

| Citation              | Species                                                       | Experimental Design                                                                                                                                                                                                                                                                                     | Evidence of Change in Kisspeptin(Kiss-1) Expression (CKE)                                                                                                                                                                                                                                                                                                                                                                                                                            | Evidence of Change in GnRH/LH/FSH Levels (CGL)                                                                                                                                                                                                                                                      | CKE observed? | CGL observed? | Relevant Notes                                                                                                                                                                                                                                                                                                              |
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| Adachi et al., 2007   | Adult Wistar-Imamichi strain rats( <i>Rattus norvegicus</i> ) | Organisms were ovariectomized(OVX) and castrated(CAS). Females received either a low E2(20 ug/mL estradiol in peanut oil) or high E2 treatment (crystalline E2 enough to produce 514.1 pg/ml of E2 levels). Males received either a testosterone(T) or high E2 treatment.; 14 hr-post estrogen exposure | significant increase in percentage of AVPV KiSS-1 mRNA-positive neurons with c-Fos immunoreactivity was significantly higher in the high E2-treated animals at 14:00 hr compared to 9:00 hr (60% c-fos activity in the AVPV KiSS-1 cells; 45% c-fos activity in the ARC KiSS-1 cells)<br>-significant increase of # of activated ARC KiSS-1 cells in Low-E2 exposure (100 vs 500 cells)<br>-significant difference of c-Fos immunoreactivity between high and low estrogen treatment | -increase of plasma LH present at 14:00 hr                                                                                                                                                                                                                                                          | Yes           | Yes           |                                                                                                                                                                                                                                                                                                                             |
|                       |                                                               | High E2-treated rats received either a brain injection of anti-rat metastin/kisspeptin monoclonal antibody or normal mouse IgG. LH assay done afterwards for LH surge.                                                                                                                                  | -significant increase in relative expression of KiSS-1 post OVX and high(2% vs 0.1%) and low estrogen (2.3% vs 0.1%) exposure in AVPV KiSS-1 cells<br>-significant decrease in relative expression of KiSS-1 cells post OVX and high estrogen exposure (2% vs 12%) and CAS and high estrogen exposure (2% vs 8%) in ARC KiSS-1 cells.                                                                                                                                                | -At hr 14, significant increase of plasma LH compared to anti-rat metastin treatment. LH surge present from 10:00hr - 18:00hr.<br>-No LH surge present in anti-rat metastin antibody treatment.                                                                                                     | Yes           | Yes           |                                                                                                                                                                                                                                                                                                                             |
| Alcin et al., 2013    | Adult Rhesus Monkey ( <i>Macaca mulatta</i> )                 | adult OVX rhesus monkeys divided into 4 experimental groups with empty-filled pills for 28 days, E2-filled pills for 28 days, empty-filled pills for 28 days and progesterone(P)-filled pills for the last 14 days, or E2-filled pills for 28 days and P-filled pills for the last 14 days              | -increased expression of Kiss1 due to OVX<br>-Abolished Kiss1 expression in the ARC Kiss-1 neurons with E2 treatments<br>-Decreased Kiss1 expression but not to the level that E2 treatment did in P-filled treatment                                                                                                                                                                                                                                                                | -OVX increases LH levels to 7 ng/mL.<br>-E2 replacement alone or in combination with P resulted in a marked suppression in circulating LH levels(2 ng/mL). P treatment alone had no effect.                                                                                                         | Yes           | Yes           | -kisspeptin neurons in the ARC of the hypothalamus appears to play a major role in mediating these feedback actions of steroids in non-primate species<br>-ovariectomised monkeys had largely expressed Kiss1 neurons whereas in intact animals, the kisspeptin cell bodies were small in size                              |
| Clarkson et al., 2008 | Mice ( <i>Mus musculus</i> )                                  | Control Mice given implants of 17B-estradiol(1ug/20 g of body weight) which is then injected. 6 days later, mice received an injection of estradiol benzoate (1ug/20g of body weight) or vehicle control at 9 am. mice were treated with progesterone as well (500 ug/20 g of body weight).             | -Kisspeptin neurons were found to express c-FOS in each of the OVX+E mice with mean percentage values of 33 ± 4%, 24 ± 4%, and 35 ± 3% of kisspeptin neurons with c-FOS in the AVPV, rPVpo, and cPVpo, respectively while in the vehicle, none of the kisspeptin neurons expressed c-FOS<br>-the percentage of kisspeptin neurons expressing c-FOS was similar (30 -40%) between Gpr54-null and wild-type mice across the RP3V                                                       | 54 ± 7% of rPOA GnRH neurons were positive for c-FOS in OVX+E mice. All mice in the OVX+E group exhibited an LH surge compared with significantly lower levels of LH in the OVX + V group<br>-Mice exhibiting highest GnRH/c-FOS co expression also exhibited highest kisspeptin/c-FOS coexpression | Yes           | Yes           | -strong correlation found between the percentage of c-FOS-positive kisspeptin neurons and percentage of c-FOS positive GnRH neurons<br>-kisspeptin-GPR54 signaling is essential for GnRH neuron activation that initiates ovulation<br>-Estrogen induces 30-40% Kiss1 c-fos expression in both the Gpr54-null and wild type |
|                       |                                                               | Gpr54 knockout mice with above procedure                                                                                                                                                                                                                                                                | -the percentage of kisspeptin neurons expressing c-FOS was similar (30 -40%) between Gpr54-null and wild-type mice across the RP3V                                                                                                                                                                                                                                                                                                                                                   | -no GnRH neurons expressed c-FOS in either Gpr54-null or Kiss1-null mice                                                                                                                                                                                                                            | Yes           | No            |                                                                                                                                                                                                                                                                                                                             |
|                       |                                                               | Kiss1 knockout mice with above procedure                                                                                                                                                                                                                                                                | -the percentage of kisspeptin neurons expressing c-FOS was similar (30 -40%) between Gpr54-null and wild-type mice across the RP3V                                                                                                                                                                                                                                                                                                                                                   | -no GnRH neurons expressed c-FOS in either Gpr54-null or Kiss1-null mice                                                                                                                                                                                                                            | Yes           | No            |                                                                                                                                                                                                                                                                                                                             |

|                                      |                                            |                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                                                                                                                                                    |     |     |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
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| D'Angelmont de Tassigny et al., 2007 | 2-4 month old Mice ( <i>Mus musculus</i> ) | -Kiss1 knockout mice generated via alternative splicing<br>-Kisspeptin-10 (100 uL of 10 uM mouse kisspeptin-10 in 0.1 M PBS) exposure to transgenic and wild-type mice                                                                                              | -100 uL of 10 uM mouse kisspeptin-10 in 0.1 M PBS<br>-kisspeptin knockout mice                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | -significantly lower plasma FSH levels compared with wild type (pre-exposure)<br>-significantly lower LH levels in mutant males and females during proestrus however not with diestrus and estrus mice (pre exposure)<br>-injection of kisspeptin in mutant mice causes significantly higher levels of plasma LH with Kiss-1 null mice shower a higher response                                                    | Yes | Yes | kisspeptin mice are viable and healthy with no apparent abnormalities however fail to undergo sexual maturation<br>-thread-like uteri, small ovaries, and do not produce mature Graafian follicles<br>-small testes and spermatogenesis arrest mainly at the early haploid spermatid stage<br>-reduced gonadotropin and sex steroid levels                                                                                                                                                                                                                                                                                                                                                                                                      |
