

Kisspeptin Physiology in the Human

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BROAD GOALS

The overall goals of this protocol are to 1) investigate the neuroendocrine control of reproduction, 2) investigate the physiology of kisspeptin administration in the human.

I. BACKGROUND & SIGNIFICANCE

A Shifting Paradigm in Reproductive Endocrinology: Discovery of a GPR54 as a Key Modulator of GnRH Secretion

The hypothalamic hormone GnRH sits atop the reproductive cascade of all mammals as a central driver of pituitary gonadotropin secretion. Its commanding role in this biological hierarchy allows it to control pulsatile gonadotropin secretion, modulate gonadal steroid feedback, and bring about full fertility in the adult. Yet, what turns on GnRH secretion at the time of puberty? And once it is turned on, what sustains the physiological amplitude and frequency so essential for normal reproductive function?

To find modulators of GnRH secretion, we utilized genetic tools in patients with GnRH deficiency (i.e., idiopathic hypogonadotropic hypogonadism [IHH]), a clinical syndrome defined by low sex steroids in the setting of low gonadotropins that results from the failure of a normal pattern of pulsatile GnRH secretion^{1,2}. In 2003, we discovered that, **in both the human and the mouse**, mutations in *GPR54* cause hypogonadotropic hypogonadism which can be corrected by the administration of exogenous GnRH³. More recently, we identified an activating mutation in *GPR54* in a girl with central precocious puberty⁴. Taken together, these loss- and gain-of-function mutations demonstrate that the kisspeptin/GPR54 pathway is a genetic determinant and important gatekeeper of pubertal function in the human.

Kisspeptin: the Ligand for GPR54

The ligand for GPR54 is encoded by the gene *KISS1*⁵⁻⁷. Its full length gene product, kisspeptin, is a 154-amino-acid protein that is proteolytically processed to a 54-amino-acid peptide, kisspeptin 68-121, initially named metastin based on its ability to inhibit tumor metastasis. Smaller C-terminal fragments of 14, 13, and 10 amino acids can also activate GPR54. The 10-amino-acid (decapeptide) isoform of kisspeptin (kisspeptin 112-121) is the isoform used in this protocol. Although the terms “kisspeptin” and “metastin” can both be found throughout the literature, there have been recent proposals to standardize the nomenclature to “kisspeptin.” The term “kisspeptin” will be used throughout this protocol.

Kisspeptin is a Powerful Stimulator of GnRH-Induced Gonadotropin Release *in vivo*

Kisspeptin peptides are the most powerful stimulators of gonadotropin secretion on a molar basis that have ever been studied in mammalian species including rodents,⁸⁻¹⁴ sheep,¹⁴ and monkeys.¹⁵

- The stimulatory effect of kisspeptin is extraordinarily potent, as intracerebral doses as low as 1 fmol evoke significant LH responses.⁸
- The effects of kisspeptin on LH secretion can be completely abrogated by a GnRH antagonist.^{8,15} Thus, kisspeptin acts to stimulate GnRH release, which in turn stimulates LH secretion.

- Kisspeptin is unable to stimulate LH release when given to *Gpr54* knockout mice, suggesting that the stimulatory effects of this peptide are mediated only through its cognate receptor.¹⁶
- However, kisspeptin is able to stimulate LH release when given to *Kiss1* knockout mice, demonstrating its powerful stimulating effects even in the face of abiding hypogonadism.¹⁶
- Kisspeptin may have a subtle effect on gonadotropin secretion directly at the pituitary. This is a controversial point in the literature and the effect is modest at best, predominantly limited to synergism with GnRH.¹⁷
- In hyperprolactinemic mice, which exhibit a form of acquired hypogonadotropism, kisspeptin is able to restore GnRH and LH release.¹⁸

What does the Discovery of Kisspeptin Mean from a Physiologic Perspective?

Probe of the GnRH Neuron

Because kisspeptin is a powerful stimulus for GnRH secretion, administration of kisspeptin offers a method to directly interrogate the function of GnRH neurons in humans, just as GnRH administration has served as a probe of pituitary gonadotrope function in humans for decades. We hypothesize that to respond to kisspeptin with an increase in serum LH humans must have a certain “threshold” number of GnRH neurons positioned properly within the hypothalamus, adequate GnRH synthetic capacity, and normal secretory mechanisms of GnRH and LH. Thus, in pathophysiologic states that compromise the integrity of the GnRH neurons, the response to kisspeptin would be reduced or attenuated. Therefore, our goal is to use kisspeptin administration as the first direct probe of GnRH neuronal integrity in human reproductive biology to gain new insights into GnRH neuronal function (or dysfunction) in a variety of pathophysiological conditions; insights that will refine our ongoing gene discovery efforts for these conditions.

Kisspeptin Administration to Humans:

The following section 1) lists the various isoforms, doses, and modes of the worldwide experience with administration of kisspeptin in human studies to date, and 2) summarizes key findings from these studies.

Kisspeptin 112-121 (the 10-amino-acid isoform used in this protocol) has been given by our group and others in the following scenarios:

A) Single Boluses (doses: 0.01 – 32 nmol/kg; routes: intravenous (IV), subcutaneous (SC))

- Healthy men¹⁹⁻²²
- Healthy women (including those taking oral contraceptives and with progestin implants)^{19,23,24}
- Healthy postmenopausal women²⁴
- Patients with low testosterone and type 2 diabetes mellitus²²
- Patients with IHH^{25,26,27,28}

B) Multiple Boluses (doses: 0.24 – 2.4 nmol/kg IV q1-2 hours for up to 5 boluses)

- Patients with IHH^{25,28,29}

C) Brief Infusions (doses: 0.02-0.72 nmol/kg/hr IV for 30 min followed by 0.01-0.36 nmol/kg/hr IV for 60 min)

- Healthy cycling women¹⁹

C) Long Infusions (doses: 1.1 – 9.5 nmol/kg/hr IV for 7 – 24 hours)

- Healthy cycling women³⁰
- Healthy men^{20,31}
- Healthy postmenopausal women³¹
- Patients with low testosterone and type 2 diabetes mellitus²²
- Patients with IHH^{26,29,32}
- Women with hyperprolactinemia³³

In addition, the 54-amino-acid isoform of kisspeptin (kisspeptin 68-121) has also been given in the following scenarios:

A) Single Boluses (doses: 0.15-12.8 nmol/kg SC)

