Imperial College London

The use of the hormone kisspeptin in *in vitro* fertilisation (IVF) treatment

Version 9 1st February 2015

MAIN SPONSOR: Imperial College London
FUNDERS: Medical Research Council (MRC)
STUDY COORDINATION CENTRE: Imperial College London
Protocol Code Number ICL/KISS/12/01
EudraCT number 2012-000154-61
Research Ethics Committee (REC) reference: 10/H0707/2

NCT No. **NCT01667406**

Protocol authorised by:

Name & Role	Date	Signature
Prof. Waljit Dhillo Professor in Endocrinology and Metabolism Imperial College London	7th 1st February 2015	

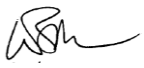
Imperial College London

The use of the hormone kisspeptin in *in vitro* fertilisation (IVF) treatment

Version 9 1st February 2015

MAIN SPONSOR: Imperial College London
FUNDERS: Medical Research Council (MRC)
STUDY COORDINATION CENTRE: Imperial College London
Protocol Code Number ICL/KISS/12/01
EudraCT number 2012-000154-61
Research Ethics Committee (REC) reference: 10/H0707/2

Protocol authorised by:

Name & Role	Date	Signature
Prof. Waljit Dhillo Professor in Endocrinology and Metabolism Imperial College London	7th 1st February 2015	

Study Management Group

Chief Investigator: Professor Stephen R. Bloom (Professor of Medicine and Head of Department of Investigative Medicine, Imperial College London)

Co-investigators: Professor Waljit Dhillo (Reader in Endocrinology and Metabolism, Department of Investigative Medicine, Imperial College London). Dr Geoff Trew (Director of the IVF Unit at Hammersmith Hospital).

Statistician: Professor Ashby (Professor of Medical Statistics and Clinical Trials, Clinical Trials Unit, Imperial College London)

Trial Management: Clinical Trials Unit, Imperial College London

Study Coordination Centre

For general queries, supply of trial documentation, and collection of data, please contact:

Study Coordinator: Professor. Waljit Dhillo
Address: Department of Investigative Medicine, Hammersmith Hospital, Du Cane Road, London W12 ONN
Tel: 020 8383 2820
Fax: 0208 383 8320
E-mail: w.dhillo@imperial.ac.uk
Web address: http://www1.imperial.ac.uk/medicine/people/w.dhillo/
Randomisations: no randomisations in this study

Clinical Queries

Clinical queries should be directed to Professor. Waljit Dhillo who will direct the query to the appropriate person

Sponsor

Imperial College is the main research sponsor for this study. For further information regarding the sponsorship conditions, please contact the Research Governance Manager:

Becky Ward
Research Governance Manager
Joint Research Compliance Office
Imperial College London and Imperial College Healthcare NHS Trust
Room 5L10A
5th Floor, Lab Block
Charing Cross Hospital
Fulham Palace Road
London, W6 8RF
0203 311 0205
E-mail: becky.ward@imperial.ac.uk
www.imperial.ac.uk/clinicalgoveranceoffice

Funder

MRC / National Institute of Health Research (NIHR)

This protocol describes a study to investigate the use of the hormone kisspeptin in IVF treatment and provides information about procedures for entering participants. The protocol should not be used as a guide for the treatment of other participants; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study.

Problems relating to this trial should be referred, in the first instance, to the study coordinator Dr. Waljit Dhillo.

This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 Statutory Instrument 2004/1031 (SI 2004/1031) and the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

Table of Contents

GLOSSARY OF ABBREVIATIONS

AE	Adverse event
AMH	Anti-Mullerian hormone
AR	Adverse reaction
ARDS	Acute respiratory distress syndrome
CRF	Case Report Form
CTA	Clinical Trials Authorisation
DMEC	Data Monitoring & Ethics Committee
FSH	Follicle stimulating hormone
GCP	Good Clinical Practice
GnRH	Gonadotrophin releasing hormone
GPR54	G-Protein Coupled Receptor 54
h	Hour
hCG	Human chorionic gonadotrophin
HPG	Hypothalamic-pituitary gonadal
ICH GCP	International Conference on Harmonisation Good Clinical Practice

ICSI	Intracytoplasmic sperm injection
ICV	Intracerebroventricular
IMP	Investigational Medicinal Product
IU/L	International Unit/Litre
IVF	In vitro fertilisation
LH	Luteinising hormone
MHRA	Medicines and Healthcare Regulatory Agency
MRC	Medical Research Council
nmol/kg	Nanomole/kilogram
NICE	National Institute for Health and Clinical Excellence
NIHR	National Institute of Health Research
OHSS	Ovarian hyperstimulation syndrome
RCOG	Royal College of Obstetricians and Gynaecologists
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SD	Standard deviation
SI 2004/1031	Statutory Instrument 2004/1031
SmPC	Summary of product characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group

KEYWORDS
Infertility, hormone, kisspeptin

STUDY SUMMARY

TITLE The use of the hormone kisspeptin in IVF treatment

DESIGN Open label, dose escalation, phase 2 clinical trial investigating the administration of Kisspeptin to induce oocyte maturation in 102 women undergoing IVF treatment

AIMS To investigate whether kisspeptin can stimulate oocyte maturation in IVF therapy for infertility

OUTCOME MEASURES Primary outcome measure: Oocyte maturation

POPULATION Study participants will be women needing IVF treatment attending the IVF unit at Imperial College Healthcare National Health Service Trust.

ELIGIBILITY Eligibility for the study will be determined at screening which will comprise a medical history, routine physical examination and basic investigations (including full blood count, urea and electrolytes, liver function tests, thyroid function tests, plasma glucose, lipid profile and electrocardiogram).