| D'Angelmont de Tassigny et al., 2008 | Mice ( <i>Mus musculus</i> )               | -adult male wild-type and gpr54 knockout mice exposed to various concentrations of kisspeptin-10(0, 0.5, 5, 50, or 500 nm) or untreated.<br>-investigate effects of continuous kisspeptin stimulation to 50 nm kisspeptin-10 for 6 hr with refresh media every hour | 0.5 nm kisspeptin-10                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | -increase in GnRH released but non-significant in controls(10 pg/mL)                                                                                                                                                                                                                                                                                                                                               | Yes | No  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                      |                                            |                                                                                                                                                                                                                                                                     | 5 nm kisspeptin-10                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | -increase in GnRH released but non-significant in controls(11 pg/mL)                                                                                                                                                                                                                                                                                                                                               | Yes | No  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                      |                                            |                                                                                                                                                                                                                                                                     | 50 nm kisspeptin-10                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | -non-significant increase in GnRH released in controls(18 pg/mL)<br>-GnRH release decreased after 5 and 6h of continuous kisspeptin-10 exposure to levels significantly lower than the first 4 hours but not statistically different from initial level of GnRH release in absence of kisspeptin-10.<br>-suggests desensitization of gpr54 to kisspeptin-10                                                        | Yes | Yes |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|                                      |                                            |                                                                                                                                                                                                                                                                     | 500 nm kisspeptin-10                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | -significant increase in GnRH released in controls(35 pg/mL). When tested with gpr-54 knockouts, non-significant increase in GnRH(10 pg/mL)                                                                                                                                                                                                                                                                        | Yes | Yes |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Dhillon et al., 2005                 | Human Males                                | double-blind cross over study where human males received a 90-min iv infusion of kisspeptin-54(4 pmol/kg*min)                                                                                                                                                       | 90-min iv infusion of kisspeptin-54(4 pmol/kg*min)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | -kisspeptin-54 infusion significantly increases plasma LH(10.8 U/L vs 4.2 U/L; p < 0.001) and FSH (3.9 U/L vs 3.2 U/L; p < 0.001)                                                                                                                                                                                                                                                                                  | Yes | Yes |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Dubois et al., 2015                  | Adult Mice ( <i>Mus musculus</i> )         | -generated ERalpha knockout mice via Cre recombinase.<br>-ovariectomized mice treated with sesame oil or E2 dissolved in sesame oil(1 mg/mL) exposure<br>-long term OVX-E2 exposure at the same concentration                                                       | -While E2 significantly suppresses Kiss1 mRNA to undetectable levels in WT mice, it had no effect on Kiss1 mRNA expression in the ARC of ERalpha KO mice.<br>-in the AVPV, Kiss1 mRNA levels were low in the WT mice and undetectable in KO mice 7 days after OVX. E2 significantly increased Kiss1 mRNA to higher levels in WT mice compared with vehicle-treated mice. E2 is able to stimulate a low level of detectable Kiss1 mRNA expression in ERalpha KO mice which did not differ from basal Kiss1 mRNA levels in vehicle-treated WT mice. | -LH levels were elevated in WT mice after OVX and were significantly decreased with E2 treatment<br>-KO mice exhibited significantly lower LH levels than WT mice after OVX and E2 treatment significantly decreased LH levels to undetectable levels in KO mice<br>-E2 is able to exert negative feedback action on LH in KO and WT mice.<br>-E2 induces LH surges in OVX WT mice however not in ERalpha KO mice. | Yes | Yes | -estradiol activation of ERalpha in kisspeptin neurons in the ARC suppresses GnRH/LH secretion whereas E2 activation of ERalpha in kisspeptin neurons in the anteroventral periventricular nucleus mediates the release of GnRH/LH surges(positive feedback)<br>-E2 stimulated LH surges in WT mice and no effect in ERalphaKO mice<br>-Kiss1 mRNA completely gone after E2 treatment however normal in ERalpha knockout<br>-this paper signifies there's multiple methods of which estrogen can reduce LH levels<br>-there may be existence of a redundant negative feedback pathways may exist<br>-suggestions that GABA afferents to the GnRH neurons may be involved and POMC expressing neurons as targets of E2 negative feedback actions |

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| Dungan et al., 2007 | Adult female Mice ( <i>Mus musculus</i> )                     | -GPR54 KO mice were generated by retroviral mutagenesis.<br>-mice received intraperitoneal injections of either the vehicle alone (100 ul of sterile 0.9% saline) or 0.1 nmol of kisspeptin-54 to test if the mice do not express GPR54 mRNA                   | -0.1 nmol of kisspeptin-54 intraperitoneal injection                                                                                                                                                                                                                       | -significant increase in Serum LH levels in WT organisms when exposed to kisspeptin-54 treatment(1.6 ng/mL vs 0.1 ng/mL; p<0.01).<br>-GPR54 knockout mice did not experience any changes when exposed to kisspeptin-54 treatment | Yes | Yes    | The Role of Kisspeptin–GPR54 Signaling in the Tonic Regulation and Surge Release of Gonadotropin-Releasing Hormone/Luteinizing Hormone |
| Dungan et al., 2007 | Adult female Mice ( <i>Mus musculus</i> )                     | -GPR54 KO mice were generated by retroviral mutagenesis.<br>-OVX mice and examined serum LH levels 7 days later, then implanted with empty or E-filled capsules<br>-experiment 3 tests whether GPR54 signaling mediates positive feedback of E on LH secretion | -When exposed to estrogen, there was an abolishment of Kiss1 cells in both WT and GPR54 KO in the ARC.<br>-In the AVPV, WT experienced a significant increase of Kiss1 cells whereas GPR54 KO experienced a significant increase to levels equal to OVX WT pre-E exposure. | -significant decrease in serum LH levels when exposed to estrogen (0.1 vs 1.3 ng/mL; p<0.01) for WT mice<br>-no changes in serum LH levels for GPR54 mice.                                                                       | Yes | Yes    | The Role of Kisspeptin–GPR54 Signaling in the Tonic Regulation and Surge Release of Gonadotropin-Releasing Hormone/Luteinizing Hormone |
| Felip et al., 2008  | Adult Male Sea bass ( <i>Centropristis striata</i> )          | injection of sbsKiss-1 and Kiss-2 intramuscular administration of 250 ng/g of body weight                                                                                                                                                                      | -kisspeptin exposure of seabass Kiss1 (250 ng/g of body weight)                                                                                                                                                                                                            | -Kiss1 significantly increases LH levels at 120 minutes post injection (p<0.05)<br>-no significant increases in FSH levels                                                                                                       | Yes | No/Yes | sbs=seabass                                                                                                                            |
|                     |                                                               |                                                                                                                                                                                                                                                                | -kisspeptin exposure of seabass Kiss2 (250 ng/g of body weight)                                                                                                                                                                                                            | -Kiss-2 significantly increases FSH and LH levels 120 and 60 minutes post injection(p<0.05)                                                                                                                                      | Yes | Yes    |                                                                                                                                        |
|                     | Adult Wistar-Imamichi strain rats( <i>Rattus norvegicus</i> ) | injection of sbsKiss-1 and Kiss-2 icv(100 pmol) and ip (15ug)                                                                                                                                                                                                  | ICV administration of sbsKiss-1 (100 pmol/rat)                                                                                                                                                                                                                             | -Significantly increases LH and FSH levels at 15 and 30 minutes post injection (P<0.05)                                                                                                                                          | Yes | Yes    |                                                                                                                                        |
|                     |                                                               |                                                                                                                                                                                                                                                                | ICV administration of sbsKiss-2 (100 pmol/rat)                                                                                                                                                                                                                             | -Significantly increases LH levels at 15 post injection (P<0.05)<br>-no significant increases in FSH levels                                                                                                                      | Yes | No/Yes |                                                                                                                                        |
|                     |                                                               |                                                                                                                                                                                                                                                                | IP administration of sbskiss-1 (15ug/rat)                                                                                                                                                                                                                                  | -significantly increases LH levels at 15 minutes post injections (P<0.05)<br>-no significant increases in FSH levels                                                                                                             | Yes | No/Yes |                                                                                                                                        |
|                     |                                                               |                                                                                                                                                                                                                                                                | IP administration of sbskiss-2(15ug/rat)                                                                                                                                                                                                                                   | -no significant increases in LH or FSH levels                                                                                                                                                                                    | Yes | No     |                                                                                                                                        |
|                     |                                                               | Intracerebroventricular injections to varying doses of kisspeptin-54(1 fmol, 10 fmol, 0.1 pmol, 1 pmol, 10 pmol, 0.1 nmol, 0.375 nmol, 1.25 nmol, 2.5 nmol, and 5 nmol) and kisspeptin-10 injections of kisspeptin-54                                          | 1 nmol kisspeptin 10 exposure for 30 minutes                                                                                                                                                                                                                               | significant increase in LH levels(2.8 ng/mL vs 0.25 ng/mL; p<0.01)                                                                                                                                                               | Yes | Yes    |                                                                                                                                        |
|                     |                                                               |                                                                                                                                                                                                                                                                | 1 nmol kisspeptin 54 exposure for 30 minutes                                                                                                                                                                                                                               | significant increase in LH levels(3 ng/mL vs 0.25 ng/mL; p<0.01)                                                                                                                                                                 | Yes | Yes    |                                                                                                                                        |