- Healthy women (in all phases of the menstrual cycle)^{34,35}
- Women with hypothalamic amenorrhea^{36,37}
- Women undergoing ovulation induction³⁸
- Women with or at risk of ovarian hyperstimulation syndrome^{39,40}

B) Multiple boluses (dose: 6.4 nmol/kg SC for up to 8 weeks)

- Healthy women^{41,42}
- Women with hypothalamic amenorrhea^{36,37}

C) Brief Infusions (doses: 0.0075-2.4 nmol/kg/hr IV for 1.25-3.75 hours)

- Healthy men^{43,44,45,46}

D) Long Infusions (doses: 0.01 – 1 nmol/kg IV and SC for up to 10 hours)

- Healthy cycling women⁴⁷
- Women with hypothalamic amenorrhea⁴⁸

While a variety of modes of administration, and specific protocol details have been used in these studies using two peptide isoforms of kisspeptin, several “big picture messages” are emerging.

First, while kisspeptin can be a powerful stimulus for GnRH release in humans, this does not occur in all physiological circumstances as shown by our group and others.^{20,21,34,43} For example, women in the follicular phase of the menstrual cycle do not respond or respond nominally to a single bolus of kisspeptin as compared to the robust response seen in luteal phase women.^{23,24,49}

Second, circulating sex steroids influence kisspeptin responsiveness. For example, as noted above, healthy women in different phases of the menstrual cycle have different responses to kisspeptin. However, the effects of sex steroids are complex: hypogonadal women with hypothalamic amenorrhea appear to have a robust response to kisspeptin,^{36,37} whereas we have demonstrated that hypogonadal postmenopausal women have negligible responses to kisspeptin.³¹ Thus while both groups of women are hypogonadal, they have remarkably different responses to kisspeptin.

Third, stimulation of the reproductive endocrine axis can be achieved by both boluses and infusions of kisspeptin. Taking healthy men as an example, the effects of a bolus of kisspeptin are straightforward: a bolus of kisspeptin stimulates a pulse of LH secretion. The effects of

kisspeptin infusions are more complex. We have administered infusions of kisspeptin in another protocol (#2011P001564 “Elucidating Kisspeptin Physiology by Blocking Kisspeptin Signaling”), the goal of which is to *suppress* the reproductive endocrine axis using *long* infusions of kisspeptin to achieve desensitization of the kisspeptin receptor. We found that 24-hour infusions of kisspeptin to healthy men produced a 3-phase response: LH first rose (stimulation), then reached a plateau (steady state), then began to fall (desensitization). This stimulation of the reproductive endocrine axis by infusions of kisspeptin has also been observed in other studies.^{19,31,43,48} Because the goal of this protocol is *stimulate* the reproductive endocrine axis, *shorter* infusions of kisspeptin (up to 12 hours) will be used in this protocol.

Fourth, infusions of kisspeptin may be the only effective way to stimulate the reproductive endocrine axis in some pathophysiologic states. For example, we have demonstrated that patients with IHH have failed to respond to single and multiple boluses of kisspeptin.²⁵ However, a genotypic subset of individuals with IHH responded when given a kisspeptin infusion of 8 hours duration.³² Thus, the mode of administration of kisspeptin can influence kisspeptin responsiveness. Additional studies, such as those planned under this protocol, are needed to understand how genotype influences responsiveness to both boluses and infusions of kisspeptin.

Therefore, to fully explore GnRH neuronal biology in both physiologic and pathophysiologic states, the goal of this protocol is to stimulate GnRH secretion by administering the 10-amino-acid isoform of kisspeptin:

- as both boluses and short infusions
- using a range of doses
- using IV and SC routes of administration
- in conjunction with manipulation of the circulating sex-steroid milieu (either by administering sex steroids such as estrogen, progesterone, or testosterone, or by temporarily withholding a subject’s hormone-replacement therapy)
- in a variety of subject populations, including healthy men and women and patients with disorders of GnRH secretion/action.

Kisspeptin Safety Data in Humans:

Kisspeptin has a track record of safety in humans. It has been given to over 150 subjects, both healthy volunteers and patients with reproductive conditions, in studies at 3 centers using 2 different isoforms at a wide range of doses.^{19-21,34,36,37,43}

Importantly, no serious adverse events related to kisspeptin have occurred in our studies or, to our knowledge, in studies conducted by other investigators. Kisspeptin has been given to women undergoing ovulation induction for *in vitro* fertilization which has resulted in 12 successful pregnancies with no adverse effects^{38,50} Kisspeptin has also been given with no adverse effects to subjects with medical conditions such as type 2 diabetes, hypothyroidism, hypertension, hyperlipidemia, and anxiety/depression, and who were taking prescription medications for these conditions.⁵¹ (SB Seminara, unpublished results, J. George, personal communication, March 24, 2013, and C. Jayasena, personal communication, June 15, 2013). Full details are available in the Kisspeptin Investigator Brochure.

Design of the Study

To assess the physiologic response to the IV or SC administration of the C-terminal decapeptide of human kisspeptin (kisspeptin 112-121), frequent blood sampling (2-52 h) will be

performed to provide detailed neuroendocrine characterization of endogenous LH secretion prior to kisspeptin administration and to evaluate the response after kisspeptin administration.

II. SPECIFIC AIM

To employ exogenous administration of kisspeptin to directly interrogate human GnRH neurons *in vivo* using GnRH-induced LH secretion as a surrogate marker of adequate GnRH neuronal fate specification, migration, hormone synthesis, secretion, and action.

Hypothesis: In patients with IHH, repetitive stimulation with kisspeptin is needed before a response is seen.

III. SUBJECT SELECTION

The goal of this protocol is to administer kisspeptin to: men and women with hypogonadotropic hypogonadism (n= 24: 12 males, 12 females to have 10 males and 10 females complete the study).

All subjects will meet the following criteria:

History:

- 18 years or older,
- confirmed diagnosis by low sex steroids in the setting of low or inappropriately normal gonadotropins,
 - o If needed to verify clinical criteria, additional diagnostic labs and imaging tests may be performed including but not limited to: ferritin, prolactin, sex steroids, FSH, LH and brain MRI.
- all medical conditions stable and well controlled,
- no prescription medications known to affect reproductive endocrine function for at least 2 months or for 5 half-lives of the drug (whichever is shorter) except for medications used to treat the subject's reproductive condition,
- no history of a medication reaction requiring emergency medical care,
- no illicit drug use,
- no excessive alcohol consumption (<10 drinks/week),
- for women, not breastfeeding and not pregnant,
- if applicable, able to undergo appropriate washout from hormone therapy.