TREATMENT All of the women in this study will undergo a standard follicle stimulating hormone (FSH) / gonadotrophin releasing hormone (GnRH) antagonist IVF protocol which is used for women undergoing IVF treatment at Hammersmith Hospital under the supervision of Dr Geoff Trew (Director of the IVF Unit). The only difference will be that women taking part in the study will have a single

subcutaneous injection of kisspeptin in place of human chorionic gonadotrophin (hCG) in order to trigger oocyte maturation.

DURATION We will aim to complete the study in 3 years

1. INTRODUCTION

1.1 BACKGROUND

Infertility is commonly defined as the inability to conceive after two years of regular unprotected sexual intercourse (1). Infertility has a high prevalence; it is estimated that around one in six UK couples have difficulty conceiving i.e. approximately 3.5 million couples (1). The inability to have children can be devastating, and has important implications for mental, social, and reproductive health.

In healthy women, the reproductive cycle is controlled by the hypothalamic-pituitary gonadal (HPG) axis. GnRH released from the hypothalamus stimulates the release of the gonadotrophins, luteinising hormone (LH) and FSH from the anterior pituitary gland. These gonadotrophins in turn regulate ovarian function and oocyte maturation and ovulation which are driven by an LH surge. Recently kisspeptin has been shown to be responsible for the endogenous LH surge, via the stimulation of hypothalamic GnRH release (6). After ovulation, progesterone produced by the corpus luteum prepares the endometrium for embryo implantation. Normal function of the corpus luteum is dependent on the pituitary secretion of LH. IVF treatment uses the exogenous administration of hormones to stimulate ovarian follicular development and oocyte maturation. FSH injections are used to stimulate ovarian follicular development and endogenous LH production is blocked in order to prevent premature ovulation. Once 3 dominant follicles have developed, hCG is administered to stimulate oocyte maturation. The oocytes are collected and fertilised with sperm in vitro. The embryo is then replaced into the endometrial cavity to allow implantation and pregnancy to occur.

IVF treatment is widely and successfully used to allow infertile couples to conceive and is now approved by the National Institute for Health and Clinical Excellence (NICE). However, the most common serious complication of IVF is ovarian hyperstimulation syndrome (OHSS), a potentially life threatening condition which has significant morbidity and mortality (2). OHSS is a systemic disease resulting from the release of vasoactive products from hyper stimulated ovaries. This potentially life threatening condition can cause massive ovarian enlargement, ovarian torsion, ascites, hydrothorax, liver dysfunction, thromboembolism, electrolyte imbalance, renal failure and acute respiratory distress syndrome (ARDS) and death (2). Mild forms of OHSS are common, occurring in 1 in 3 of all IVF cycles, whilst almost 1 in 10 IVF cycles are complicated by moderate or severe OHSS (3).

The major cause of OHSS is the use of hCG in current IVF protocols for oocyte maturation (4). Human chorionic gonadotrophin binds to the LH receptor and therefore mimics the actions of endogenous LH. However, hCG has a much longer circulating half life than LH; this results in overstimulation of the corpus luteum which is the underlying cause of OHSS (12). Human chorionic gonadotrophin circulates for up to a week after injection (13), whereas with the physiological stimulus, the LH surge only lasts for 48 hours (14). This overstimulation of the corpus luteum also results in an environment more hostile to embryo implantation (5). A more physiological method for oocyte maturation in IVF treatment should prevent overstimulation of the corpus luteum and thus the subsequent development of OHSS (5).

Kisspeptin is a neuropeptide which stimulates the HPG axis. Kisspeptin offers a novel approach in IVF therapy to improve pregnancy rates and avoid the complication of OHSS. Kisspeptin is the endogenous agonist of the G protein-coupled receptor, GPR54 (19-22). The kisspeptin / GPR54 system plays a critical role in the hypothalamic regulation of the HPG axis. Inactivating GPR54 mutations in humans have been shown to cause hypogonadotrophic hypogonadism (absence of sexual maturation with low sex hormones and gonadotrophins) and failure of pubertal development (23-24). GPR54 or kisspeptin deficient mice have a similar phenotype with abnormal sexual development, low circulating gonadotrophin concentrations, and infertility (23, 25, 26). Furthermore, administration of kisspeptin potently stimulates activity of the HPG axis. Acute intracerebroventricular (ICV) or peripheral administration of kisspeptin to rodents or primates potently stimulates gonadotrophin release (10, 27). Kisspeptin stimulates the release of a limited pool of endogenous GnRH, which results in release of endogenous gonadotrophins. (9-11). We have recently demonstrated for the first time that acute administration of kisspeptin to healthy male and female volunteers with normal reproductive function, and in women with infertility due to hypothalamic amenorrhea potently stimulates the HPG axis without

side effects (7, 8, 33). The evidence to date demonstrates that the kisspeptin / GPR54 system is critical for normal pubertal development and reproductive function.

Kisspeptin is a critical upstream regulator of GnRH neurones necessary for normal reproductive function. Kisspeptin is found in hypothalamic regions important in the regulation of the HPG axis (9). In rodents, hypothalamic kisspeptin neurons are activated during proestrus (ie the time of the cycle just prior to ovulation), and blockade of the actions of hypothalamic kisspeptin with a specific monoclonal antibody abolishes the proestrus LH surge, suggesting that kisspeptin signalling is required for a normal menstrual cycle (28). Recent evidence demonstrates that kisspeptin is the physiological regulator of the LH surge (6) and exogenous administration of kisspeptin has been shown to induce ovulation in rats and sheep (31, 32). In keeping with this, our previous human studies demonstrate that kisspeptin stimulates LH release most potently in the pre-ovulatory phase of the menstrual cycle in females with regular normal menstrual cycles (8).

