Data Protection Officer upon registration of the study. The Study Centre has arrangements in place for the secure storage and processing of the study data which comply with the University of Birmingham policies.

The System incorporates the following security countermeasures:

Physical security measures: restricted access to the building, supervised onsite repairs and storages of back-up tapes/disks are stored in a fire-proof safe.

Logical measures for access control and privilege management: including restricted accessibility, access controlled servers, separate controls used non-identifiable data etc.

Network security measures: including site firewalls, antivirus software, separate secure network protected hosting etc.

System Management: the System shall be developed by the MedSciNet and will be implemented and maintained by MedSciNet.

System Design: the system shall comprise of a database and a data entry application with firewalls, restricted access, encryption and role based security controls.

Operational Processes: the data will be processed and stored by MedSciNet, then securely transferred to the Study Centre (University of Birmingham).

Data processing: Statisticians will have access to anonymised data.

System Audit: The System shall benefit from the following internal/external audit arrangements:

- Internal audit of the system
- Periodic IT risk assessments

Data Protection Registration: The University of Birmingham has Data Protection Registration to cover the purposes of analysis and for the classes of data requested. The University's Data Protection Registration number is Z6195856.

10.6. Archiving

It is the responsibility of the PI to ensure all essential trial documentation and source documents (e.g. signed ICFs, Investigator Site Files, Pharmacy Files, participants' hospital notes, copies of CRFs etc.) at their site are securely retained for at least 25 years. MedSciNet will ensure all data is securely retained for at least 25 years.

11. QUALITY CONTROL AND QUALITY ASSURANCE

11.1. Site Set-up and Initiation

All PIs will be asked to sign the necessary agreements including a Site Signature & Delegation Log between the PI and the CTU, and supply a current CV and GCP certificate to BCTU. All site staff who are performing trial specific tasks are required to sign the Site Signature and Delegation Log, which details which tasks have been delegated to them by the PI.

Prior to commencing recruitment, each recruiting site will undergo a process of initiation, either a meeting or a teleconference, at which key members of the site research team are required to attend, covering aspects of the trial design, protocol procedures, adverse event reporting, collection and reporting of data and record keeping. Sites will be provided with an Investigator Site File and a Pharmacy File containing essential documentation, instructions, and other documentation required for the conduct of the trial. The BCTU trials team must be informed immediately of any change in the site research team.

11.2. Monitoring

The monitoring requirements for this trial have been developed following trial specific risk assessment by BCTU and as documented in the monitoring plan.

11.3. Onsite Monitoring

For this trial we will monitor sites in accordance with the trial risk assessment and monitoring plan. Any monitoring activities will be reported to the trials team and any issues noted will be followed up to resolution. Additional on-site monitoring visits may be triggered, for example by poor CRF return, poor data quality, low SAE reporting rates, excessive number of participant withdrawals or deviations (also defined in the monitoring plan). Investigators will allow the LOCI trial staff access to source documents as requested. The monitoring will be conducted by BCTU.

11.4. Central Monitoring

Trials staff will check incoming ICFs and CRFs for compliance with the protocol, data consistency, missing data and timing at a frequency and intensity determined by the Data Management Plan. Sites will be sent DCFs requesting missing data or clarification of inconsistencies or discrepancies.

11.5. Audit and Inspection

The Investigator will permit trial-related monitoring, audits, ethical review, and regulatory inspection(s) at their site, providing direct access to source data/documents. The investigator will comply with these visits and any required follow up. Sites are also requested to notify BCTU of any relevant inspections.

11.6. Notification of Serious Breaches

Sites may be suspended from further recruitment in the event of serious and persistent non-compliance with the protocol and/or GCP, and/or poor recruitment. Any major problems identified may be reported to the Trial Management Group, Trial Steering Committee, and the REC. This includes reporting serious breaches of GCP and/or the trial protocol to the REC and MHRA.

In accordance with Regulation 29A of the Medicines for Human Use (Clinical Trials) Regulations 2004 and its amendments, the Sponsor of the trial is responsible for notifying the licensing authority in writing of any serious breach of the conditions and principles of GCP in connection with that trial or the protocol relating to that trial, within 7 days of becoming aware of that breach.