Physical Examination:

- normal blood pressure, (systolic BP < 140 mm Hg, diastolic < 90 mm Hg).

Laboratory Studies:

- white blood cell, platelet counts, and TSH between 90% of the lower limit and 110% of the upper limit of the reference range,
- prolactin below 110% of the upper limit of the reference range with the exception of participants with hyperprolactinemia who by definition will have elevated levels of prolactin.
- hemoglobin
 - o women: no less than 0.5 gm/dL below the lower limit of the reference range for normal women,
 - o men:
 - on adequate testosterone replacement therapy: normal male reference range,

- off testosterone replacement therapy: no lower than 0.5 gm/dL below the lower limit of the reference range for normal women (> 11.5 gm/dL in our laboratory), as men with hypogonadism off treatment have low serum testosterone levels which causes hemoglobin concentrations to fall to the female range,
- BUN, creatinine not elevated,
- AST, ALT <2x upper limit of reference range,
- for women, negative serum hCG pregnancy test at the time of screening (additional urine pregnancy test will be conducted prior to drug administration).

Additional criteria based on subject population:

1. Men and women with hypogonadotropic hypogonadism (n= 120: 80 males, 40 females to have 40 males and 20 females complete the study). All subjects will have confirmed diagnosis by low sex steroids in the setting of low or inappropriately normal gonadotropins. If needed to verify clinical criteria, additional diagnostic labs and imaging tests may be performed including but not limited to: ferritin, prolactin, sex steroids, FSH, LH and brain MRI.

IV. SUBJECT ENROLLMENT

Subjects will be recruited from postings and advertisements, from patients in the Reproductive Endocrine Associates, and from a pool of subjects who previously participated in research studies in the REU and have given written consent to be contacted about future studies. We will also use internet postings including CR.NET, Clinicaltrials.gov, and the REU web site so that interested subjects may contact us about the study. We will also utilize the REU database and the Partners RPDR or the Biobank Portal to identify patients with reproductive disorders. Partners RPDR or the Biobank Portal will be utilized to identify patients who are part of the Partners Biobank or within the Partners system who may be eligible for this protocol. We may also use identified information from the RPDR or from the Electronic Medical Record on consented Biobank subjects to review charts to determine eligibility. Clinical providers from other units at MGH may also directly alert the study team about potential subjects. We will review medical records to determine if these patients are potential candidates for study participation. Potential subjects identified through RPDR or by their clinical providers, will first be contacted through their clinical provider by phone, email, letter, or in person. Participants who are enrolled in the Biobank will have had provided consent per the Partners Biobank IRB approved protocol.

Our study will collaborate with the Partners Biobank to contact potential study subjects using a re-contact letter to be sent from the Biobank staff. Patients who consent to participate in the Partners Biobank provide consent to be re-contacted by the Biobank in the future for additional information/samples, and/or to be asked if they would be interested in joining other research studies. The Partners Biobank will send an invitation letter, co-signed by the Biobank PI and our PI, and opt-in or opt-out letter to patients. The Biobank will also provide us with an identified data set that includes patient MRNs, names, phone numbers and mailing addresses for all recontacted patients. Ten business days after the Biobank sends the recontact letters to patients, we will call each patient on the list (who did not choose to opt-out) to ask whether they would like to participate in our study. We may provide the Biobank with a list of the response status for each patient so that the Biobank may measure the success rate for the recontact process.

Pre-screening

Participants may be asked to complete a list of preliminary questions, in person or via phone, that will help us determine their eligibility. Identifiable information for individuals who are not eligible will be stored separately from collected health information.

The consent process will take place prior to the commencement of study procedures and will in all instances include a complete explanation of the study by one of the study medical professionals (physicians, physician assistants, or nurse practitioners.) Study staff will go over the purpose of the study, study procedures, potential risks of participation, and alternatives to participation. Before signing a PHRC-approved consent form, potential participants will be given the opportunity to ask any questions that they may have concerning the study to the study investigators. The voluntary nature of participation in these studies will be underscored. It will be made clear that a decision not to participate will in no way affect the relationship of patient to physician, investigator, or institution regarding the ongoing provision of care. Finally, a copy of the signed consent form containing all the information outlined above as well as contact information for the physician/investigator will be provided to the study participant.

Consent may be obtained either in-person at Massachusetts General Hospital or remotely. In all instances, the consent form will be sent to the subject by mail or electronically in advance of the consent process and all steps of the consent process as described above will be completed.

When consent is obtained in-person, subject will sign the consent form in the presence of the study investigator after all parts of the consent process are completed. Study investigator will sign the consent form at the time of the visit as well.

When consent is obtained remotely, study investigator will complete the consent process, as described above, over the phone. Subject will sign the consent form after all parts of the consent process are completed. The subject will have the option to sign the consent form using Adobe eSign or to sign using a handwritten signature on a physical copy of the consent form.

- If subject signs using Adobe eSign, study investigator will confirm subject's identity before signature is obtained. The investigator who conducted the call will sign the consent form upon receipt using Adobe eSign as well. A PDF file of the consent form and Adobe eSign audit report will be saved in the subject's file.
- If a physical copy of the consent form is signed, subject will return the consent form to study team via mail or at next in-person visit. The study investigator who conducted the call will sign the consent form upon receipt.

In all instances when consent is obtained remotely, study investigator will confirm that subject has signed the consent form prior to start of study activities. A copy of the signed consent form will be sent to the subject by mail or electronically. Study team will use encrypted email communication to send the signed consent form electronically. The method of signature and date and time of the phone call will be noted in the subject's file.

V. STUDY PROCEDURES

General Outline of Protocol

The goal of these studies is to assess the GnRH response to kisspeptin in patients with reproductive disorders. GnRH cannot be measured in the peripheral circulation, and so LH will be measured as a surrogate marker of GnRH secretion. LH is secreted by the pituitary in direct response to GnRH, and there is a well-established one-to-one concordance between GnRH and LH pulses.^{52,53}

The protocol will consist of up to 4 visits:

- 1) An outpatient screening visit,
- 2) An outpatient visit for placement of GnRH pump,
- 3) Admission to the Clinical Research Center (CRC) to assess the subject's response to kisspeptin and GnRH,
- 4) If applicable, a follow-up outpatient visit.

