

RESPONSE TO REVIEW: The reviewers requested clarification on following:

Human Studies:

--Why this protocol will be used and how the doses were defined: This grant challenges the dogma that patients with IHH are GnRH "deficient." Rather this grant hypothesizes that patients with IHH are relatively kisspeptin resistant and that 1) repetitive and/or 2) higher doses of kisspeptin can overcome this resistance. Thus, the protocol allows for the administration of kisspeptin IV every 2 hours over 40 hours (= 20 doses total). Moreover, the protocol employs a range of doses spanning 2 log orders. The doses chosen were based upon 10 years of human investigation (funded by this grant). The lowest dose (0.313 ng/kg), when given as a single IV bolus, results in a GnRH-induced LH response in healthy volunteers but not patients with IHH. The other doses are half log order increments above this dose. With this study design, the PI can evaluate the effects of both dose and time in repetitive kisspeptin administration.

--Genetic heterogeneity of the human subjects with IHH that is expected to increase the variability of the results: This grant hypothesizes kisspeptin resistance may supersede the underlying genetics of IHH/KS patients. In other words, genetics is not deterministic and patients with mutations in several different genes may respond to kisspeptin. This concept is supported by the Preliminary Data.

--Absence of genetic studies in Aim 1 to correlate them to the phenotyping to be done: The PI is Director of a National Center for Translational Research in Reproduction and Infertility which supports genetic investigations of patients with IHH/KS. Thus, the PI has access to all genetics data and will create those linkages, as shown in the Preliminary Data of this proposal.

--Potential role of kisspeptin resistance in delayed puberty and IHH: Up until now, when a child presents with delayed puberty, there have been no diagnostic tests available that can predict whether that child will enter spontaneous puberty by age 18 or not. If a child with delayed puberty responds to a single bolus of kisspeptin, he/she is NOT KISSPEPTIN RESISTANT; that child is predicted to enter puberty spontaneously. If a child with delayed puberty does not respond to a single bolus of kisspeptin, he/she IS KISSPEPTIN RESISTANT. That child is predicted to receive a diagnosis of IHH/KS by age 18.

Non-human primate studies:

--Shortcoming in the design of Aim 1: A deep characterization of the timing of sexual maturation in male macaques was performed in this funding cycle and has now been published.

--Value of the studies in Aim 2b: There has never before been an analysis of the role of dynorphin in the non-human primate, the animal which best approximates human puberty and reproduction. The proposed studies are critical to understanding the role of kisspeptin, neurokinin B, and dynorphin as a complex, interacting network that regulates reproduction and fertility.

--Lack of spatial resolution where dynorphin is released: This has been clarified in the body of the grant.

Both human and non-human primate studies:

--Inconsistent consideration of sex as a biologic variable was inconsistent: With one exception in the non-human primate, all studies in this grant include both males and females: 1) As shown by Dr. [REDACTED], co-Investigator, the hypothalamic circuitry regulating GnRH secretion is the same in male and female non-human primate and includes kisspeptin, NKB, and dynorphin. 2) As shown by the PI, males and females (including healthy volunteers and patients) show remarkable concordance in their responses to kisspeptin. Male and female healthy volunteers respond to a single kisspeptin IV bolus. Males and females with IHH do not. Boys and girls with constitutional delay of puberty (CDP) perform similarly in a kisspeptin stimulation test. 3) Although IHH and CDP are more prevalent in males, this is not thought to represent an intrinsic difference in the signals that trigger sexual maturation but rather a threshold effect of the signals that are present.

PLAN FOR THIS RESEARCH PROJECT GOING FORWARD, E.G., RE-ORGANIZATION OF THE WORK FOR A RESUBMISSION OR NEW GRANT SUBMISSION: The pink sheets for this grant did not point to major flaws in the grant's organization. Rather, the reviewers asked for clarification on topics, including the human protocols, underlying genetics, and relevance of the dynorphin studies in the non-human primate. With interim support, PI and her team will 1) maintain support for the entire kisspeptin administration program, which is a key component of this R01 and also Dr. [REDACTED]'s K23, 2) employ the PI's study team including [REDACTED] (Program Manager) and [REDACTED] (Clinical Research Coordinator) and 3) continue to gather preliminary data to support each of the hypotheses in this proposal. Since the original submission, more human data has been generated and this is included in this mini-grant application to ECOR.