For the purposes of this regulation, a "serious breach" is a breach which is likely to affect;

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial

Sites are therefore requested to notify the Trials office of any suspected trial-related serious breach of GCP and/or the trial protocol. Where the Trials Office is investigating whether or not a serious breach has occurred sites are also requested to cooperate with the Trials Office in providing sufficient information to report the breach to the MHRA where required and in undertaking any corrective and/or preventive action.

12. END OF TRIAL DEFINITION

The end of trial is defined as the date of last data capture. This will allow sufficient time for the completion of protocol procedures, data collection and data input. The BCTU trial team will notify the main REC, MHRA and RGT within 90 days of the end of trial. Where the trial has terminated early, the Trials Office will inform the MHRA and REC within 15 days of the end of trial. The Trials Office will provide them with a summary of the clinical trial report within 12 months of the end of trial. A copy of the end of trial notification as well as the summary report will be sent to MHRA and REC.

13. STATISTICAL CONSIDERATIONS

13.1. Sample Size

Just under two thousand participants (n=1,992) will provide greater than 99% power (at p=0.05) to detect differences in the letrozole vs clomifene comparison (assuming rates of 39% vs 29% respectively for rate of live birth ≥34 weeks) and the same power to detect differences in the metformin vs placebo comparison (also assuming rates of 39% vs 29%, respectively). Adjusting for a worst case scenario of 5% attrition, the total number required will be 2,100 participants. The high rate of power (>99%) has been chosen to ensure we will have 90% power (p=0.05 and 1050 participants) to answer the further question of letrozole plus metformin vs clomifene plus metformin (the latter comparison assuming rates of 44% and 34%, respectively). The basis for the proportions used in these calculations is provided in the next paragraph.

The live birth rates used in the sample size calculations was taken from our systematic review (Table 8). We used the estimate in the clomifene alone group (24%) as the base estimate for these calculations as this was by far the largest group involving 36 trial groups and 2299 women – it was also the most conservative estimate (lowest rate), which was important as information was only available on the rate of pregnancy and not live birth rate. A 10% absolute increase was identified in our clinician survey as minimally important so we chose this as the difference we wanted to detect when comparing clomiphene with letrozole or with the addition of metformin (i.e. both increased to 34%). We assumed using both letrozole and metformin would have an additive effect, i.e. increased to 44%. Overall, this amounted to an assumption of 29% versus 39% in the two main comparisons (letrozole vs clomifene and metformin vs placebo) when we take into account the factorial design (i.e. the overall rates at the margins). No interaction of the effect is assumed in these calculations as the biological mechanism of these agents is considered sufficiently different and – to our knowledge – is unlikely to have a pronounced effect on outcome.

Table 8: Meta-analysis of clinical pregnancy rates in the four arms of the proposed 2x2 factorial design trial.

Drug	Letrozole	Clomifene
Metformin	**49%** (95% CI 39% to 63%, 1 trial arm, 67 women)	**39%** (95% CI 36% to 42%, 9 trial arms, 635 women)
No metformin	**35%** (95% CI 33% to 38%, 8 trial arms, 832 women)	**24%** (95% CI 23% to 25%, 36 trial arms, 2,299 women)

13.2. Analysis of Outcome Measures

A separate Statistical Analysis Plan will be produced and will provide a more comprehensive description of the planned statistical analyses. A brief outline of these analyses is given below.

The primary comparisons will be composed of (i) those randomised to letrozole versus those randomised to clomifene; (ii) those randomised to metformin versus those randomised to placebo and (iii) randomised to letrozole plus metformin versus those randomised to clomifene plus metformin. The three comparisons are considered distinct questions and will be reported separately; hence no adjustment for multiple testing across comparisons is proposed.