|                     |                                                               |                                                                                                                                                                                                                                                                | 1 fmol kisspeptin-54 exposure for 30 minutes                                                                                                                                                                                                                               | significant increase in LH levels(1.5 ng/mL vs 0.5 ng/mL; p<0.01)                                                                                                                                                                | Yes | Yes    |                                                                                                                                        |
|                     |                                                               |                                                                                                                                                                                                                                                                | 10 fmol kisspeptin-54 exposure for 30 minutes                                                                                                                                                                                                                              | significant increase in LH levels(3.5 ng/mL vs 0.5 ng/mL; p<0.001)                                                                                                                                                               | Yes | Yes    |                                                                                                                                        |
|                     |                                                               |                                                                                                                                                                                                                                                                | 10 pmol kisspeptin-54 exposure for 30 minutes                                                                                                                                                                                                                              | significant increase in LH levels(2.75 ng/mL vs 0.5 ng/mL; p<0.001)                                                                                                                                                              | Yes | Yes    |                                                                                                                                        |
|                     |                                                               |                                                                                                                                                                                                                                                                | 1.2 nmol kisspeptin-54 exposure for 30 minutes                                                                                                                                                                                                                             | significant increase in LH levels(3 ng/mL vs 0.5 ng/mL; p<0.001)                                                                                                                                                                 | Yes | Yes    |                                                                                                                                        |
|                     |                                                               |                                                                                                                                                                                                                                                                | 50 pmol kisspeptin54 and acyline (GnRH antagonist) exposure                                                                                                                                                                                                                | no significant increase in LH or FSH levels                                                                                                                                                                                      | Yes | No     |                                                                                                                                        |

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| Gottsch et al., 2004           | Adult male Mice ( <i>Mus musculus</i> )                                  | (0.05 nmol) or aCSF to compare acyline and control mice.                                                                                                                                                                                                                                                                                                                                                              | 50 pmol kisspeptin 54 exposure                                                                                                                                                                                                                                                                                               | significant increase in LH(4.2 ng/mL vs 0.2 ng/mL; P<0.001) and FSH levels (16 ng/mL vs 8 ng/mL; P<0.001)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Yes | Yes |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Gutierrez-Pascual et al., 2007 | Pituitaries from Wistar-Imamichi strain rats( <i>Rattus norvegicus</i> ) | kisspeptin-10 exposure at various concentrations                                                                                                                                                                                                                                                                                                                                                                      | Kisspeptin-10 exposure(10 <sup>-10</sup> M)                                                                                                                                                                                                                                                                                  | -significant increase in LH secretion (p<0.05)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Yes | Yes |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|                                |                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                       | Kisspeptin-10 exposure (10 <sup>-8</sup> M)                                                                                                                                                                                                                                                                                  | -2-fold significant increase in LH secretion with this concentration being the peak concentration (p<0.05)<br>-Kisspeptin-10 exposure with GnRH causes a synergistic increase in LH secretion (p<0.05)<br>--4hr exposure still had a significant increase in LH secretion but less so than GnRH (p<0.05)                                                                                                                                                                                                                                                                                                                                                                                    | Yes | Yes |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
|                                |                                                                          |                                                                                                                                                                                                                                                                                                                                                                                                                       | Kisspeptin-10 exposure (10 <sup>-6</sup> M)                                                                                                                                                                                                                                                                                  | -significant increase in LH secretion (p<0.05)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Yes | Yes |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Hu et al., 2015                | Female Sprague Dawley rats ( <i>Rattus norvegicus</i> )                  | -kisspeptin antisense or rAAV-EGFP intranuclear injection (5e12) were infused into the ARC or AVPV nucleus over 10 minutes                                                                                                                                                                                                                                                                                            | -kisspeptin antisense injection                                                                                                                                                                                                                                                                                              | -significant decrease in LH surge amplitude after ARC-kisspeptin-AS injection<br>-significant decrease in proestrus LH surge in the AVPV (only 1 rat exhibited a surge on the proestrus)<br>-LH pulse interval was significantly increased in the ARC                                                                                                                                                                                                                                                                                                                                                                                                                                       | Yes | Yes | -knockout in the AVPV resulted in significant delay in vaginal opening and first vaginal estrous, abnormal estrous cyclicity, and reduction in the occurrence of LH surges<br>-32% reduction of kisspeptin in the ARC had no effect on the onset of puberty but resulted in abnormal estrous cyclicity and decreased LH pulse frequency<br>-suggests AVPV has a greater control in pubertal timing and ARC is important in normal pulsatile LH secretion in rats                                                                                                                                                                                                         |
| Irwig et al., 2005             | Adult male Sprague-Dawley rats ( <i>Rattus norvegicus</i> )              | -experiment 1: rats were divided into three groups which received either subcutaneous saline and intracerebroventricular aCSF (n = 5), subcutaneous saline and intracerebroventricular kisspeptin-52 (n = 5), or subcutaneous acyline and intracerebroventricular kisspeptin-52. 0.5nmol of kisspeptin-52 given.<br>-experiment 2: rats received kisspeptin-52(0.5 nmol) or vehicle for Fos induction in GnRH neurons | -kisspeptin injection treatment of 0.5 nmol of kisspeptin-52<br>-castration in male rat significantly increased expression of Kiss1 mRNA in the ARC nucleus as compared with intact controls<br>-increase was also reflected by increase in # of identifiable Kiss-1 neurons and increase in per cell control of Kiss-1 mRNA | -LH concentrations were significantly increased in the saline plus kisspeptin 52 group as compared with the saline plus aCSF group at 1hr post ICV injection (4 ng/mL vs 0.2 ng/mL; p<0.02)<br>-kisspeptin did not significantly increase LH or FSH concentrations with GNRH antagonist acyline (0.2 ng/mL; 6 ng/mL)<br>-serum levels of FSH were not significantly different among treatment groups at 1 hour post ICV injection (10 ng/mL vs 8 ng/mL)<br>-serum levels of LH (15 ng/mL vs 0.2 ng/mL; p<0.002) and FSH (40 ng/mL vs 10 ng/mL; p<0.002) were significantly increased in kisspeptin-52 group 2 hours post injection<br>-kisspeptin induced fos activity in GnRH neurons(86%) | Yes | Yes | -kisspeptin-52 increased the serum levels of LH hormones<br>-GnRH antagonist blocked the kisspeptin-52 induced increase in LH hormone<br>-86% of the GnRH neurons coexpressed Fos 2 h after the kisspeptin-52 challenge, whereas fewer than 1% of the GnRH neurons expressed Fos following injection of vehicle alone<br>-77% of GnRH neurons coexpress GPR54 mRNA<br>-found significantly higher levels in the arcuate nucleus of castrates<br>-GnRH are direct targets for regulation by kisspeptins and that Kiss-1 mRNA is regulated by gonadal hormones, suggesting that KiSS-1 neurons play an important role in the feedback regulation of gonadotropin secretion |

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| Jayasena et al., 2014  | Human women                                                          | Following superovulation with recombinant FSH and administration of GnRH antagonist, 53 women were administered a single dose of kisspeptin-54(1.6 nmol/kg; n=2, 3.2 nmol/kg; n=3, 6.4 nmol/kg; n=24, 12.8nmol/kg; n=24)                                                                                                     | kisspeptin-54 exposure @ various concentrations                                                                                                                                                                                                                                      | -post 12 hours, all treatments experienced an LH surge                                                                                                                                                                                                                                                                                                                                                                  | Yes     | Yes | Unequal sample sizes                                                                                                                                                                                    |