The major cause of OHSS in current IVF protocols is the pharmacological use of hCG which is used for oocyte maturation (4). Since kisspeptin stimulates the release of physiological levels of GnRH and the consequent release of endogenous gonadotrophins (9-11) it should lead to a physiological LH surge which would be dependent on the sensitivity of the HPG axis of each woman. A more physiological approach for oocyte maturation in IVF treatment would prevent overstimulation of the corpus luteum and OHSS in IVF treatment (5). The significant advantage of kisspeptin over current treatments is that its effects would depend on the sensitivity of an individual's HPG axis. This would result in a more physiological LH surge and oocyte maturation during IVF treatment, thereby improving safety and efficacy of IVF treatment (5).

GnRH antagonists are widely used in IVF protocols to suppress the increase in endogenous LH which would otherwise cause premature ovulation (29). These agents bind to GnRH receptors in the pituitary gland and competitively inhibit the actions of endogenous GnRH. The onset of the suppression of pituitary gonadotrophin release is rapid, as is the recovery of pituitary function after cessation. This makes GnRH antagonists ideal agents for short term pituitary suppression (29). In addition since pituitary function recovers almost immediately when GnRH antagonist is withdrawn, they allow the potential use of physiological stimulators of endogenous gonadotrophins (e.g. kisspeptin) in IVF protocols to generate a more physiological LH surge and oocyte maturation in IVF protocols (29).

We hypothesise that use of kisspeptin in place of hCG in IVF protocols will result in the physiological release of the endogenous releasable pool of GnRH and subsequent gonadotrophin secretion; this in turn will lead to oocyte maturation.

The aim of the current study is to provide proof of concept that administration of kisspeptin can induce oocyte maturation in infertile women undergoing IVF treatment.

1.2 RATIONALE FOR CURRENT STUDY

Research question: Can kisspeptin stimulate oocyte maturation in IVF therapy for infertility ?

Hypothesis: kisspeptin will stimulate oocyte maturation in IVF therapy for infertility

Infertility affects one in six couples in the UK (1). IVF treatment is now widely and successfully used to enable infertile couples to conceive. Approximately 45,000 cycles of IVF are performed in Britain each year. However, IVF treatment can result in the potentially life threatening condition, OHSS (2). Mild forms of OHSS occur in approximately 1 in 3 of all IVF cycles, whilst approximately 1 in 10 IVF cycles result in moderate or severe OHSS (3). The major cause of OHSS is the pharmacological use of hCG to stimulate oocyte maturation in current IVF protocols (4). The development of a more physiological stimulus for oocyte maturation should avoid this dangerous side effect and improve the safety and efficacy of IVF treatment (5). Kisspeptin is a hormone which stimulates reproductive hormone secretion in animals and is responsible for the endogenous LH surge which results in ovulation (6). In keeping

with this, exogenous administration of kisspeptin has been shown to induce ovulation in rats and sheep (31, 32). We have conducted the first studies of kisspeptin administration to humans; in which we have shown that acute administration of kisspeptin potently stimulates reproductive hormone secretion in healthy men and women (7, 8), as well as women with infertility due to hypothalamic amenorrhea (33). Importantly, we have demonstrated that healthy women with regular menstrual cycles are most sensitive to the effects of kisspeptin immediately prior to ovulation (8). Recent evidence demonstrates that kisspeptin stimulates the release of a limited pool of endogenous GnRH which results in the release of endogenous gonadotrophins (9-11). Therefore, the significant advantage of kisspeptin over current treatments is that its effects would depend on the sensitivity of an individual's HPG axis. This would result in a more physiological LH surge and oocyte maturation during IVF treatment, thereby improving safety and efficacy of IVF treatment (5).

2. STUDY OBJECTIVES

To investigate whether kisspeptin can stimulate oocyte maturation in IVF therapy for infertility.

3. STUDY DESIGN

Type of study: Open label, dose escalation, phase 2 clinical trial investigating the administration of Kisspeptin to induce oocyte maturation in 90 women undergoing IVF treatment

Duration: the treatment that is specific to this study will only be the single injection of kisspeptin and blood sample following kisspeptin injection subsequently. The rest of the IVF protocol is part of standard care.

Number and type of patients recruited: 90 women undergoing IVF treatment for infertility

3.1 STUDY OUTCOME MEASURES

Primary outcome: To investigate whether kisspeptin can stimulate oocyte maturation in IVF therapy for infertility.

Secondary outcome measures:
Rates of OHSS
Plasma kisspeptin levels
Serum LH
Serum FSH
Serum Estradiol
Ovarian follicular number and size
Oocyte quality
Embryo quality
βhCG concentration 12 to16 days after embryo transfer
Clinical pregnancy rate
Fertilisation rate
Pregnancy outcomes
Live births

4. PARTICIPANT ENTRY

4.1 PRE-RANDOMISATION EVALUATIONS

Study participants will be women needing IVF treatment for male factor infertility requiring intracytoplasmic sperm injection (ICSI), attending the IVF unit at Queen Charlottes and Chelsea Hospital, Imperial College Healthcare NHS Trust. Eligibility for the study will be determined at screening which will comprise a medical history, routine physical examination and basic investigations (including full blood count, urea and electrolytes, liver function tests, thyroid function tests, plasma glucose, lipid profile and electrocardiogram). Participants will be free to withdraw from the study at any time.

4.2 INCLUSION CRITERIA

- Aged 18 – 34 years
- Body mass index between 18 and 29 kg/m2
- Stable body weight for at least 3 months
- Normal early menstrual cycle follicular phase serum FSH concentration (≤12iu/l)
- Serum anti-Mullerian hormone (AMH) > 40pmol/L or antral follicle count (AFC) on ultrasound scanning > 23 follicles

or those women who have had a good follicular response in a previous IVF cycle in the previous 6 months with a dose of FSH of 150iu

- No more than one previous IVF treatment cycle
- Both ovaries intact

4.3 EXCLUSION CRITERIA

- History of any medical, psychological or other condition, or use of any medications, including over-the-counter products, which, in the opinion of the investigators, would either interfere with the study or potentially cause harm to the volunteer
- Without access at home to a telephone, or other factor likely to interfere with ability to participate reliably in the study
- Treatment with an investigational drug within the preceding 2 months
- Donated blood during the preceding 3 months or intention to do so before the end of the study
- Previous poor response to IVF treatment

4.4 WITHDRAWAL CRITERIA

It will be made clear to participants that they will be free to withdraw from the study at any time without providing any reason and this will not affect their standard care in any way.