In the first instance, for all three comparisons, all analyses will be based on the intention to treat principle, i.e. all participants will be analysed in the treatment group to which they were randomised irrespective of compliance or other protocol deviation. For all outcome measures, appropriate summary statistics will be presented by group (e.g. proportions/percentages, mean/standard deviation or median/interquartile range). All outcomes will be presented with point estimates (e.g. relative risks, incident rate ratios, hazard ratios, mean differences) and 95% confidence intervals. Treatment effects will be adjusted for the minimisation variables listed in section 6.2 as well as both group allocations (from the factorial randomisation) by including them in the regression models (see section 13.2.2).

For the primary outcome and the key secondary outcomes, we will incorporate a hierarchical approach to statistical testing (via analysis methods described below).[22] That is, if the primary outcome is statistically significant ($p<0.05$) we will proceed to test the key secondary outcomes

(pregnancy loss) without any adjustment for the overall rate of type I error (i.e. the test will be conditional and only performed if there is rejection of the primary outcome null hypothesis). Safety outcomes will be subject to statistical testing without adjustment for multiple testing as adjustment for multiplicity is counterproductive for considerations of safety.[16] Exploratory secondary outcome will not be subject to statistical testing and will be presented for supporting evidence only (significance will not be inferred from the confidence interval width).

13.2.1. Primary Outcome Measure

The primary outcome is the proportion of women randomised who experience a live birth at and beyond 34 weeks of gestation. The denominator of this proportion will be all women randomised and the numerator will be those women who have conceived during the six ovulation induction cycles (maximum 240 days) and have gone on to have a live birth at or beyond 34 weeks. A log-binomial regression model will be used to calculate the adjusted relative risk and 95% confidence interval. Statistical significance of the treatment group parameter will be determined from the p-value generated by the model

13.2.2. Secondary and Safety Outcome Measures

For dichotomous outcome (e.g. pregnancy loss), relative risks and 95% confidence intervals will be generated in the same fashion as the primary outcome. Poisson regression will be used for count data (number of ovulation induction cycles to live birth), linear regression for continuous data (e.g. birth weight) and a Cox Proportional Hazard (PH) model (provided the assumptions of proportionality are met) for time to event data (time from randomisation to pregnancy). For the key secondary outcomes (if tested, see above) and safety outcomes the p-values from the treatment group parameters in the models will be used to determine statistical significance.

For the secondary pregnancy outcomes (such as ongoing pregnancy) the analysis population will be women who went on to achieve confirmed pregnancy. For neonatal outcomes and complication rates, twin babies – in the first instance – will both be counted in the analysis population. The effect of this will be explored through sensitivity analysis. In the event of twin babies having different pregnancy outcomes, for example one live birth and one pregnancy loss, both the events will be counted in the separate categories, i.e. they will contribute to both a live birth event and a pregnancy loss event.

13.2.3. Subgroup Analyses

Subgroup analyses will be restricted to the primary outcome only. BMI (<30 and ≥30) is the single key subgroup of interest (i.e. we propose to be able to draw firm conclusion about any differential effect with respect to this variable only). Other subgroup analyses will be considered exploratory. These will be limited to the same variables used in the minimisation algorithm (apart from centre; see section 6.2) and androgen excess confirmed clinically or through laboratory testing. Tests for interaction will be performed by including the treatment group by subgroup interaction parameter in the statistical model. A p-value for this parameter will be produced. Given there is only one key subgroup of interest, no adjustments will be made for multiple testing on this p-value.

13.2.4. Missing Data and Sensitivity Analyses

Every attempt will be made to collect full follow-up data on all study participants; it is thus anticipated that missing data will be minimal. Participants with missing primary outcome data will not be included in the primary analysis in the first instance. This presents a risk of bias, and sensitivity analyses will be undertaken to assess the possible impact of the risk. In brief, this will include a multiple imputation (MI) approach. Full details will be included in the Statistical Analysis Plan.

13.3. Planned Interim Analysis

Interim analyses of safety and efficacy for presentation to the independent DMC will take place during the study. The committee will meet prior to study commencement to agree the manner and timing of such analyses but this is likely to include the analysis of the primary and major secondary outcomes and full assessment of safety (SAEs) at least at annual intervals. Criteria for stopping or modifying the study based on this information will be ratified by the DMC. Details of the agreed plan will be written into the Statistical Analysis Plan. Further details of DMC arrangements are given in section 14.5.