| Kauffman et al., 2007  | Adult Wistar-Imamichi strain rats( <i>Rattus norvegicus</i> )        | adult rats were either left intact or gonadectomized(GNX) and implanted with 17 $\beta$ -estradiol or nothing. Adult female rats were OVX and given estradiol in sesame oil(5 ug) with a second injection of estradiol(50 ug)                                                                                                | -In the AVPV Kiss1 neurons, E2 causes a significant increase in Kiss1 cells and mRNA only in female rats. Overall increase in Kiss1 cells in female controls.<br>-In the ARC Kiss1 neurons, E2 causes a significant decrease in Kiss1 cells and mRNA regardless of treatment or sex. | -significantly reduced LH and FSH levels post GNX and estrogen introduction                                                                                                                                                                                                                                                                                                                                             | Yes     | Yes | Sexual Differentiation of Kiss1 Gene Expression in the Brain of the Rat<br>sexual differentiation in Kiss1 expression in AVPV but not in the ARC                                                        |
| Kauffman et al., 2007  | Mice ( <i>Mus musculus</i> )                                         | GPR54 KO mice were generated via retroviral mutagenesis. GnRH neuronal activation measured in GPR54 knockout mice and WT through centrally infused exposure to kisspeptin-54 (1 nmol) or vehicle                                                                                                                             | -kisspeptin exposure<br>-increase in Kiss1 neurons in the AVPV nucleus compared with WT males                                                                                                                                                                                        | -In control mice, 85% of GnRH neurons contained nuclear fos activity after kisspeptin treatment whereas vehicle control had <1% of GnRH neurons showed Fos activity<br>-<2% of GnRH neurons showed Fos activity in GPR54 KO mice post exposure.<br>-WT males infused with kisspeptin had elevated levels of LH(4 ng/mL) compared with all other groups whereas GPR54 KO mice did not show an increase in LH(0.5 ng/mL). | Yes     | Yes | The Kisspeptin Receptor GPR54 Is Required for Sexual Differentiation of the Brain and Behavior                                                                                                          |
| Kinoshita et al., 2005 | Adult Female Wistar-Imamichi strain rats( <i>Rattus norvegicus</i> ) | -Animals were OVX and either received a varying dose of E2 to produce a low (35.8 pg/mL) or high (514.1 pg/mL) E2 level. Others received nothing.<br>-control rats also received an injection of anti rat metastin or PBS(non immunized mouse IgG) into the third ventricle to determine the effects of metastin on LH surge | -non-significant change in relative expression of Kiss-1 compared to OVX controls<br>-injection of metastin                                                                                                                                                                          | -injection of metastin @ 2 nmol caused a surge-like LH secretion in low-level E2-primed female rats but not in the OVX<br>-local injection of metastin into the POA induced a sustained surge-like increase in plasma LH level in E2-primed OVX rats                                                                                                                                                                    | No(Yes) | Yes | -low estrogen treatment did not induce a change in KiSS1 expression however Kiss1 injection did induce a change in plasma LH along with estrogen. OVX alone with metastin could not produce a LH surge. |
|                        |                                                                      |                                                                                                                                                                                                                                                                                                                              | -increased KiSS-1 expression due to OVX<br>-injection of metastin @ 2 nmol into the 3rd ventricle                                                                                                                                                                                    | -infusion of specific monoclonal antibody @ 1ul/h completely blocked the proestrus LH surge seen in control animals.<br>-estrous cycles were disrupted in female rats receiving POA infusion of anti rat metastin                                                                                                                                                                                                       | Yes     | Yes |                                                                                                                                                                                                         |

|                      |                                    |                                                                                                                                                                                                                                                                                                              |                                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |     |     |                                                                                                                                                                                                                                                                                                                                                                            |
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| Kirilov et al., 2013 | Adult Mice ( <i>Mus musculus</i> ) | -GnRH neuron-specific deletion of Gpr54 to assess the role of GnRH neurons generated using mouse embryonic stem cells<br>-tested 10nM kisspeptin response with control and mutant mice                                                                                                                       | -100nmol kisspeptin via i.p. exposure           | -In control mice, 9 of 14 GnRH neurons from 3 mice responded to 100-nM kisspeptin whereas in mutant mice, none of the 19 GnRH neurons from 4 mice responded to the kisspeptin treatment.<br>-given 100 nmol kisspeptin, adult control male mice experienced a significant sevenfold increase in LH secretion(4.5 ng/mL) 10 minutes later. No effect was seen in mutant mice.<br>-significant decrease in basal FSH levels in GPR54 mutants. No significant effect seen in basal LH levels.          | Yes | Yes | -GPR-54 null mice failed to reproduce, had reduced teste sizes, and were infertile<br>-when the GPR-54 mice were rescued, fertility and everything was restored                                                                                                                                                                                                            |
| Lapatto et al., 2007 | Mice ( <i>Mus musculus</i> )       | GPR54 KO mice generated via neomycin/G418 resistance cassette. Mice injected with 50 nmol of metastin 43-52<br><br>Kiss1 KO mice generated via neomycin/G418 resistance cassette. Mice injected with 50 nmol of metastin 43-52                                                                               | -injection of mouse metastin 43-52              | -FSH levels in Gpr54 KO mice(males: 1.5ng/mL; females:3.3 ng/mL) were significantly lower than those in WT mice(males:20.5 ng/mL; females:6 ng/mL).<br>-only males had significantly lower LH levels(0.05ng/mL) compared to WT mice(0.2 ng/mL).<br>-exposure to mouse metastin, Gpr54 KO mice failed to increase in gonadotropins.                                                                                                                                                                  | Yes | No  | -both Kiss1 and GPR54 knockout mice were viable but infertile and have abnormal sexual maturation<br>-Kiss1 mice experienced more variable hypogonadism as half showed gonadal weight comparable with controls but half had similar levels to Gpr54 KO mice<br>-it is to be expected that Gpr54 KO mice fail to see an increase in gonadotropins due to metastin exposure. |
|                      |                                    |                                                                                                                                                                                                                                                                                                              | -injection of mouse metastin 43-52              | -Female FSH levels of KO mice(3.8 ng/mL) were lower than those in WT mice(6 ng/mL) but not statistically different. Male KO mice FSH levels(1.9 ng/mL) were significantly lower than WT males(20.5 ng/mL).<br>-exposure to mouse metastin, saw a significant increase of LH and FSH levels comparable to controls.<br>-The subgroup of Kiss1 knockout females with higher gonadal weights had less robust gonadotropin responses to metastin than Kiss1 knockout females with lower gonadal weights | Yes | Yes |                                                                                                                                                                                                                                                                                                                                                                            |
| Lents et al., 2008   | Gilts ( <i>Percina evides</i> )    | -prepubertal gilts were fitted with i.c.v. cannula and indwelling jugular catheters. Animals were randomly assigned to receive 0, 10, or 100 ug kisspeptin in saline -, prepubertal gilts, fitted with indwelling jugular catheters, randomly received 0, 1, 2.5, or 5 mg kisspeptin in saline intravenously | 10 ug kisspeptin ICV treatment                  | -significant increase in LH and FSH levels (P<0.001)                                                                                                                                                                                                                                                                                                                                                                                                                                                | Yes | Yes |                                                                                                                                                                                                                                                                                                                                                                            |
|                      |                                    |                                                                                                                                                                                                                                                                                                              | 100 ug kisspeptin ICV treatment                 | -significant increase in LH and FSH levels (P<0.001) with concentration causing larger effect.                                                                                                                                                                                                                                                                                                                                                                                                      | Yes | Yes |                                                                                                                                                                                                                                                                                                                                                                            |
|                      |                                    |                                                                                                                                                                                                                                                                                                              | 1 mg kisspeptin IV treatment                    | -significant increase in LH (P<0.05)<br>-no significant increase seen in FSH levels                                                                                                                                                                                                                                                                                                                                                                                                                 | Yes | Yes |                                                                                                                                                                                                                                                                                                                                                                            |
|                      |                                    |                                                                                                                                                                                                                                                                                                              | 2.5 mg kisspeptin IV treatment                  | -significant increase in LH (P<0.05)<br>-no significant increase seen in FSH levels                                                                                                                                                                                                                                                                                                                                                                                                                 | Yes | Yes |                                                                                                                                                                                                                                                                                                                                                                            |