5. RANDOMISATION AND ENROLMENT PROCEDURE

5.1 RANDOMISATION OR REGISTRATION PRACTICALITIES

This is an open label, dose escalation, phase 2 clinical trial investigating the administration of Kisspeptin to induce oocyte maturation – there will therefore be no randomisation as all women will receive kisspeptin

5.2 UNBLINDING

This is an open label, dose escalation, phase 2 clinical trial investigating the administration of Kisspeptin to induce oocyte maturation and all women will receive at least one dose of kisspeptin. Women will receive either one, or two doses of kisspeptin at least 8 hours apart. Women will be advised that the first injection will be kisspeptin for all women, but the second injection received may be either kisspeptin or saline.

6. TREATMENTS

6.1 TREATMENT ARMS

All of the women in this study will undergo a standard FSH/GnRH antagonist IVF protocol with luteal phase supplementation which is used for women undergoing IVF treatment at Hammersmith Hospital under the supervision of Dr Geoff Trew (Director of the IVF Unit) (please see appendix 2). The only difference will be that women taking part in the study will have either one or two subcutaneous injections of kisspeptin in place of hCG in order to trigger oocyte maturation. This injection will be administered by two experienced physicians at the Hammersmith Hospital or in the participant's home according to the patients preference.

The patients will be administered with the first dose of kisspeptin 12-24 hours after the last injection of FSH/GnRH antagonist for final oocyte maturation. Women will be randomised to receive either a single dose of kisspeptin (9.6nmol/kg), or two doses of kisspeptin 8-12 hours apart. The optimal dosing regimen of kisspeptin will be the dose of kisspeptin which causes the highest level of oocyte maturation.

Since kisspeptin causes a rapid and potent rise in LH release following sc injection we would expect that kisspeptin will induce oocyte maturation at a similar timing as when hCG or GnRH are used to trigger oocyte maturation which is 32-36 hours after injection (13, 16, 17, 18). In order to determine the changes in reproductive hormones which occur during oocyte maturation, participants will be given two options for blood testing following injection of kisspeptin. These options will be discussed at screening, and participants are entirely free to decline overnight blood sampling without altering their study participation or clinical care:

Option 1. Overnight blood sampling: Participants opting for overnight blood sampling will be admitted to the Wellcome / NIHR Clinical Research Facility (CRF), Hammersmith Hospital. Blood will be sampled for measurement of plasma LH, FSH, progesterone, kisspeptin and oestradiol. Total blood loss will not exceed 100ml, and blood sampling will not be performed more frequently than every 15min.

Option 2. Less intensive blood testing: In participants who do not opt into overnight blood sampling, plasma LH, FSH, progesterone and oestradiol will be measured immediately before kisspeptin injection, on up to six occasions within the first 24h following kisspeptin injection (according to participant availability), and then at the time of oocyte retrieval.

These hormone measurements will determine the time course following kisspeptin injection at which an endogenous LH surge occurs which will be used to confirm the timing of oocyte retrieval.

This study will determine the potential of kisspeptin as a trigger for oocyte maturation in IVF treatment.

6.2 DOSE MODIFICATIONS FOR TOXICITY

Not applicable

6.3 PREMEDICATION

Not applicable

6.4 INTERACTION WITH OTHER DRUGS

The only medication which participants should avoid is the oral contraceptive pill or hormone replacement therapy. All patients attending for IVF treatment will be asked to stop these medications as part of their standard care before IVF treatment whether they participate in the study or not.

6.5 DISPENSING AND ACCOUNTABILITY

The pharmacy at Hammersmith will be responsible for storing and dispensing the study drug and full accountability documentation of the Investigational Medicinal Product (IMP) will be kept in pharmacy.

7. PHARMACOVIGILANCE

7.1 DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. *An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, whether or not considered related to the IMP.*

Adverse Reaction (AR): all untoward and unintended responses to an IMP related to any dose administered. *All AEs judged by either the reporting investigator or the sponsor as having reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.*

Unexpected Adverse Reaction: an AR, the nature or severity of which is not consistent with the applicable product information (eg investigator's brochure for an unapproved investigational product or summary of product characteristics (SmPC) for an authorised product*). When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected. Side effects documented in the SmPC which occur in a more severe form than anticipated are also considered to be unexpected.*

Serious Adverse Event (SAE) or **Serious Adverse Reaction:** any untoward medical occurrence or effect that at any dose

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Suspected Unexpected Serious Adverse Reaction (SUSAR): any suspected adverse reaction related to an IMP that is both unexpected and serious.

7.2 CAUSALITY

Most adverse events and adverse drug reactions that occur in this study, whether they are serious or not, will be expected treatment-related toxicities due to the drugs used in this study. The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in the table below.

If any doubt about the causality exists the local investigator should inform the study coordination centre who will notify the Chief Investigators. The pharmaceutical companies and/or other clinicians may be asked to advise in some cases.

In the case of discrepant views on causality between the investigator and others, all parties will discuss the case. In the event that no agreement is made, the MHRA will be informed of both points of view.

Relationship	Description
Unrelated	There is no evidence of any causal relationship
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to

	the event (e.g. the participant's clinical condition, other concomitant treatments).
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

7.3 REPORTING PROCEDURES

All adverse events will be reported. Depending on the nature of the event the reporting procedures below will be followed. A flowchart is given below to aid in the reporting procedures.