The total amount of blood drawn across all parts of the study will not exceed PHRC guidelines.

Protocol Details

1. Outpatient Screening Visit:

The screening visit will include a complete history and physical examination and blood tests to ensure subjects meet the inclusion criteria. The total volume of blood drawn during screening is 18 ml.

Some subjects may travel from a distance to participate. For subjects traveling long distances to participate, the screening history may be obtained by phone. Documentation of a physical examination performed by a local physician within the previous 12 months may be used in lieu of an in-person physical examination; in this case, a physical examination will also be performed by a study physician at the CRC visit. To obtain physical examination results performed off-site, subjects will be given an MGH "authorization for disclosure of medical information from another facility" form. If a physical examination is performed solely for the purposes of this study, study funds will pay for the cost of the physical examination. Screening laboratory studies will be performed at MGH on a blood sample shipped by the subject or at a local laboratory site (e.g. LabCorp, Quest, etc.); study funds will pay for all phlebotomy and shipping costs. Laboratory studies already performed within the previous 6 months will be in lieu of repeating screening studies unless abnormal.

2. Pituitary "priming":

Subjects may be asked to undergo pituitary "priming" with GnRH. One consequence of impaired GnRH secretion is that, similarly to a muscle that undergoes atrophy with disuse, the pituitary gland may secrete LH only weakly in response to GnRH. Because we will rely on measurement of LH as a reflection of GnRH secretion, it is essential that the pituitary respond normally to GnRH. Fortunately, a normal pituitary response to GnRH can be restored in patients with hypogonadotropic hypogonadism by administering GnRH to "prime" the pituitary gland⁵⁴. This involves using a pump device to deliver GnRH, as intermittent administration of GnRH is essential to achieving a normal response⁵⁵. The MGH Reproductive Endocrine Unit has a long history of using pump devices to administer pulsatile GnRH to achieve pituitary priming (as well as restoration of reproductive function and fertility; MGH protocol 1999-P-003752), and these well-established methods are used in our protocol.

- Priming: Priming is achieved by administering pulsatile GnRH via an infusion pump for at least 4 days.
 - o Pump placement: The subcutaneous catheter for the pump will be placed by a study physician or by the research subject after proper instruction from a study physician before the scheduled date of a CRC admission. This may be done as a separate outpatient visit to the Reproductive Endocrine Unit or in the research subject's home.

- o Pump settings: The pump (Crono “F” pump) will be programmed to administer 25 ng/kg SC q2h as per long-standing protocols (e.g., MGH IRB 1999-P-003752). The subject will be instructed on when and how to turn the pump on and off, how to care for the pump, and who and when to call if problems arise. The pump will administer GnRH at least 4 days and up to 14 days.

3. Admissions to Clinical Research Center:

The general structure of visits is:

- a period of q10 minute blood sampling without intervention to assess endogenous hormone secretion,
- administration of kisspeptin followed by q10 minute blood sampling to assess the response to kisspeptin, and
- administration of GnRH followed by q10 minute blood sampling to assess the response to GnRH.

The precise details of these visits will fall within the following parameters:

- Frequent blood draws (3-5 mL) X up to 52 hours; such that the total amount of blood drawn during the CRC admissions does not exceed 550mL.
- Kisspeptin administration as one or more IV or SC boluses and/or an IV infusion
 - o The dose of each kisspeptin bolus will not exceed 13.19 mcg/kg (10 nmol/kg)
 - o Kisspeptin boluses will be administered no more frequently than once an hour
- GnRH will be given at doses ranging from 2.5 to 250 ng/kg (usually 25-75 ng/kg), no more frequently than hourly.

Justification of doses:

- The largest bolus dose of kisspeptin to be given (13.1 mcg/kg = 10 nmol/kg) is equal the largest IV dose of kisspeptin 112-121 used in human studies in the literature to date.¹⁹
- GnRH doses of 25-75 ng/kg give LH responses in the physiological range in most subjects. Under protocols in the Reproductive Endocrine Unit, subjects have been given GnRH at doses up to 800 ng/kg every 2 hours for up to 12 months with no significant adverse effects.^{56,57}

Females will undergo a pregnancy test at screening and again at admission prior to receiving kisspeptin. Subjects will receive isovolumetric replacement with saline during blood sampling. Kisspeptin will be administered by a CRC RN. Vital signs (HR, BP, RR, and T) will be measured every 1-4 hours throughout the frequent sampling portions of the protocol and every 10 minutes for up to 30 minutes after kisspeptin administration.

Termination criteria will include: 1) blood pressure > 180 mmHg systolic or >105 mmHg diastolic at any point during the protocol; 2) development or suspicion of an allergic or serious adverse reaction; 3) positive pregnancy test; 4) temperature $\geq 101.5^{\circ}\text{F}$ at any point before kisspeptin administration. Any subject who develops high blood pressure or an allergic reaction will receive follow-up inpatient medical care until the issue is resolved, with subsequent outpatient and/or phone follow-up as needed. The CRC is fully equipped to handle medical emergencies, with trained staff, a bag-valve mask in the room, a crash cart on the floor, and rapid access to a physician code team.

4. Follow-up phone calls:

All participants will receive a post-treatment phone call within 48 h of discharge from the Clinical

Research Center. This phone call will document any adverse events. If there are any significant adverse events, these will be evaluated with a follow-up clinic visit. An additional post-treatment phone call will be made 5-7 days after discharge from the Clinical Research Center.

5. Final follow-up study visit (under specific circumstances):

When a significant adverse event is reported, subjects will be asked to attend a follow-up visit. To date, we have not detected any consistent subjective complaints, physical examination findings, or laboratory abnormalities at follow-up visits. If any test result is abnormal, the subject will be followed until screening tests (CBC, BUN, creatinine, liver function studies) return to the ranges specified in eligibility criteria.

For subjects traveling long distances to participate, the interval history may be acquired in a phone conversation. Documentation of a physical examination performed by a local physician may be used in lieu of a follow-up physical examination. To obtain physical examination results performed off-site, subjects will be given an MGH "authorization for disclosure of medical information from another facility" form. If a physical examination is performed solely for the purposes of this study, study funds will pay for the cost of the physical examination. Follow-up laboratory studies will be performed at MGH on a blood sample mailed from the subject or at a local laboratory site (e.g. LabCorp, Quest, etc.); study funds will pay for all phlebotomy and shipping costs.