|                      |                                    |                                                                                                                                                                                                                                                                                                              | 5 mg kisspeptin IV treatment                    | -significant increase in LH (P<0.05)<br>-no significant increase seen in FSH levels                                                                                                                                                                                                                                                                                                                                                                                                                 | Yes | Yes |                                                                                                                                                                                                                                                                                                                                                                            |
|                      |                                    |                                                                                                                                                                                                                                                                                                              | kisspeptin-10 treatment @ 14 -log M for 4 hours | -non-significant increase in LH secretion                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Yes | No  |                                                                                                                                                                                                                                                                                                                                                                            |

|                            |                                                                                             |                                                                                                                                                                                                                                                         |                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |     |        |
|----------------------------|---------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|--------|
| Luque et al., 2011         | primary pituitary cell cultures from female baboons ( <i>Papio anubis</i> , 7-12 yr of age) | -exposure to Kisspeptin-10 at various concentrations<br>-exposure to estradiol at 10 nM                                                                                                                                                                 | kisspeptin-10 treatment @ 12 -log M for 4 hours        | -significant increase in LH secretion in a dose-dependent manner                                                                                                                                                                                                                                                                                                                                                                                                                                 | Yes | Yes    |
|                            |                                                                                             |                                                                                                                                                                                                                                                         | kisspeptin-10 treatment @ 10 -log M for 4 hours        | -significant increase in LH secretion in a dose-dependent manner                                                                                                                                                                                                                                                                                                                                                                                                                                 | Yes | Yes    |
|                            |                                                                                             |                                                                                                                                                                                                                                                         | kisspeptin-10 treatment @ 8 -log M for 4 hours         | -significant increase in LH secretion in a dose-dependent manner<br>-For the first 24 hours, LH secretion was significantly higher than control. After 48 hours, non-significantly higher secretion however still higher.<br>-synergistic increase of LH secretion with estrogen and kisspeptin-10 exposure. Estrogen alone also significantly increases LH levels.                                                                                                                              | Yes | Yes    |
|                            |                                                                                             |                                                                                                                                                                                                                                                         | kisspeptin-10 treatment @ 6 -log M for 4 hours         | -significant increase in LH secretion in a dose-dependent manner                                                                                                                                                                                                                                                                                                                                                                                                                                 | Yes | Yes    |
|                            |                                                                                             |                                                                                                                                                                                                                                                         |                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |     |        |
| Messenger et al., 2005     | Mice ( <i>Mus musculus</i> )                                                                | -gpr54 was knocked out of a transgenic mice line.<br>-injection of kisspeptin @ 100 uL of 10 uM mouse kisspeptin in 0.1 M PBS via i.p.                                                                                                                  | kisspeptin treatment(100 uL of 10 uM mouse kisspeptin) | -mice experienced a robust and significant crease in plasma LH and FSH in response to kisspeptins whereas mutant mice(gpr54 knockout mice) failed to mount any response                                                                                                                                                                                                                                                                                                                          | Yes | Yes    |
|                            | 2-3 year old Ile de France ewes ( <i>Ovis aries</i> )                                       | -sheep were OVX and treated with estradiol via s.c. to reduce GnRH/LH secretion for 1 month. Afterwards, animals were treated intracerebroventricularly for 4 hours with human kisspeptin diluted in sterile, pyrogen-free saline (3ul/min & 0.2ug/min) | kisspeptin treatment(0.2 ug/min)                       | -kisspeptin infusion in the third ventricle induced a rapid and sustained increase in LH secretion in all four animals in experiment group but none in the control group. High levels reached during first 2 hours and progressive decline towards end.<br>-CSF sampling showed abrupt increase in GnRH secretion in the CSF at start of kisspeptin infusion. LH secretion began decreasing before the end of peptide infusion period; however, GnRH secretion persisted(decreased sensitivity). | Yes | Yes    |
| Narayanaswamy et al., 2016 | Human females                                                                               | subcutaneous infusion of kisspeptin-54 at varying doses (0.1, 0.3, and 1 nmol/kg/hr)                                                                                                                                                                    | 0.1 nmol/kg/hr kisspeptin-54                           | -non-significant increase in mean serum LH<br>-significant increase in mean serum FSH (p <0.05)                                                                                                                                                                                                                                                                                                                                                                                                  | Yes | No/Yes |
|                            |                                                                                             |                                                                                                                                                                                                                                                         | 0.3 nmol/kg/hr kisspeptin-54                           | -significant increase in mean serum LH (p<0.001)<br>-significant increase in mean serum FSH (p <0.001)                                                                                                                                                                                                                                                                                                                                                                                           | Yes | Yes    |
|                            |                                                                                             |                                                                                                                                                                                                                                                         | 1 nmol/kg/hr kisspeptin-54                             | -significant increase in mean serum LH (p<0.001)<br>-significant increase in mean serum FSH (p <0.001)                                                                                                                                                                                                                                                                                                                                                                                           | Yes | Yes    |
|                            |                                                                                             | Central intracerebroventricular administration of Kiss-1 peptide into the cerebral lateral ventricle(1 nmol/10 uL) or vehicle saline. immature rats                                                                                                     | Kiss-1 exposure                                        | -significant increase in FSH levels in both males and females compared to vehicle control with peak at 15 minutes                                                                                                                                                                                                                                                                                                                                                                                | Yes | Yes    |

|                               |                                                                |                                                                                                                                                                                                                             |                                                                                                                                                                         |                                                                                                                                                                      |     |        |                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-------------------------------|----------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|--------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Navarro et al., 2005          | Adult Wistar-Imamichi strain rats ( <i>Rattus norvegicus</i> ) | Central intracerebroventricular administration of Kiss-1 peptide into the cerebral lateral ventricle (1 nmol/10 uL) or vehicle saline. adult rats                                                                           | Kiss-1 exposure                                                                                                                                                         | -significant increase in FSH levels in male adults compared to vehicle control at 60 minutes only. Time course shows that significant increase begins at 30 minutes. | Yes | Yes    | -ip and iv injection of Kiss-1 significantly stimulated FSH secretion in vivo.<br>-Kiss-1 failed to elicit basal FSH release directly at the pituitary level although it moderately enhanced GnRH-stimulated FSH secretion in vitro<br>-blockade of endogenous GnRH actions abolishes Kiss-1 ability to elicit FSH secretion                                                                                                            |