7.3.1 Non serious AR/AEs

All such toxicities, whether expected or not, will be recorded in the toxicity section of the relevant case report form and sent to the study coordination centre within one month of the form being due.

7.3.2 Serious AR/AEs

Fatal or life threatening SAEs and SUSARs will be reported on the day that the investigators are aware of the event. The SAE form asks for nature of event, date of onset, severity, corrective therapies given, outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). The responsible investigator (Dr Waljit Dhillo) will sign the causality of the event. Additional information should be sent within 5 days if the reaction has not resolved at the time of reporting.

SAEs

An SAE form will be completed and faxed to the Imperial College Joint Research Compliance Office for review.

SUSARs

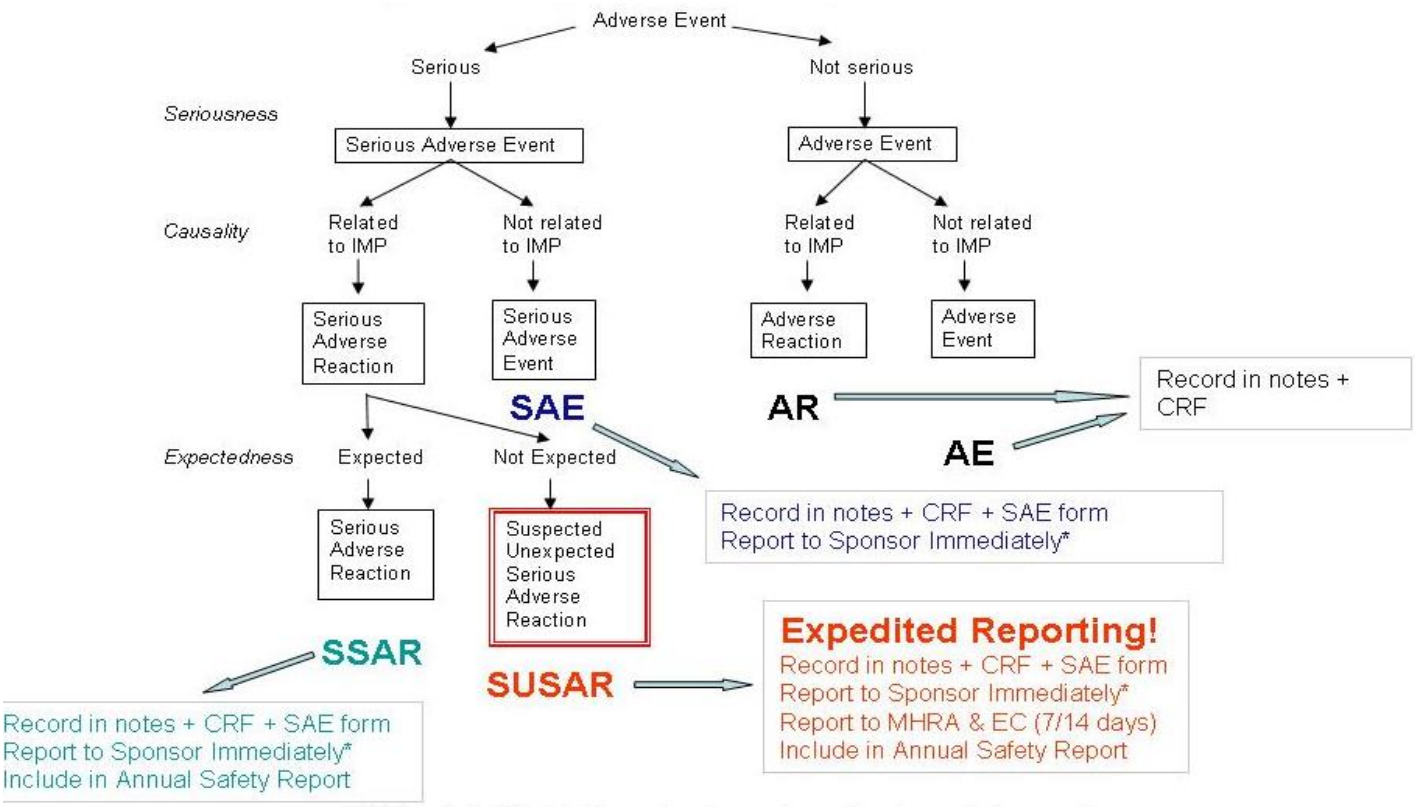
In the case of serious, unexpected and related adverse events, the staff involved in the study will:

Complete the SAE case report form & send it immediately (within 24 hours, preferably by fax), signed and dated to Dr Waljit Dhillo together with relevant treatment forms and anonymised copies of all relevant investigations.

Or

Contact the Dr Waljit Dhillo by phone and then send the completed SAE form to Dr Waljit Dhillo within the following 24 hours as above.

Dr Waljit Dhillo will notify the MHRA, main REC and Sponsor of all SUSARs occurring during the study according to the following timelines; fatal and life-threatening within 7 days of notification and non-life threatening within 15 days. All investigators will be informed of all SUSARs occurring throughout the study.



* Unless identified in the protocol as not requiring immediate reporting

Contact details for reporting SAEs and SUSARs
Fax: 0208 383 8320, attention Dr Waljit Dhillo
Please send SAE forms to: Dr Waljit Dhillo
Tel: 020 8383 2820 (Mon to Fri 09.00 – 17.00)

8. ASSESSMENT AND FOLLOW-UP

Each participant will be in the study for the period of their IVF treatment, which will be no longer than eight weeks. Following this if the patient becomes pregnant they will have routine antenatal follow up for the duration of their pregnancy and delivery at Queen Charlottes' Hospital. There will be no additional interventions needed during the pregnancy related to the study but we will follow up patients until the birth of their child.