Where does the kisspeptin come from and how is it prepared?

Ten grams of GMP grade kisspeptin 112-121 have been synthesized by the NIH and are available for our use. Although this material is of the highest quality, a second check on this peptide will also be performed, with testing for sterility and pyrogenicity. The peptide is undergoing ongoing stability testing in collaboration with Henry Keutmann, M.D. of the MGH Endocrine Unit who has purified and analyzed several peptides used by the Reproductive Endocrine Unit in the past and in consultation with members of the Research Pharmacy Investigational New Drug/Peptide Division. The kisspeptin is prepared and stored by Research Pharmacy for the entirety of the protocol. On the day of the study the dose of kisspeptin will be prepared by Research Pharmacy, brought by study staff to a CRC nurse for refrigerated storage until use.

Where does the GnRH come from and how is it prepared?

GMP grade GnRH is synthesized by Polypeptide Labs. Although this material is of the highest quality, a second check on this peptide will also be performed, with testing for sterility and pyrogenicity.

Use of Samples and Data:

Any excess sera from the overnight frequent sampling study will be coded and stored in the REU lab. Each sample will be given a unique identifying code and will be stored for improving existing assays as well as developing new assays. Use of these specimens will be targeted at furthering our understanding of reproductive disorders and improving the management of subjects with these disorders. IRB approval will be obtained if samples are used in the future for purposes not described in the protocol.

Information will be stored in the Progeny database. Each study participant is assigned an identification number. This number becomes the unique identifier for the patient in the database. Only one table in the Progeny database houses the patient's name and unique identifier. The database is maintained on a MGH server subdirectory. Passwords are required to enter the

server. Access to the subdirectory is restricted to members of the research team. The Progeny database is password-protected.

All data (including data from other Reproductive Endocrine Unit studies they have completed or will participate in) may be collectively used for ongoing research and to better our understanding of reproductive disorders. This includes results utilizing results from genetic studies performed under protocol 1999-P-00006955 "Molecular Basis of Inherited Reproductive Disorders". Genetic screening results may be used to help interpret response to kisspeptin administration. IHH subjects participating in the present proposal will be given the option of also participating in the genetics study if they have not done so already.

VI. BIOSTATISTICAL ANALYSIS

Assays

Given the long-term ongoing nature of this study, the assay methodologies employed to characterize the biochemical profile (both reproductive and metabolic) will be based on standard accepted methods as established by the MGH Pathology department and the Reproductive Endocrine Reference Laboratory. As such these methods may include immunoassays, measurement using standard laboratory platforms, radioimmunoassays, and mass spectroscopy. We anticipate that these methods will continue to evolve as the diagnostics field changes.

Data analysis

The hypothalamic-pituitary axis will be analyzed using the following parameters:

- a) Arithmetic mean levels for LH and FAS based on q10 min blood sampling
Analysis of pulsatile hormone secretion of LH and FAS using a modification of the Santen and Bardin Method ⁶⁰ augmented by deconvolution analysis ²¹. If evidence of endogenous GnRH secretion is present, GnRH pulse frequency will be determined by calculating the number of LH and FAS pulses during the sampling interval. LH pulse amplitude will be calculated from the difference between the nadir and peak hormone levels.
- b) FSH and sex steroid levels from 2-h sample pools will be compared using analysis of variance with repeated measures to assess their responsiveness to kisspeptin.

VII. RISKS AND DISCOMFORTS

The risks involved with this study are associated with the frequent blood sampling , administration of kisspeptin and GnRH, and any additional diagnostic testing.

Blood Draws and IV Lines:

There is a slight discomfort associated with the placement of IV lines and a small risk of infection at blood draw sites. There is a slight chance that an IV line placed will fail. In the event this occurs, a new IV line will be placed. Subjects may faint from blood drawing or develop bruise or irritation at the site of the blood draw. In addition, it is possible that subjects could develop anemia from blood-drawing. Subjects will be instructed to avoid donating blood or having large blood draws for 8 weeks before and after the study.

Subcutaneous administration:

There is a possibility of a reaction at the site of SC administration including: pain, tenderness, warmth, itching, swelling, irritation, redness, or bruising.

Investigational New Drug (Kisspeptin IND, also known as, Metastin 45-54 IND # 74, 977 and 74,978):

IND # 74,977 is for women and IND # 74,978 is for men; therefore, this protocol is performed under both INDs.

Four findings point to the safety of kisspeptin:

- 1) Kisspeptin is a peptide that is produced endogenously.
- 2) Blood levels of kisspeptin rise 7,000- to 10,000-fold across human gestation.⁶¹ This clearly does not have long-term effects on women or their later reproductive capacity.
- 3) We have performed two years of pre-clinical toxicology testing giving kisspeptin to rats and dogs, and have not observed untoward effects.
- 4) Our group has acquired substantial experience with human administration of kisspeptin. To date, we have administered kisspeptin 112-121 to over 80 healthy volunteers, over 40 patients with IHH, and 8 patients with hyperprolactinemia and we have observed no adverse effects related to kisspeptin. Subjects are monitored closely during their CRC admission, and no subjective symptoms have been reported or abnormal vital signs observed. At the outpatient follow-up visit 1-2 weeks after the CRC admission, some subjects reported fatigue that lasted no more than 1 day after the CRC admission. Otherwise, subjects have reported no abnormal subjective symptoms, and there have been no abnormal physical examination findings or laboratory abnormalities.

These points in combination with the experience of other groups with human kisspeptin administration demonstrate the safety and tolerability of kisspeptin.

Investigational New Drug (GnRH IND #93,353)

Administration of GnRH has been a cornerstone of physiologic investigation in the Reproductive Endocrine Unit here at MGH for the past 30 years. We have accumulated a large clinical experience and have treated many GnRH deficient men without any significant complications. In this protocol, the administration of Kisspeptin and GnRH will be separated by at least 1 hour; they would not be given simultaneously. In pre-clinical studies examining the stability of kisspeptin in human plasma, the half-life of kisspeptin is 55 seconds, with >99% of kisspeptin 112-121 degraded within 30 minutes²¹. Similarly, the *in vivo* half-life of kisspeptin in humans is 4 minutes.⁶²

VIII. POTENTIAL BENEFITS

There are no direct benefits to patients that are anticipated from the administration of kisspeptin. The only benefit from participating in this study comes from the altruistic contribution to furthering our understanding of the biology of human reproduction.