|                               |                                                                | dose-response analysis of effects of centrally administered Kiss-1 in pubertal male rats (10 nmol, 1 nmol, 500 pmol, 100 pmol, 10 pmol, 100 fmol)                                                                           | 1 fmol, 10 fmol, 100 fmol Kiss-1 exposure                                                                                                                               | -no/non-significant increase in FSH and LH levels                                                                                                                    | Yes | No     |                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                               |                                                                |                                                                                                                                                                                                                             | 10 pmol Kiss-1 exposure                                                                                                                                                 | -significant increase in LH levels but non-significant increase in FSH levels                                                                                        | Yes | No/Yes |                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                               |                                                                |                                                                                                                                                                                                                             | 100 pmol Kiss-1 exposure                                                                                                                                                | -significant increase in LH and FSH levels but less significant FSH levels compared to peak concentrations                                                           | Yes | Yes    |                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                               |                                                                |                                                                                                                                                                                                                             | 1 nmol, 10 nmol Kiss-1 exposure                                                                                                                                         | -significant increase in LH and FSH levels with peak at 10 nmol (20 ng/mL for FSH and 8 ng/mL for LH @ 10 nmol)                                                      | Yes | Yes    |                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Neal-Perry et al., 2009       | Female Sprague Dawley rats ( <i>Rattus norvegicus</i> )        | -OVX'd young and middle-aged rats exposed to estradiol benzoate/progesterone and kisspeptin-10 (10 nM)                                                                                                                      | -Kisspeptin 10 treatment (10 nM)                                                                                                                                        | -kisspeptin exposure restores LH surge in middle-aged rats. Kisspeptin-10 had no effect with the LH surge for young rats.                                            | Yes | Yes    | -middle-aged rats exhibiting delayed and attenuated LH surges have reduced levels of Kiss1 mRNA in the anterior hypothalamus under estrogen-positive feedback conditions                                                                                                                                                                                                                                                                |
| Pielecka-Fortuna et al., 2008 | Adult Female Mice ( <i>Mus musculus</i> )                      | -transgenic female mice in which green fluorescent protein was genetically targeted to GnRH neurons OVX'd, given various concentrations of kisspeptin (0.1, 1, 10, 20, and 100 nM) and/or 0.526 ug estradiol in sesame oil. | 0.1 nM                                                                                                                                                                  | non-significant increase in firing rate response of GnRH neurons to kisspeptin                                                                                       | Yes | No     | and estradiol significantly potentiated the response<br>-Kisspeptin greatly increases GnRH/LH release and GnRH neuron firing activity and may be involved in estradiol feedback, but the neurobiological mechanisms for these actions are unknown<br>-EC50 of OVX and OVX+E approximately the same (3.6 nM and 4.5 nM)                                                                                                                  |
|                               |                                                                |                                                                                                                                                                                                                             | 1 nM                                                                                                                                                                    | non-significant increase in firing rate response of GnRH neurons to kisspeptin                                                                                       | Yes | No     |                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                               |                                                                |                                                                                                                                                                                                                             | 10 nM                                                                                                                                                                   | significant increase in firing rate response of GnRH neurons to kisspeptin                                                                                           | Yes | Yes    |                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                               |                                                                |                                                                                                                                                                                                                             | 20 nM                                                                                                                                                                   | significant increase in firing rate response of GnRH neurons to kisspeptin                                                                                           | Yes | Yes    |                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                               |                                                                |                                                                                                                                                                                                                             | 100 nM                                                                                                                                                                  | significant increase in firing rate response of GnRH neurons to kisspeptin                                                                                           | Yes | Yes    |                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Richard et al., 2008          | Sprague Dawley rats ( <i>Rattus norvegicus</i> )               | OVX'd and injected with 10 ug of E2 every 2 days for 3 weeks                                                                                                                                                                | -Kiss1 mRNA significantly reduced in OVX rats but E2 restores it to levels similar to mestroestrous.                                                                    | -OVX causes a significant increase in LHB. E2 restores LHbeta levels to those similar to mestroestrous.                                                              | Yes | Yes    | -PPT = propylazotriol<br>-DPN = diarylpropionitrile<br>-GPR54 expression positively regulated by GnRH and negatively controlled by chronic exposure to E2<br>--administration of the selective oestrogen receptor alpha ligand propylpyrazotriol, but not the selective ERb ligand diarylpropionitrile, mimics this effect of Kiss-1 expression<br>-increasing the E2 concentration causes a significant increase in Kiss-1 mRNA levels |
|                               |                                                                | OVX'd and tested ERalpha and ERbeta agonists where rats were injected daily (s.c.) for 3 days with 1 mg of PPT (ERalpha agonist), 1 mg of DPN (ERbeta agonist), or 1 mg of PPT and DPN                                      | -OVX abolishes Kiss-1 mRNA levels. PPT significantly increases Kiss-1 mRNA levels to levels above Mestroestrous.                                                        | -OVX causes a significant increase in LHB. PPT restores LHbeta levels to those similar to mestroestrous.                                                             | Yes | Yes    |                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                               |                                                                |                                                                                                                                                                                                                             | -OVX abolishes Kiss-1 mRNA levels. DPN is unable to induce any changes in Kiss-1 mRNA levels. significantly increases Kiss-1 mRNA levels to levels above Mestroestrous. | -OVX causes a significant increase in LHB. DPN increases LHbeta levels above original OVX.                                                                           | No  | No     |                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|                               |                                                                |                                                                                                                                                                                                                             | -OVX abolishes Kiss-1 mRNA levels. PPT with DPN significantly increases Kiss-1 mRNA levels to levels above Mestroestrous.                                               | -OVX causes a significant increase in LHB. PPT with DPN restores LHbeta levels to those similar to mestroestrous.                                                    | Yes | Yes    |                                                                                                                                                                                                                                                                                                                                                                                                                                         |



|                       |                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |     |     |                                                                                                                                                                                         |
|-----------------------|--------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|-----|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Seminara et al., 2006 | juvenile male Rhesus Monkey ( <i>Macaca mulatta</i> )                                            | -continuous administration of metastin 45-54 (100 ug/h)<br>-post 4-5 wk of intermittent priming w/ GnRH to confirm pituitary responsiveness to GnRH has been upregulated, iv intermittent infusion of GnRH was interrupted and began injections of metastin 45-54 (10ug)                                                                                           | kisspeptin treatment(100ug/hr)                                                                                                     | -continuous metastin infusion desensitizes GPR54 and fails to induce LH pulses with injections of metastin afterwards<br>-initial introduction of metastin caused a significant increase of LH 2-fold of that to GnRH for 3 hrs before progressive decline to levels similar of controls(10.6 ng/mL peak)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Yes | Yes |                                                                                                                                                                                         |
| Shibata et al., 2007  | Adult Male Rhesus Macaques ( <i>Macaca mulatta</i> )                                             | castrated adult males were implanted with four or five testosterone-filled pills.                                                                                                                                                                                                                                                                                  | -testosterone exposure causes a significant reduction in kisspeptin mRNA levels in the mediobasal hypothalamus(1.3 vs 0.5; p<0.01) | -testosterone supplement causes a suppression of LH and FSH secretion across a 7 week period with significant suppression beginning at week 6.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Yes | Yes |                                                                                                                                                                                         |