8.1 LOSS TO FOLLOW-UP

The patients who are lost to follow from this study will be contacted using their telephone and address details supplied at the time of entry into the study to ensure safety. As far as data analysis an intention to treat analysis will be carried out for this study and sensitivity analysis will be used in data analysis to take account of the small number of drop outs that may occur in the study.

8.2 TRIAL CLOSURE

The study will stop recruiting patients when the optimal dose of kisspeptin required to trigger oocyte maturation has been determined. The optimal dose of kisspeptin will be the dose of kisspeptin which causes oocyte maturation in >90% of women (n=15). However, we will continue to follow up all women in the study until they have completed their pregnancy.

9. STATISTICS AND DATA ANALYSIS

Sample size calculation: For the primary endpoint of oocyte maturation in the proposed patient population, there is no directly relevant data that has been published previously upon which sample sizes estimates could be based. However, there is evidence in the literature (13) of a relationship between successful oocyte maturation and rises in luteinising hormone (LH) which can be used as a basis for sample size calculations for this study. In that study, successful oocyte maturation occurs following a mean rise in LH of 106IU/L at 4hours following a GnRH agonist trigger in a FSH/GnRH antagonist IVF protocol. Our previous dose finding studies of kisspeptin on LH release in normal women in the preovulatory phase of their menstrual cycle show that kisspeptin (12.8nmol/kg) can increase LH by up to 148IU/L with an estimated SD of 78IU/L. Using the 2-group t-test approach, we have calculated that with 13 patients in for each dose of kisspeptin tested will have at least 90% power to detect a difference of 148IU/L amongst any 2 groups (2-sided 5% significance level without correction for multiplicity and common standard deviation of 78 IU/L) (nQuery Advisor Version 5.0. Statistical Solutions, Cork, Ireland). This n number is similar to that used in our recent studies of kisspeptin on LH release in normal women in the preovulatory phase of their menstrual cycle (n=8) to show a significant effect of kisspeptin on LH release compared to controls (8). It is anticipated that the drop-out rate will be less than 10% (based on similar previous studies in these highly motivated infertile patients). Therefore, fifteen women for each of the 6 doses of kisspeptin (0.4, 0.8, 1.6, 3.2, 6.4 and 12.8nmol/kg) administered will be recruited to allow for dropouts during the study and each woman will only participate in the study once. As such a total of 90 women will be recruited to the study.

Data analysis for the study:
The statistical data analysis will be carried out by Imperial Clinical Trials Unit under the supervision of Professor Ashby (Professor of Medical Statistics and Clinical Trials, Imperial College London). An intention to treat analysis will be carried out for this study and sensitivity analysis will be used in data analysis to take account of the small number of drop outs that may occur in the study. The primary analysis of the primary endpoint (oocyte maturation) will employ a logistic model to characterise the relationship between dose of kisspeptin and oocycte maturation. Additionally Bayesian analysis methods will be employed to characterise the relationship between dose of kisspeptin, LH and oocyte maturation.

10. MONITORING

10.1 RISK ASSESSMENT
A risk assessment will be carried out according to the sponsor's standard operating procedures (SOPs).

10.2 MONITORING AT LOCAL SITE
Imperial College will risk assess the project and monitoring will be carried out according to the sponsors SOPs.

11. REGULATORY ISSUES

11.1 CLINICAL TRIALS AUTHORISATION (CTA)
A Clinical Trials Authorisation from the UK Competent Authority will be obtained.

11.2 ETHICS APPROVAL
The Study Coordination Centre has submitted an ethics application to the Hammersmith and Queen Charlotte's Research Ethics Committee. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

11.3 CONSENT
Consent to enter the study will be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent will be obtained. The right of the participant to refuse to participate without giving reasons will be respected. After the participant has entered the trial the clinician remains free to give alternative treatment to that

specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so will be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

11.4 CONFIDENTIALITY

Participants' identification data will be required for the registration process. The Study Coordination Centre will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

11.5 INDEMNITY

Imperial College holds Public Liability ("negligent harm") and Clinical Trial ("non-negligent harm") insurance policies which apply to this trial.

11.6 SPONSOR

Imperial College London will act as the main sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

11.7 FUNDING

An MRC Developmental Clinical Studies grant application has been submitted to fund this study (outcome July 2010). This is an academic study and the investigators will not be paid for recruiting patients into this study.

11.8 AUDITS AND INSPECTIONS

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP.

12. TRIAL MANAGEMENT

A Trial Management Group (TMG) will be appointed and will be responsible for overseeing the progress of the trial. The day-to-day management of the trial will be co-ordinated by Professor Waljit Dhillo. In addition a Data Monitoring Committee will be convened (for further details please see the Data Monitoring & Ethics Committee (DMEC) Standard Operating Procedure enclosed in with this application.

13. PUBLICATION POLICY

All publications and presentations relating to the study will be authorised by the Trial Management Group. The first publication of the trial results will be in the name of the Trial Management Group, if this does not conflict with the journal's policy. If there are named authors, these will include at least the trial's Chief Investigator, Statistician and Trial Coordinator. Members of the TMG and the Data Monitoring Committee will be listed and contributors will be cited by name if published in a journal where this does not conflict with the journal's policy.

14. REFERENCES

1. HFEA facts and figures http://www.hfea.gov.uk/en/406.html.
2. RCOG Guideline No.5. The Management of Ovarian Hyperstimulation Syndrome. Sept 2006.
3. Delvigne A, Rozenberg S. Epidemiology and prevention of ovarian hyperstimulation syndrome (OHSS).Hum Reprod Update. 2002 8(6):559-77.
4. Golan A, Ron-el R, Herman A, Soffer Y, Weinraub Z, Caspi E. Ovarian hyperstimulation syndrome: an update review. Obstet Gynecol Surv. 1989 44(6):430-40.
5. Macklon NS, Stouffer RL, Giudice LC, Fauser BC. The science behind 25 years of ovarian stimulation for in vitro fertilization. Endocr Rev. 2006; 27(2):170-207.