13.4. Planned Final Analyses

The primary analysis for the study will occur once all participants have completed all assessments and corresponding outcome data has been entered onto the study database and validated as being ready for analysis.

13.5. Health Economic Evaluation

If letrozole with or without metformin is superior to clomifene and results in more live births ≥ 34 weeks of gestation, significant economic implications may be seen for the health sector. For example, letrozole with or without metformin may result in fewer multiple pregnancies and pregnancy complications as well as fewer neonatal complications compared with clomifene alone. However, the additional cost of changing to letrozole with or without metformin would need to be justified and shown to provide good value for the public health care resources, which could be more effectively spent elsewhere in the health system. An economic evaluation is, therefore, required to assess the cost-effectiveness of letrozole and the added value of metformin in the management of ovulation induction for women with PCOS and infertility.

Resource use data will be collected prospectively from the NHS and Personal Social Service (PSS) perspective, through case report forms in order to estimate the overall cost of drug administration, management of subfertile PCOS women, and follow-up care up to the primary endpoint of 6 menstrual cycles or 28 days post birth. We will also explore the private costs incurred as a result of the intervention by collecting data through self-report questionnaires.

The main resource categories related to ovulation induction that will be monitored include:

1. Drug administration

2. Resource use associated with adverse events and complications, such as multiple pregnancy, preterm birth and neonatal complications

3. Resource use associated with outpatient or emergency visits and hospital admissions until final discharge, for example if a pregnancy or neonatal complication occurs

4. Contacts with community and social care services, such as GP, practice nurse, and fertility specialists

5. Time-off work and other private expenses

In order to value health care resource use to estimate the overall cost of each trial-arm, unit costs will be applied to each resource item. Information on unit costs will be obtained from key UK national sources, such as the NHS reference costs, the Unit Costs of Health and Social Care,[17] the British National Formulary, and the Office for National Statistics.

Given the potential impact of subfertility on physical and, particularly, psychological health, health-related quality of life data will be obtained based on participants responses to the EQ-5D-5L at baseline and at each clinical review.[18] A preference-based index of health-related quality of life will be derived using the recently published English value set, and Quality-Adjusted Life-Years (QALYs) will be calculated using the area under the curve approach.

Economic analysis: A trial-based economic evaluation will explore the cost-effectiveness of letrozole versus clomifene and the value of adding metformin for ovulation induction in women with subfertility and PCOS. The principal outcome for the economic evaluation will be the achievement of a live birth ≥ 34 weeks or more. A secondary analysis reporting results in terms of cost per QALY will also be carried out. The health economic analysis will perform an independent analysis which is likely to be different to the analysis of the clinical endpoints. The most appropriate approach for the economic analysis of factorial trials is the within-the-table analysis[19] Other approaches to the analysis will be explored in sensitivity analysis. The advantage of factorial trials is that they can provide increased statistical power between treatments, but they can raise challenges for economic evaluations where interactions are likely. Interactions may arise due to non-compliance, or pharmacokinetic, biological or behavioural mechanisms. Such factors can have a multiplicative effect, where the effect of say, letrozole and metformin in combination is equal to the product (not the sum) of the individual effects of letrozole and metformin, thus there will be an interaction on a natural scale that risks being misinterpreted if the analysis adopted the standard logistical regression approach to multiplicative effects. The within-the-table analysis assumes that the interventions are mutually exclusive i.e. the costs and effects of inducing ovulation with letrozole will be influenced by the inclusion of metformin and vice versa, and therefore each trial arms are treated separately with this approach. The most cost-effective option will be determined using principles of dominance and extended dominance. The analysis will follow the recommendations of Dakin and Gray for the analysis of factorial trials.[19]

The distribution of costs and outcomes and missing data, censoring and correlations between costs and outcomes will be explored. Multiple imputation will be used for missing data which will include

dummy variables for each factor and a full set of interaction terms as predictors of missing data. Suitable regression approaches will be used for adjustment for baseline imbalances.