| Smith et al., 2007    | Adult Corriedale Ewes ( <i>Ovis aries</i> )                                                      | -in vitro test: pituitary cells from OVX and ovary-intact ewes given human kisspeptin-10 or vehicle treatment (control).<br>-in vivo test: HPD (hypothalamus-pituitary disconnection) ewes were OVX, given GnRH(250 ng in 2 hourly pulses). At 1100 h, 100 ug of mouse kisspeptin-10 administered via jugular cannula. Controls were OVX and hypo-pit intact ewes. | human kisspeptin treatment                                                                                                         | -in vitro, kisspeptin treatment resulted in an 80% increase in concentration of LH from primary cultures of pituitary cells taken from ewes during follicular phase of estrous cycle<br>-no effect of kisspeptin on LH concentrations in cultures from pituitary glands take from either the luteal or OVX ewes<br>-in all cell cultures, regardless of whether it was previously treated with kisspeptin, GnRH caused an increase in LH concentrations<br>-10 <sup>-10</sup> -10 M Kisspeptin is minimum concentration to induce a significant increase in LH in vitro.<br>-in all 4 in vivo experiments, no effect of kisspeptin on LH concentrations in HPD ewes(hypothalamus-pituitary disconnect)<br>-in OVX hypothalamus-pituitary intact animals, kisspeptin treatment resulted in 4-fold increase in LH concentrations (100ug) | Yes | Yes | important thing to note is they exposed kisspeptin to the pituitary which you shouldn't expect to see a response as GPR-54 is expressed in the GnRH neurons located in the hypothalamus |
|                       | Bovine pituitary glands from 1 month old Holstein intact male calves( <i>Bos taurus taurus</i> ) | kisspeptin-10 exposure @ 10, 100, 1000, 10,000 nM                                                                                                                                                                                                                                                                                                                  | 10 nM kisspeptin-10 exposure                                                                                                       | non-significant increase in plasma LH levels(105% vs 100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Yes | No  |                                                                                                                                                                                         |
|                       |                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                    | 100 nM kisspeptin-10 exposure                                                                                                      | non-significant increase in plasma LH levels(115% vs 100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Yes | No  |                                                                                                                                                                                         |
|                       |                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                    | 1000 nM kisspeptin-10 exposure                                                                                                     | significant increase in plasma LH levels (125% vs 100%; p<0.05)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Yes | Yes |                                                                                                                                                                                         |
|                       |                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                    | 10000 nM kisspeptin-10 exposure                                                                                                    | significant increase in plasma LH levels (130% vs 100%; p<0.05)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Yes | Yes |                                                                                                                                                                                         |
|                       | Bovine pituitary glands from 8 month old Holstein intact male calves( <i>Bos taurus taurus</i> ) | kisspeptin-10 exposure @ 10, 100, 1000, 10,000 nM                                                                                                                                                                                                                                                                                                                  | 10 nM kisspeptin-10 exposure                                                                                                       | non-significant increase in plasma LH levels(110% vs 100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Yes | No  |                                                                                                                                                                                         |
|                       |                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                    | 100 nM kisspeptin-10 exposure                                                                                                      | non-significant increase in plasma LH levels(110% vs 100%)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Yes | No  |                                                                                                                                                                                         |
|                       |                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                    | 1000 nM kisspeptin-10 exposure                                                                                                     | significant increase in plasma LH levels (130% vs 100%; p<0.05)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Yes | Yes |                                                                                                                                                                                         |
|                       |                                                                                                  |                                                                                                                                                                                                                                                                                                                                                                    | 10000 nM kisspeptin-10 exposure                                                                                                    | significant increase in plasma LH levels (140% vs 100%; p<0.05)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Yes | Yes |                                                                                                                                                                                         |

|                       |                                                                                          |                                                                          |                                      |                                                                                                                                                                                                                                                                                                                |     |        |                                                                                                                                  |
|-----------------------|------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|--------|----------------------------------------------------------------------------------------------------------------------------------|
| Suzuki et al., 2008   | Porcine pituitary glands from 6-month old triple crossbred barrows ( <i>Sus scrofa</i> ) | kisspeptin-10 exposure @ 10, 100, 1000, 10,000 nM                        | 10 nM kisspeptin-10 exposure         | non-significant increase in plasma LH levels(110% vs 100%)                                                                                                                                                                                                                                                     | Yes | No     |                                                                                                                                  |
|                       |                                                                                          |                                                                          | 100 nM kisspeptin-10 exposure        | significant increase in plasma LH levels (130% vs 100%; p<0.05)                                                                                                                                                                                                                                                | Yes | Yes    |                                                                                                                                  |
|                       |                                                                                          |                                                                          | 1000 nM kisspeptin-10 exposure       | significant increase in plasma LH levels (135% vs 100%; p<0.05)                                                                                                                                                                                                                                                | Yes | Yes    |                                                                                                                                  |
| Thompson et al., 2004 | Male Wistar-Imamichi strain rats( <i>Rattus norvegicus</i> )                             | intracerebroventricular administration of varying doses of kisspeptin-10 | 0.1 nmol kisspeptin-10 exposure      | non-significant increase in plasma LH (1 ng/mL vs 0.2 ng/mL) and FSH levels (14 ng/mL vs 13 ng/mL)                                                                                                                                                                                                             | Yes | No     | -increase plasma LH and FSH in a dose-dependent manner.<br>-kiss10 does not influence LH or FSH release from pituitary fragments |
|                       |                                                                                          |                                                                          | 0.3 nmol kisspeptin-10 exposure      | non-significant increase in plasma LH (2 ng/mL vs 0.2 ng/mL) and FSH levels (20 ng/mL vs 13 ng/mL)                                                                                                                                                                                                             | Yes | No     |                                                                                                                                  |
|                       |                                                                                          |                                                                          | 1 nmol kisspeptin-10 exposure        | significant increase in plasma LH (5 ng/mL vs 0.2 ng/mL; p<0.05) and FSH levels (24 ng/mL vs 13 ng/mL)                                                                                                                                                                                                         | Yes | Yes    |                                                                                                                                  |
|                       |                                                                                          |                                                                          | 3 nmol kisspeptin-10 exposure        | significant increase in plasma LH(9 ng/mL vs 0.2 ng/mL; p<0.05) but non-significant increase in FSH levels(20 ng/mL vs 13 ng/mL)                                                                                                                                                                               | Yes | Yes    |                                                                                                                                  |
|                       |                                                                                          |                                                                          | 3 nmol kisspeptin-10 exposure 10 min | -significant increase in plasma LH levels (2 ng/mL vs 1ng/mL; p<0.05) but no increase in plasma FSH levels(18 ng/mL vs 18ng/mL)                                                                                                                                                                                | Yes | No/Yes |                                                                                                                                  |
|                       |                                                                                          |                                                                          | 3 nmol kisspeptin-10 exposure 20 min | -significant increase in plasma LH levels (3 ng/mL vs 1ng/mL;p<0.01) but non-significant increase in plasma FSH levels(21 ng/mL vs 18ng/mL)                                                                                                                                                                    | Yes | No/Yes |                                                                                                                                  |
|                       |                                                                                          |                                                                          | 3 nmol kisspeptin-10 exposure 60 min | -significant increase in plasma LH levels (5 ng/mL vs 1ng/mL; p<0.001) and plasma FSH levels(33 ng/mL vs 18ng/mL; p<0.01)                                                                                                                                                                                      | Yes | Yes    |                                                                                                                                  |