6. Clarkson J, d'Anglemont de Tassigny X, Moreno AS, Colledge WH, Herbison AE. Kisspeptin-GPR54 signaling is essential for preovulatory gonadotropin-releasing hormone neuron activation and the luteinizing hormone surge. J Neurosci. 2008 28(35):8691-7.
7. Dhillo WS, Chaudhri OB, Patterson M, Thompson EL, Murphy KG, Badman MK et al. Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. J Clin Endocrinol Metab. 2005; 90(12):6609-15.
8. Dhillo WS, Chaudhri OB, Thompson EL, Murphy KG, Patterson M, Ramachandran R et al. Kisspeptin-54 stimulates gonadotropin release most potently during the preovulatory phase of the menstrual cycle in women. J Clin Endocrinol Metab. 2007; 92(10):3958-66.
9. Irwig MS, Fraley GS, Smith JT, Acohido BV, Popa SM, Cunningham MJ et al. Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. Neuroendocrinology. 2004; 80(4):264-72.
10. Thompson EL, Patterson M, Murphy KG, Smith KL, Dhillo WS, Todd JF et al. Central and peripheral administration of kisspeptin-10 stimulates the hypothalamic-pituitary-gonadal axis. J Neuroendocrinol. 2004; 16(10):850-8.
11. Messager S, Chatzidaki EE, Ma D, Hendrick AG, Zahn D, Dixon J et al. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. Proc Natl Acad Sci U S A. 2005; 102(5):1761-6.
12. Damewood MD, Shen W, Zacur HA, Schlaff WD, Rock JA, Wallach EE. Disappearance of exogenously administered human chorionic gonadotropin. Fertil Steril. 1989; 52(3):398-400.
13. Fauser BC, de Jong D, Olivennes F, Wramsby H, Tay C, Itskovitz-Eldor J et al. Endocrine profiles after triggering of final oocyte maturation with GnRH agonist after cotreatment with the GnRH antagonist ganirelix during ovarian hyperstimulation for in vitro fertilization. J Clin Endocrinol Metab. 2002 Feb;87(2):709-15.
14. Hoff JD, Quigley ME, Yen SS. Hormonal dynamics at midcycle: a reevaluation. J Clin Endocrinol Metab. 1983 Oct;57(4):792-6.
15. Kol S. Luteolysis induced by a gonadotropin-releasing hormone agonist is the key to prevention of ovarian hyperstimulation syndrome. Fertil Steril. 2004 Jan;81(1):1-5.
16. Humaidan P, Bredkjaer HE, Bungum L, Bungum M, Grøndahl ML, Westergaard L et al. GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: a prospective randomized study. Hum Reprod. 2005; 20(5):1213-20.
17. Kolibianakis EM, Schultze-Mosgau A, Schroer A, van Steirteghem A, Devroey P, Diedrich K et al. A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocyte maturation instead of HCG in patients undergoing IVF with GnRH antagonists. Hum Reprod. 2005; 20(10):2887-92.
18. Griesinger G, Diedrich K, Devroey P, Kolibianakis EM. GnRH agonist for triggering final oocyte maturation in the GnRH antagonist ovarian hyperstimulation protocol: a systematic review and meta-analysis. Hum Reprod Update. 2006; 12(2):159-68.
19. Kotani M, Detheux M, Vandenbogaerde A, Communi D, Vanderwinden JM, Le Poul E et al. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. J Biol Chem 2001; 276(37):34631-34636.
20. Lee JH, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE et al. KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. J Natl Cancer Inst 1996; 88(23):1731-1737.
21. Muir AI, Chamberlain L, Elshourbagy NA, Michalovich D, Moore DJ, Calamari A et al. AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. J Biol Chem 2001; 276(31):28969-28975.
22. Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K et al. Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. Nature 2001; 411(6837):613-617.
23. Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK et al. The GPR54 gene as a regulator of puberty. N Engl J Med 2003; 349(17):1614-1627. 24. de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proc Natl Acad Sci U S A 2003; 100(19):10972-10976.
25. Lapatto R, Pallais JC, Zhang D, Chan YM, Mahan A, Cerrato F et al. Kiss1-/- mice exhibit more variable hypogonadism than Gpr54-/- mice. Endocrinology. 2007; 148(10):4927-36.
26. d'Anglemont de Tassigny X, Fagg LA, Dixon JP, Day K, Leitch HG, Hendrick AG et al. Hypogonadotropic hypogonadism in mice lacking a functional Kiss1 gene. Proc Natl Acad Sci U S A. 2007 Jun 19;104(25):10714-9.