IX. MONITORING AND QUALITY ASSURANCE

Prior to initiation of the protocol, the Partners Human Research Committee (PHRC) will have reviewed and approved the protocol and consent form for this study. The PHRC reviews the progress of the trial at least annually following initial approval of the protocol and consent form, and may require the Investigator to provide information following reports of adverse events and/or protocol amendments.

The Investigator and all key personnel involved in this study have completed the Collaborative IRB Training Initiative (CITI), a mandatory tutorial on the responsible conduct of human subject research. This tutorial covers MGH policy and federal regulations governing human subject research commonly known as Good Clinical Practices. The tutorial and institutional policies and procedures governing the conduct of human subjects research are available on the MGH

Internet website and may be accessed by the Investigator and key study personnel if questions arise.

Adverse Event (AE) Reports:

The Investigator will report adverse events experienced by study subjects in accordance with PHRC guidelines to the PHRC and the FDA.

Unanticipated and Serious Adverse Event Reports:

As per regulations CFR 21 Part 312, the Investigator will report Unanticipated and Serious Adverse Events to the FDA in accordance with the PHRC and FDA reporting guidelines. The Investigator will report Unanticipated and Serious Adverse Events to the PHRC in accordance with PHRC reporting guidelines. In addition, SAEs that occur within 30 days after a subject's active participation in the study and that are categorized as definitely, probably, or possibly related to the study will be reported to the PHRC.

Investigational New Drug (IND): Kisspeptin IND #s 74, 977 and 74,978, GnRH IND #93,353

The Principal Investigator, who is also the kisspeptin IND holder, will notify the FDA and all participating investigators in a written IND safety report of any adverse experience associated with the use of the drug that is both serious and unexpected or any finding from tests in laboratory animals that suggest a significant risk for human subjects, including reports of mutagenicity, teratogenicity, or carcinogenicity. Each notification will be made as soon as possible and no greater than 15 calendar days after the initial receipt of the information. Each written notification will be submitted on an FDA Form 3500A. The Principal Investigator will also notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days after the initial receipt of the information.

On an annual basis as part of the update to the study IND, the Principal Investigator will submit to the FDA: A summary of all IND safety reports submitted during the past year, a list of all subjects who died during the participation in the investigation, listing cause of death for each, a list of subjects who dropped out during the course of the investigation in association with any adverse experience, whether or not thought to be drug related.

Investigational New Drug (GnRH IND #93,353)

The Principal Investigator, who is also the kisspeptin IND holder, will notify the FDA and all participating investigators in a written IND safety report of any adverse experience associated with the use of the drug that is both serious and unexpected or any finding from tests in laboratory animals that suggest a significant risk for human subjects, including reports of mutagenicity, teratogenicity, or carcinogenicity. Each notification will be made as soon as possible and no greater than 15 calendar days after the initial receipt of the information. Each written notification will be submitted on an FDA Form 3500A. The Principal Investigator will also notify the FDA by telephone or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the drug as soon as possible but no later than 7 calendar days after the initial receipt of the information.

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Monitoring:

The Principal Investigator is responsible for oversight of the trial. The study will also be monitored by an independent Data Safety Monitoring Board that will meet once a year.

The DSMB responsibilities will include:

- Review of the research protocol, informed consent documents and plans for safety and data monitoring of the study. This is to determine the risks and benefits to research subjects, protection and safety of the subjects and to offer suggestions for improving the study design.
- Review of all adverse events (expected, unexpected and serious), enrollment, dropout rates and protocol deviations conducted to ensure subject safety. The Board will be provided with reports of serious adverse events as they occur and may request additional information from the Investigator in the course of its review.
- Evaluate the progress of the trial, including periodic assessments of data quality/completeness, recruitment goals, protocol adherence, accrual and retention of participants and other factors that may affect the study outcome. Protect confidentiality of the study participants, trial data and results of the monitoring.
- Weigh efficacy and adverse events to determine if the trial should continue as originally designed, should be changed or should be stopped based on the data.

A copy of the minutes will be distributed to the DSMB members, the Investigator and the PHRC. The minutes will reflect which of the following three determinations the DSMB may make as a result of its review: 1) the study may continue without modification/amendment; 2) the study should be amended; 3) the study should terminate.

REFERENCES

1. Hoffman AR, Crowley WF, Jr. Induction of puberty in men by long-term pulsatile administration of low-dose gonadotropin-releasing hormone. *N Engl J Med* 1982;307:1237-41.
2. Seminara SB, Hayes FJ, Crowley WF, Jr. Gonadotropin-releasing hormone deficiency in the human (idiopathic hypogonadotropic hypogonadism and Kallmann's syndrome): pathophysiological and genetic considerations. *Endocr Rev* 1998;19:521-39.
3. Seminara SB, Messager S, Chatzidaki EE, et al. The GPR54 gene as a regulator of puberty. *N Engl J Med* 2003;349:1614-27.
4. Teles MG, Bianco SD, Brito VN, et al. A GPR54-activating mutation in a patient with central precocious puberty. *N Engl J Med* 2008;358:709-15.
5. Ohtaki T, Shintani Y, Honda S, et al. Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature* 2001;411:613-7.
6. Muir AI, Chamberlain L, Elshourbagy NA, et al. AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. *J Biol Chem* 2001;276:28969-75.
7. Kotani M, Detheux M, Vandenbogaerde A, 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;276:34631-6.
8. Gottsch ML, Cunningham MJ, Smith JT, et al. A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. *Endocrinology* 2004;145:4073-7.
9. Thompson EL, Patterson M, Murphy KG, et al. Central and peripheral administration of kisspeptin-10 stimulates the hypothalamic-pituitary-gonadal axis. *J Neuroendocrinol* 2004;16:850-8.