|                       |                                                                                          |                                                                          | 10 nmol kisspeptin exposure          | -non-significant increase in LH-releasing hormone(150 ng/mL vs 100 ng/mL)<br>-non-significant increase in plasma LH or FSH levels at both 20 min and 60 min post injection                                                                                                                                     | Yes | No     |                                                                                                                                  |
|                       |                                                                                          |                                                                          | 30 nmol kisspeptin exposure          | -significant increase in LH-releasing hormone(170 ng/mL vs 100 ng/mL; p<0.05)<br>-non-significant increase in plasma LH or FSH levels at both 20 min and 60 min post injection                                                                                                                                 | Yes | No/Yes |                                                                                                                                  |
|                       |                                                                                          |                                                                          | 100 nmol kisspeptin exposure         | -significant increase in LH-releasing hormone(200 ng/mL vs 100 ng/mL; p<0.001)<br>-significant increase in plasma LH levels at 20 minutes post injection(6ng/mL vs 0.5 ng/mL; p<0.05) but not 60 min post injection<br>-non-significant increase in plasma FSH levels at both 20 min and 60 min post injection | Yes | No/Yes |                                                                                                                                  |
|                       |                                                                                          |                                                                          | 0.1 nmol Kisspeptin-10 exposure      | -no increase in plasma LH levels                                                                                                                                                                                                                                                                               | Yes | No     |                                                                                                                                  |

|                                       |                                                                                   |                                                                                                                                                                         |                                                                                                                                                                                                                                                                   |                                                                                                                                                          |     |        |                |
|---------------------------------------|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------|-----|--------|----------------|
| Thompson et al., 2006                 | Adult Male Wistar-Imamichi strain rats ( <i>Rattus norvegicus</i> )               | subcutaneous administration of kisspeptin @ 0.1, 0.3, 1.0, and 50 nmol of Kiss10, Kiss14, and Kiss54 along with continuous Kiss-54 administration for 13 days @ 50 nmol | 0.3 nmol Kisspeptin-10 exposure                                                                                                                                                                                                                                   | -no increase in plasma LH levels                                                                                                                         | Yes | No     |                |
|                                       |                                                                                   |                                                                                                                                                                         | 1 nmol Kisspeptin-10 exposure                                                                                                                                                                                                                                     | -no increase in plasma LH levels                                                                                                                         | Yes | No     |                |
|                                       |                                                                                   |                                                                                                                                                                         | 50 nmol Kisspeptin-10 exposure                                                                                                                                                                                                                                    | -non-significant increase in plasma LH levels                                                                                                            | Yes | No     |                |
|                                       |                                                                                   |                                                                                                                                                                         | 0.1 nmol Kisspeptin-14 exposure                                                                                                                                                                                                                                   | -no increase in plasma LH levels                                                                                                                         | Yes | No     |                |
|                                       |                                                                                   |                                                                                                                                                                         | 0.3 nmol Kisspeptin-14 exposure                                                                                                                                                                                                                                   | -no increase in plasma LH levels                                                                                                                         | Yes | No     |                |
|                                       |                                                                                   |                                                                                                                                                                         | 1 nmol Kisspeptin-14 exposure                                                                                                                                                                                                                                     | -no increase in plasma LH levels                                                                                                                         | Yes | No     |                |
|                                       |                                                                                   |                                                                                                                                                                         | 50 nmol Kisspeptin-14 exposure                                                                                                                                                                                                                                    | -non-significant increase in plasma LH levels                                                                                                            | Yes | No     |                |
|                                       |                                                                                   |                                                                                                                                                                         | 0.1 nmol Kisspeptin-54 exposure                                                                                                                                                                                                                                   | -non-significant increase in plasma LH levels                                                                                                            | Yes | No     |                |
|                                       |                                                                                   |                                                                                                                                                                         | 0.3 nmol Kisspeptin-54 exposure                                                                                                                                                                                                                                   | -non-significant increase in plasma LH levels                                                                                                            | Yes | No     |                |
|                                       |                                                                                   |                                                                                                                                                                         | 1 nmol Kisspeptin-54 exposure                                                                                                                                                                                                                                     | -significant increase in plasma LH levels (6ng/mL vs 0.5 ng/mL; p<0.001)                                                                                 | Yes | Yes    |                |
|                                       |                                                                                   |                                                                                                                                                                         | 50 nmol Kisspeptin-54 exposure                                                                                                                                                                                                                                    | -significant increase in plasma LH levels (8ng/mL vs 0.5 ng/mL; p<0.001)<br>-no significant change in plasma LH or FSH levels post-long term sc exposure | Yes | Yes    |                |
|                                       |                                                                                   |                                                                                                                                                                         | 50 nmol Kisspeptin-54 exposure; 1 day                                                                                                                                                                                                                             | -significant increase in plasma LH levels (2.7 ng/mL vs 1.1 ng/mL; p<0.001)<br>-non-significant increase in plasma FSH levels                            | Yes | No/Yes |                |
|                                       |                                                                                   |                                                                                                                                                                         | 50 nmol Kisspeptin-54 exposure; 2 day                                                                                                                                                                                                                             | -no significant change in plasma LH or FSH levels (slight decrease in plasma FSH)                                                                        | Yes | No     |                |
| 50 nmol Kisspeptin-54 exposure; 3 day | -no significant change in plasma LH or FSH levels (slight decrease in plasma FSH) | Yes                                                                                                                                                                     | No                                                                                                                                                                                                                                                                |                                                                                                                                                          |     |        |                |
| Tomikawa et al., 2012                 | Adult Mice ( <i>Mus musculus</i> )                                                | OVXed and implanted with E2-filled pill @ 200 ug/mL                                                                                                                     | -E2 treatment causes a significant increase in kisspeptin signal intensity in the AVPV kisspeptin neurons (200 vs 50 & 120 vs 20)<br>-E2 treatment causes a significant decrease in kisspeptin signal intensity in the ARC kisspeptin neurons (50 vs 5 & 75 vs 5) | -significant increase in plasma LH concentrations (2.1 ng/mL vs 0.4 ng/mL; p<0.05)                                                                       | Yes | Yes    | arbitrary unit |