27. Plant TM, Ramaswamy S, Dipietro MJ. Repetitive activation of hypothalamic G protein-coupled receptor 54 with intravenous pulses of kisspeptin in the juvenile monkey (Macaca mulatta) elicits a sustained train of gonadotropin-releasing hormone discharges. Endocrinology. 2006; 147(2):1007-13
28. Kinoshita M, Tsukamura H, Adachi S, Matsui H, Uenoyama Y, Iwata K et al. Involvement of central metastin in the regulation of preovulatory luteinizing hormone surge and estrous cyclicity in female rats. Endocrinology 2005; 146(10):4431-4436.
29. DiLuigi AJ, Nulsen JC. Effects of gonadotropin-releasing hormone agonists and antagonists on luteal function. Curr Opin Obstet Gynecol. 2007; 19(3):258-65.
30. Staessen C, Camus M, Bollen N, Devroey P, Van Steirteghem AC. The relationship between embryo quality and the occurrence of multiple pregnancies. Fertil Steril. 1992 Mar;57(3):626-30.
31. Matsui H, Takatsu Y, Kumano S, Matsumoto H, Ohtaki T. Peripheral administration of metastin induces marked gonadotropin release and ovulation in the rat. Biochem Biophys Res Commun. 2004 Jul 23;320(2):383-8.
32. Caraty A, Smith JT, Lomet D, Ben Saïd S, Morrissey A, Cognie J, Doughton B, Baril G, Briant C, Clarke IJ. Kisspeptin synchronizes preovulatory surges in cyclical ewes and causes ovulation in seasonally acyclic ewes. Endocrinology. 2007 Nov;148(11):5258-67.
33. Jayasena CN, Nijher GM, Chaudhri OB, Murphy KG, Ranger A, Lim A, Patel D, Mehta A, Todd C, Ramachandran R, Salem V, Stamp GW, Donaldson M, Ghatei MA, Bloom SR, Dhillo WS. Subcutaneous injection of kisspeptin-54 acutely stimulates gonadotropin secretion in women with hypothalamic amenorrhea, but chronic administration causes tachyphylaxis. J Clin Endocrinol Metab. 2009 Nov;94(11):4315-23.

APPENDIX 1. SUMMARY OF INVESTIGATIONS, TREATMENT AND ASSESSMENTS

All of the women in this study will undergo a standard FSH/GnRH antagonist IVF protocol with luteal phase supplementation which is used as part of routine care for women undergoing IVF treatment at Hammersmith Hospital (see appendix 2) under the supervision of Dr Geoff Trew (Director of the IVF Unit). The only difference will be that women taking part in the study will have:

1. a single subcutaneous injection of kisspeptin in place of hCG in order to trigger oocyte maturation.
2. Plasma LH, FSH, progesterone and oestradiol will be measured immediately before kisspeptin injection and then after kisspeptin injectionon up to 6 occasions during the first 24h and finally at the time of oocyte retrieval. These hormone measurements will determine the time course following kisspeptin injection at which an endogenous LH surge occurs which will be used to confirm the timing of oocyte retrieval.

All of the other procedures that the patients will have are part of routine care and would be the same for volunteers not participating in the study.

APPENDIX 2. STANDARD FSH/GNRH ANTAGONIST IVF PROTOCOL WITH LUTEAL PHASE SUPPLEMENTATION

All of the women in this study will undergo a standard FSH/GnRH antagonist IVF protocol with luteal phase supplementation which is used as part of routine care for women undergoing IVF treatment at Hammersmith Hospital (see appendix 2) under the supervision of Dr Geoff Trew (Director of the IVF Unit). The current protocol is detailed below. This will be updated according to best clinical care.

Daily subcutaneous (sc) FSH injections (Gonal F) will be commenced on day 2 of the patient's menstrual cycle. Daily GnRH antagonist administration (Cetrotide) will be used to inhibit a premature

LH surge and will be started on the 5th day of FSH injections. The patient will attend 5 days after commencing the FSH injections for a pelvic ultrasound scan (USS) to determine ovarian follicular development. The patient will be asked to attend for further scans according to follicular size and response (if the largest follicle is 10-12mm the next USS will be 3 days later, if the largest follicle is 13-15mm the next USS will be 2 days later, if the largest follicle is 16mm daily USS will be performed). When 3 ovarian follicles of ≥ 17mm have developed, the FSH and GnRH antagonist injections will be stopped. Kisspeptin will be given in place of hCG at this point in the protocol as detailed in section 6.1 of the protocol to cause oocyte maturation.
Thirty two to thirty six hours later, transvaginal ultrasound-directed oocyte retrieval will be carried out. Oocyte maturation will be determined using standard criteria (calculations of the percentage of metaphase II (MII) oocytes and fertilisation rate (number of two pronuclear (2PN) oocytes / number of metaphase oocytes injected) (15). ICSI will be carried out using fresh sperm from the male partner. The quality of the embryos will be classified as high quality (score 1), medium quality (score 2), and low quality (score 3) as previously described (30). One or two embryos of highest quality will be transferred to the uterine cavity of the patient on day 3-5 after oocyte collection. The remaining embryos will be frozen for the patient to use in future if required. Progesterone and oestradiol (at dosages recommended by the IVF team) will be started on the morning after oocyte collection until 12 weeks of pregnancy for luteal phase supplementation. After oocyte retrieval doxycycline (100mg bd) will be given for 7 days as well as analgesia as required (diclofenac suppository or paracetamol). Women will be followed up according to the standard protocol of the IVF Unit at Hammersmith Hospital. Women in whom kisspeptin does not result in oocyte maturation will receive a further IVF cycle (i.e. with hCG as a trigger for oocyte maturation) as part of their standard care. Pregnancy will be defined biochemically by a plasma βhCG concentration >10iu/l 12 to 16 days after embryo transfer. Pregnancy will be defined clinically as an intrauterine gestational sac with a heartbeat 2-3 weeks after a positive hCG test. An additional ultrasound scan may also be performed at 7 weeks if there is clinical suspicion of an ectopic pregnancy. In women with a clinical pregnancy, standard pregnancy follow up will occur at Queen Charlotte's Hospital. Pregnancy outcomes and live births will be documented. IVF clinicians will make decisions in the best clinical interest of patients to safeguard patient safety where necessary as would occur in normal clinical practice.

APPENDIX 3. DETAILS OF THE IMP

The IMP is kisspeptin-54. Doses between 0.4 and 12.8nmol/kg will be administered during this study. The IMP has been manufactured and labelled in compliance with Good Manufacturing Practice Annex 13 by Bachem Switzerland. Please see the Investigators Brochure for further details of the IMP and for storage conditions.