10. Matsui H, Takatsu Y, Kumano S, Matsumoto H, Ohtaki T. Peripheral administration of metastatin induces marked gonadotropin release and ovulation in the rat. *Biochem Biophys Res Commun* 2004;320:383-8.
11. Navarro VM, Fernandez-Fernandez R, Castellano JM, et al. Advanced vaginal opening and precocious activation of the reproductive axis by KiSS-1 peptide, the endogenous ligand of GPR54. *J Physiol* 2004;561:379-86.
12. Navarro VM, Castellano JM, Fernandez-Fernandez R, et al. Characterization of the potent luteinizing hormone-releasing activity of KiSS-1 peptide, the natural ligand of GPR54. *Endocrinology* 2005;146:156-63.
13. Navarro VM, Castellano JM, Fernandez-Fernandez R, et al. Effects of KiSS-1 peptide, the natural ligand of GPR54, on follicle-stimulating hormone secretion in the rat. *Endocrinology* 2005;146:1689-97.
14. Messenger S, Chatzidaki EE, Ma D, et al. Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. *Proc Natl Acad Sci U S A* 2005;102:1761-6.
15. Shahab M, Mastronardi C, Seminara SB, Crowley WF, Ojeda SR, Plant TM. Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates. *Proc Natl Acad Sci U S A* 2005;102:2129-34.
16. Lapatto R, Pallais JC, Zhang D, et al. Kiss1-/- mice exhibit more variable hypogonadism than Gpr54-/- mice. *Endocrinology* 2007;148:4927-36.
17. Gutierrez-Pascual E, Martinez-Fuentes AJ, Pinilla L, Tena-Sempere M, Malagon MM, Castano JP. Direct pituitary effects of kisspeptin: activation of gonadotrophs and somatotrophs and stimulation of luteinising hormone and growth hormone secretion. *J Neuroendocrinol* 2007;19:521-30.
18. Sonigo C, Bouilly J, Carre N, et al. Hyperprolactinemia-induced ovarian acyclicity is reversed by kisspeptin administration. *J Clin Invest* 2012;122:3791-5.
19. Jayasena CN, Nijher GM, Comninou AN, et al. The effects of kisspeptin-10 on reproductive hormone release show sexual dimorphism in humans. *J Clin Endocrinol Metab* 2011;96:E1963-72.
20. George JT, Veldhuis JD, Roseweir AK, et al. Kisspeptin-10 is a potent stimulator of LH and increases pulse frequency in men. *J Clin Endocrinol Metab* 2011;96:E1228-36.
21. Chan YM, Butler JP, Pinnell NE, et al. Kisspeptin resets the hypothalamic GnRH clock in men. *J Clin Endocrinol Metab* 2011;96:E908-15.
22. George JT, Veldhuis JD, Tena-Sempere M, Millar RP, Anderson RA. Exploring the pathophysiology of hypogonadism in men with type 2 diabetes: kisspeptin-10 stimulates serum testosterone and LH secretion in men with type 2 diabetes and mild biochemical hypogonadism. *Clin Endocrinol (Oxf)* 2013;79:100-4.
23. Chan YM, Butler JP, Sidhoum VF, Pinnell NE, Seminara SB. Kisspeptin administration to women: a window into endogenous kisspeptin secretion and GnRH responsiveness across the menstrual cycle. *J Clin Endocrinol Metab* 2012;97:E1458-67.
24. George JT, Anderson RA, Millar RP. Kisspeptin-10 stimulation of gonadotrophin secretion in women is modulated by sex steroid feedback. *Hum Reprod* 2012;27:3552-9.
25. Chan Y-M, Sidhoum VF, Lippincott MF, Seminara SB. Non-Kisspeptin-Responsive GnRH Neurons in Patients With Idiopathic Hypogonadotropic Hypogonadism. *ENDO* 2013, June 15, 2013.
26. Francou B et al. Normosmic congenital hypogonadotropic hypogonadism due to TAC3/TACR3 mutations: prevalence, characterization of neuroendocrine phenotypes and novel mutations. *ENDO* 2011, June 4, 2011.
27. Chan Y-M, Lippincott MF, Butler JP, et al. Exogenous Kisspeptin Administration as a Probe of GnRH Neuronal Function in Patients With Idiopathic Hypogonadotropic Hypogonadism. *The Journal of Clinical Endocrinology and Metabolism* 2014;99:E2762-E71.
28. Lippincott MF, Chan YM, Delaney A, Rivera-Morales D, Butler JP, Seminara SB. Kisspeptin Responsiveness Signals Emergence of Reproductive Endocrine Activity: Implications for Human Puberty. *J Clin Endocrinol Metab* 2016;101:3061-9.
29. Lippincott MF, Leon S, Chan YM, et al. Hypothalamic Reproductive Endocrine Pulse Generator Activity Independent of Neurokinin B and Dynorphin Signaling. *J Clin Endocrinol Metab* 2019;104:4304-18.
30. Skorupskaite K, George JT, Veldhuis JD, Millar RP, Anderson RA. Interactions Between Neurokinin B and Kisspeptin in Mediating Estrogen Feedback in Healthy Women. *J Clin Endocrinol Metab* 2016;101:4628-36.