The results of these economic analyses will be presented using cost-effectiveness acceptability frontiers to reflect decision uncertainty across different thresholds of willingness-to-pay per additional unit of outcome. Deterministic and probabilistic sensitivity analyses will be undertaken to explore the robustness of the findings to plausible variations in key assumptions and analytical methods used, and to consider the broader issue of generalisability of the study's results. Conclusions will be based on an incremental comparison between mutually exclusive treatment combinations that considers the factors as interacting treatments regardless of whether interactions are included in the analysis that estimate the mean costs and mean QALYs for each arm.

14. TRIAL ORGANISATIONAL STRUCTURE

14.1. Sponsor

The sponsor for this trial is the University of Birmingham.

14.2. Coordinating Centre

The trial coordinating centre (Trial Office) is Birmingham Clinical Trials Unit, based at the University of Birmingham.

14.3. Trial Management Group

The Trial Management Group will take responsibility for the day-to-day management of the trial, and will include the CI, statistician and trial manager. The role of the group is to monitor all aspects of the conduct and progress of the trial, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the trial itself.

14.4. Trial Steering Committee

A single TSC will be created for the LOCI trial and meet via teleconference as required depending on the needs of the trial office.

Membership and duties/responsibilities are outlined in the TSC Charter. In summary, the TSC will: provide overall oversight of the trial, including the practical aspects of the study, as well as ensuring that the study is run in a way which is both safe for the participants and provides appropriate feasibility data to the sponsor and investigators.

14.5. Data Monitoring and Ethics Committee

Data analyses will be supplied in confidence to an independent Data Monitoring and Ethics Committee (DMEC), which will be asked to give advice on whether the accumulated data from the trial, together with the results from other relevant research, justifies the continuing recruitment of further participants. The DMEC will operate in accordance with a trial specific charter. The DMEC will meet at least annually as agreed by the Committee and documented in the Charter. More frequent meetings may be required for a specific reason (e.g. safety) and will be recorded in minutes.

Additional meetings may be called if recruitment is much faster than anticipated and the DMEC may, at their discretion, request to meet more frequently or continue to meet following completion of recruitment. An emergency meeting may also be convened if a safety issue is identified. The DMEC may consider recommending the discontinuation of the trial if the recruitment rate or data quality are unacceptable or if any issues are identified which may compromise participant safety. The trial will stop early if the interim analyses showed differences between treatments that were deemed to be convincing to the clinical community.

14.6. Finance

The research costs of the trial are funded by a National Institute for Health Research (NIHR) Health Technology Assessment (HTA), reference 17/116/01, awarded to Prof Arri Coomarasamy at the University of Birmingham. The trial has been designed to minimise extra 'service support' costs for participating hospitals as far as possible. Additional costs, service support costs and excess treatment costs associated with the trial, e.g. gaining consent, are estimated in the Statement of Activities. These costs should be met by accessing the Trust's Support for Science budget via the Local Comprehensive Research Network.

15. ETHICAL CONSIDERATIONS

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects, adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964, amended at the 48th World Medical Association General Assembly, Somerset West, Republic of South Africa, October 1996 (website: http://www.wma.net/en/30publications/10policies/b3/index.html).

The trial will be conducted in accordance with the UK Policy Framework for Health and Social Care Research 2017, the applicable UK Statutory Instruments, (which include the Medicines for Human Use Clinical Trials 2004 and subsequent amendments and the General Data Protection Regulation (GDPR) 2018, and the EU Clinical Trials directive.This trial will be carried out under a Clinical Trial Authorisation in accordance with the Medicines for Human Use Clinical Trials regulations. The protocol will be submitted to and approved by the main REC prior to circulation and the start of the trial. All correspondence with the MHRA and/or REC will be retained in the Trial Master File/Investigator Site File, and an annual progress report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given by the REC, and annually until the trial is declared ended.

Before any participants are enrolled into the trial, the PI at each site is required to obtain local R&D approval/assurance. Sites will not be permitted to enrol participants until written confirmation of R&D approval/assurance is received by the BCTU trials team.

It is the responsibility of the PI to ensure that all subsequent amendments gain the necessary local approval. This does not affect the individual clinicians' responsibility to take immediate action if thought necessary to protect the health and interest of individual participants.

16. CONFIDENTIALITY AND DATA PROTECTION

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the General Data Protection Regulation, 2018.

Participants will always be identified using their unique trial identification number and initials on the Case Report Form and any correspondence between members of the BCTU and trial team. Participants will give their explicit consent for the movement of their consent form, giving permission for BCTU to be sent a copy. This will be used to perform in-house monitoring of the consent process.

The Investigator must maintain documents not for submission to BCTU (e.g. Participant Identification Logs) in strict confidence. In the case of specific issues and/or queries from the regulatory authorities, it will be necessary to have access to the complete trial records, provided that participant confidentiality is protected.

MedSciNet will maintain the confidentiality of all participant's data and will not disclose information by which participants may be identified to any third party other than those directly involved in the treatment of the participant and organisations for which the participant has given explicit consent for data transfer (e.g. sponsor). Representatives of the LOCI trial team and sponsor may be required to have access to participant's notes for quality assurance purposes but participants should be reassured that their confidentiality will be respected at all times.

17. FINANCIAL AND OTHER COMPETING INTERESTS

There are no financial or other competing interests associated with this trial protocol.

18. INSURANCE AND INDEMNITY

The University of Birmingham has in place Clinical Trials indemnity coverage for this trial which provides cover to the University for harm which comes about through the University's, or its staff's, negligence in relation to the design or management of the trial and may alternatively, and at the University's discretion provide cover for non-negligent harm to participants.

With respect to the conduct of the trial at Site and other clinical care of the participant, responsibility for the care of the participants remains with the NHS organisation responsible for the Clinical Site and is therefore indemnified through the NHS Litigation Authority.

The University of Birmingham is independent of any pharmaceutical company, and as such it is not covered by the Association of the British Pharmaceutical Industry (ABPI) guidelines for participant compensation.

19. AMENDMENTS

The decision to amend the protocol and associated trial documentation will be initiated by the TMG. As sponsor, The University of Birmingham will be responsible for deciding whether an amendment is substantial or non-substantial. Substantive changes will be submitted to REC and HRA for approval. Once this has been received, R&D departments will be notified of the amendment, and requested to provide their approval. If no response is received within 35 days, an assumption will be made that the site has no objection to the amendment and it will be implemented at the site. All amendments will be tracked in the 'Protocol Amendments' section of the protocol.

20. POST-TRIAL CARE

All patients will continue to receive standard medical care following participation in the clinical trial. There are no interventions that participant's will be prevented from accessing after their participation in the trial has been completed.

21. PUBLICATION POLICY

Results of this trial will be submitted for publication in a peer reviewed journal. The manuscript will be prepared by the CI and authorship will be determined by the trial publication policy.

Any secondary publications and presentations prepared by Investigators must be reviewed and approved by the TMG. Manuscripts must be submitted to the TMG in a timely fashion and in advance of being submitted for publication, to allow time for review and resolution of any outstanding issues. Authors must acknowledge that the trial was performed with the support of the University of Birmingham. Intellectual property rights will be addressed in the Clinical Study Site Agreement between Sponsor and site.

22. ACCESS TO FINAL DATA SET

Only the trial steering group will have access to the full trial dataset in order to ensure that the overall results are not disclosed by an individual trial site prior to the main publication. Following publication of the findings, the final trial dataset will be made available to external researchers upon approval from the trial management group and the BCTU data sharing committee in line with standard data sharing practices for clinical trial data sets.

22.1. Data sharing

Data collected from this study may be used for future PCOS-related studies, if consented for by the participant. Permission will also be sought, via written consent, to contact the participants at a later date to collect data on the babies born in the trial. This is included in the ICF.

23. REFERENCE LIST

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