31. Lippincott MF, Chan Y-M, Sidhoum VF, Seminara SB. Continuously Administered Kisspeptin as a Pseudo-antagonist of the Kisspeptin Receptor ENDO 2013, June 15, 2013:SAT-151.
32. Young J, George JT, Tello JA, et al. Kisspeptin restores pulsatile LH secretion in patients with neurokinin B signaling deficiencies: physiological, pathophysiological and therapeutic implications. *Neuroendocrinology* 2013;97:193-202.
33. Millar RP, Sonigo C, Anderson RA, et al. Hypothalamic-Pituitary-Ovarian Axis Reactivation by Kisspeptin-10 in Hyperprolactinemic Women With Chronic Amenorrhea. *J Endocr Soc* 2017;1:1362-71.
34. Dhillon WS, Chaudhri OB, Thompson EL, et al. Kisspeptin-54 stimulates gonadotropin release most potently during the preovulatory phase of the menstrual cycle in women. *J Clin Endocrinol Metab* 2007;92:3958-66.
35. Jayasena CN, Comninou AN, Veldhuis JD, et al. A single injection of kisspeptin-54 temporarily increases luteinizing hormone pulsatility in healthy women. *Clin Endocrinol (Oxf)* 2013;79:558-63.
36. Jayasena CN, Nijher GM, Abbara A, et al. Twice-weekly administration of kisspeptin-54 for 8 weeks stimulates release of reproductive hormones in women with hypothalamic amenorrhea. *Clin Pharmacol Ther* 2010;88:840-7.
37. Jayasena CN, Nijher GM, Chaudhri OB, et al. Subcutaneous injection of kisspeptin-54 acutely stimulates gonadotropin secretion in women with hypothalamic amenorrhea, but chronic administration causes tachyphylaxis. *J Clin Endocrinol Metab* 2009;94:4315-23.
38. Abbara A, Jayasena CN, Nijher GM, et al. Kisspeptin - A Novel Physiological Trigger for Oocyte Maturation in IVF Treatment. ENDO 2013, June 17, 2013:FP36-3.
39. Abbara A, Jayasena CN, Christopoulos G, et al. Efficacy of Kisspeptin-54 to Trigger Oocyte Maturation in Women at High Risk of Ovarian Hyperstimulation Syndrome (OHSS) During In Vitro Fertilization (IVF) Therapy. *J Clin Endocrinol Metab* 2015;100:3322-31.
40. Abbara A, Clarke S, Islam R, et al. A second dose of kisspeptin-54 improves oocyte maturation in women at high risk of ovarian hyperstimulation syndrome: a Phase 2 randomized controlled trial. *Hum Reprod* 2017;32:1915-24.
41. Comninou AN, Jayasena CN, Nijher GM, et al. Kisspeptin advances the menstrual cycle in healthy women. ENDO 2013, June 18, 2013.
42. Jayasena CN, Comninou AN, Nijher GM, et al. Twice-daily subcutaneous injection of kisspeptin-54 does not abolish menstrual cyclicity in healthy female volunteers. *J Clin Endocrinol Metab* 2013;98:4464-74.
43. Dhillon WS, Chaudhri OB, Patterson M, et al. Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. *J Clin Endocrinol Metab* 2005;90:6609-15.
44. Jayasena CN, Abbara A, Narayanaswamy S, et al. Direct comparison of the effects of intravenous kisspeptin-10, kisspeptin-54 and GnRH on gonadotrophin secretion in healthy men. *Hum Reprod* 2015;30:1934-41.
45. Comninou AN, Wall MB, Demetriou L, et al. Kisspeptin modulates sexual and emotional brain processing in humans. *J Clin Invest* 2017;127:709-19.
46. Izzi-Engbeaya C, Comninou AN, Clarke SA, et al. The effects of kisspeptin on beta-cell function, serum metabolites and appetite in humans. *Diabetes Obes Metab* 2018;20:2800-10.
47. Narayanaswamy S, Jayasena CN, Ng N, et al. Subcutaneous infusion of kisspeptin-54 stimulates gonadotrophin release in women and the response correlates with basal oestradiol levels. *Clin Endocrinol (Oxf)* 2016;84:939-45.
48. Abbara A, Jayasena CN, Ratnasabapathy R, et al. Continuous intravenous infusion of kisspeptin-54 stimulates pulsatile LH secretion in women with hypothalamic amenorrhea. ENDO 2013, June 17, 2013.
49. Seminara S, Chan YM, Lippincott M. Kisspeptin Administration in Postmenopausal Women. *Massachusetts General Hospital*; 2013.
50. Abbara A, Jayasena CN, Comninou AN, et al. Kisspeptin - a Novel Physiological Trigger for Oocyte Maturation in IVF Treatment Endocrine Society 2014 Oral Presentation; 2014.
51. George JT, Veldhuis JD, Tena-Sempere M, Millar RP, Anderson RA. Exploring the pathophysiology of hypogonadism in men with type 2 diabetes: Kisspeptin-10 stimulates serum testosterone and LH secretion in men with type 2 diabetes and mild biochemical hypogonadism. *Clin Endocrinol (Oxf)* 2012.
52. Levine JE, Pau KY, Ramirez VD, Jackson GL. Simultaneous measurement of luteinizing hormone-releasing hormone and luteinizing hormone release in unanesthetized, ovariectomized sheep. *Endocrinology* 1982;111:1449-55.

53. Clarke IJ, Cummins JT. The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. *Endocrinology* 1982;111:1737-9.
54. Yoshimoto Y, Moridera K, Imura H. Restoration of normal pituitary gonadotropin reserve by administration of luteinizing-hormone-releasing hormone in patients with hypogonadotropic hypogonadism. *The New England journal of medicine* 1975;292:242-5.
55. Belchetz PE, Plant TM, Nakai Y, Keogh EJ, Knobil E. Hypophysial responses to continuous and intermittent delivery of hypophthalamic gonadotropin-releasing hormone. *Science* 1978;202:631-3.
56. Abel BS, Shaw ND, Brown JM, et al. Responsiveness to a physiological regimen of GnRH therapy and relation to genotype in women with isolated hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 2013;98:E206-16.
57. Sykiotis GP, Hoang XH, Avbelj M, et al. Congenital idiopathic hypogonadotropic hypogonadism: evidence of defects in the hypothalamus, pituitary, and testes. *J Clin Endocrinol Metab* 2010;95:3019-27.
58. Jayasena CN, Comninou AN, Narayanaswamy S, et al. Acute and chronic effects of kisspeptin-54 administration on GH, prolactin and TSH secretion in healthy women. *Clin Endocrinol (Oxf)* 2014;81:891-8.
59. Melmed S, Casanueva FF, Hoffman AR, et al. Diagnosis and treatment of hyperprolactinemia: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011;96:273-88.
60. Santen RJ, Bardin CW. Episodic luteinizing hormone secretion in man. Pulse analysis, clinical interpretation, physiologic mechanisms. *J Clin Invest* 1973;52:2617-28.
61. Horikoshi Y, Matsumoto H, Takatsu Y, et al. Dramatic elevation of plasma metastin concentrations in human pregnancy: metastin as a novel placenta-derived hormone in humans. *J Clin Endocrinol Metab* 2003;88:914-9.
62. Jayasena CN, Nijher GM, Comninou AN, et al. The Effects of Kisspeptin-10 on Reproductive Hormone Release Show Sexual Dimorphism in Humans. *J Clin Endocrinol Metab*.
63. Lestishock L, Pariseau C, Rooholamini S, Ammerman S. Anaphylaxis from depot medroxyprogesterone acetate in an adolescent girl. *Obstet Gynecol* 2011;118:443-5.
64. Selo-Ojeme DO, Tillisi A, Welch CC. Anaphylaxis from medroxyprogesterone acetate. *Obstet Gynecol* 2004;103:1045-6.