REPRODUCTION
RESEARCH

# Central and peripheral administration of kisspeptin activates gonadotropin but not somatotropin secretion in prepubertal gilts

Clay A Lents, Neely L Heidorn, C Richard Barb[1] and J Joe Ford[2]

*Department of Animal and Dairy Science, College of Agricultural and Environmental Sciences, 316 Edgar L Rhodes Center for Animal and Dairy Science, The University of Georgia, 425 River Road, Athens, Georgia 30602-2771, USA, [1]Richard B Russell Agricultural Research Center, USDA-ARS, Athens, Georgia 30604-5667, USA and [2]US Meat Animal Research Center, USDA-ARS, Clay Center, Nebraska 68933, USA*

*Correspondence should be addressed to C A Lents; Email: clents@uga.edu*

## Abstract

**It is well established that kisspeptin signaling is necessary for the onset of puberty in laboratory animals. However, the role that kisspeptin may have in regulating puberty in large domestic animals is unknown. We tested the hypothesis that either central or peripheral infusion of kisspeptin would stimulate gonadotropin and GH secretion in prepubertal gilts. In experiment 1, prepubertal gilts were fitted with i.c.v. cannula and indwelling jugular catheters. Animals were randomly assigned to receive 0, 10, or 100 μg kisspeptin in saline. In experiment 2, prepubertal gilts, fitted with indwelling jugular catheters, randomly received 0, 1, 2.5, or 5 mg kisspeptin in saline intravenously. Serial blood samples were collected every 15 min for 3 h before and 5 h after infusions, and serum concentrations of LH, FSH, and GH were determined. Mean concentrations of LH and FSH remained at basal levels for control animals but were increased ($P<0.001$) for animals receiving i.c.v. infusion of kisspeptin. Area under the LH and FSH curves following i.c.v. infusion of kisspeptin increased ($P<0.001$) in a dose-dependent manner. Concentrations of GH were unaffected by i.c.v. treatment. Peripheral administration of kisspeptin increased ($P<0.05$) serum concentrations of LH but not FSH or GH. Thus, kisspeptin can activate gonadotropic but not somatotropic hormone secretion in prepubertal gilts. The present data support the concept that kisspeptin plays a role in the mechanism involved in initiating puberty in swine.**

*Reproduction* (2008) **135** 879–887

## Introduction

Gonadotropin-releasing hormone (GnRH) is secreted from hypothalamic neurons and is transported through the hypophyseal portal veins to the anterior pituitary gland where it stimulates synthesis and secretion of luteinizing hormone and follicle-stimulating hormone (LH and FSH; Schally *et al*. 1971). These pituitary gonadotropins are essential for normal gonadal development and function (Brinkley 1981, Desjardins 1981, Clarke *et al*. 1983, Gharib *et al*. 1990). In mammals, the components of the hypothalamic–pituitary–gonadal (HPG) axis are functional prior to the normal onset of puberty (Lutz *et al*. 1984, Urbanski & Ojeda 1987), which is thought to be brought about by central and peripheral changes that lead to increased activity of the GnRH pulse generator (Pelletier *et al*. 1981, Foster *et al*. 1985, Plant *et al*. 1989). However, the mechanisms within the brain that regulate the proper temporal release of GnRH from hypothalamic neurons to initiate puberty remain largely unknown.

In general, increased excitatory signals and reduced inhibitory signals to the GnRH neuronal network prompt the onset of puberty (Ojeda & Urbanski 1994). With regard to the former, kisspeptins have been implicated as potent stimulators of the gonadotropic axis (Gottsch *et al*. 2004, Dhillo *et al*. 2005). Structurally related peptides, kisspeptins, are products of the *KISS1* gene (Kotani *et al*. 2001, Ohtaki *et al*. 2001). Synthesized as a pre-prohormone, it is cleaved to liberate a 54 amino acid peptide that can be proteolytically processed (Takino *et al*. 2003) to shorter variants; all of which share the same amidated C terminus and retain full biological activity (Kotani *et al*. 2001, Ohtaki *et al*. 2001). Kisspeptin, acting through its cognate G-protein coupled receptor GPR54/KISS1R (Kotani *et al*. 2001), is thought to be an important determinant in the onset of puberty (Seminara & Kaiser 2005). Indeed, *Gpr54*/*Kiss1r* knockout mice failed to initiate puberty (Funes *et al*. 2003, Seminara *et al*. 2003) and naturally occurring mutations in *GPR54/KISS1R* result in idiopathic hypogonadotropic hypogonadism in humans (de Roux *et al*. 2003, Seminara *et al*. 2003, Semple *et al*. 2005). Expression of the *KISS1* and *GPR54/KISS1R* genes is both hormonally and developmentally regulated in the rodent (Navarro *et al*. 2004*a*, 2004*b*). Kisspeptin stimulates LH secretion (Matsui *et al*. 2004,


ISSN 1470–1626 (paper) 1741–7899 (online)
DOI: 10.1530/REP-07-0502
Online version via www.reproduction-online.org

Thompson *et al*. 2004, Navarro *et al*. 2005*b*) in a GnRH-dependent manner (Gottsch *et al*. 2004, Shahab *et al*. 2005, Arreguin- Arevalo *et al*. 2007). It has been demonstrated that kisspeptin acts directly on GnRH neurons to stimulate GnRH and gonadoroptin secretion (Thompson *et al*. 2004, Irwig *et al*. 2005, Messager *et al*. 2005). This has culminated in the establishment of a role for kisspeptin in the initiation of puberty in the rodent and primate (Shahab *et al*. 2005, Castellano *et al*. 2006). Data are available with regard to the effects of kisspeptin on LH secretion in the adult ewe (Messager *et al*. 2005, Arreguin-Arevalo *et al*. 2007, Caraty *et al*. 2007). However, the possible role of kisspeptin in mediating the onset of puberty in large domestic species, in particular the pig, has yet to be determined.

In the present study, we hypothesize that kisspeptin is an important regulator of gonadotropin secretion in the pig. To test this hypothesis, we administered kisspeptin either centrally or peripherally to prepubertal gilts and measured changes in gonadotropin secretion. Because the occurrence of puberty in the pig is directed by mechanisms in which the HPG axis regulates both gonadal function and growth (Barb *et al*. 1999), we tested the hypothesis that kisspeptin would modulate growth hormone (GH) secretion in the prepubertal gilt.

## Results

### *Experiment 1*

A treatment–time interaction ($P<0.001$) was detected for serum concentrations of LH. Prior to the i.c.v. infusion, serum concentrations of LH were similar for all animals ($0.25\pm0.03$ ng/ml). Following i.c.v. treatment, LH concentrations remained at basal levels for the control animals but were increased ($P<0.001$) for animals receiving either 10 or 100 μg kisspeptin (Figs 1 and 2). Mean concentration, peak concentration, and area under the curve (AUC) of serum LH were greater ($P<0.001$) in period 2, following i.c.v. infusion, for kisspeptin-treated animals compared with saline-treated controls (Fig. 2). Mean concentration and AUC of serum LH in period 2 were greater ($P<0.01$) for animals treated with the 100 μg dose of kisspeptin compared with the 10 μg dose (Fig. 2), but peak concentrations of serum LH in period 2 were similar (Fig. 2). All animals responded to a single i.v. bolus infusion of GnRH in period 3 with elevated serum LH (Fig. 1). Mean concentration and peak concentration of serum LH in period 3 were greater ($P<0.05$) for control animals compared with kisspeptin-treated animals (Fig. 2). Animals treated with the 10 μg dose of kisspeptin had decreased ($P<0.05$) AUC

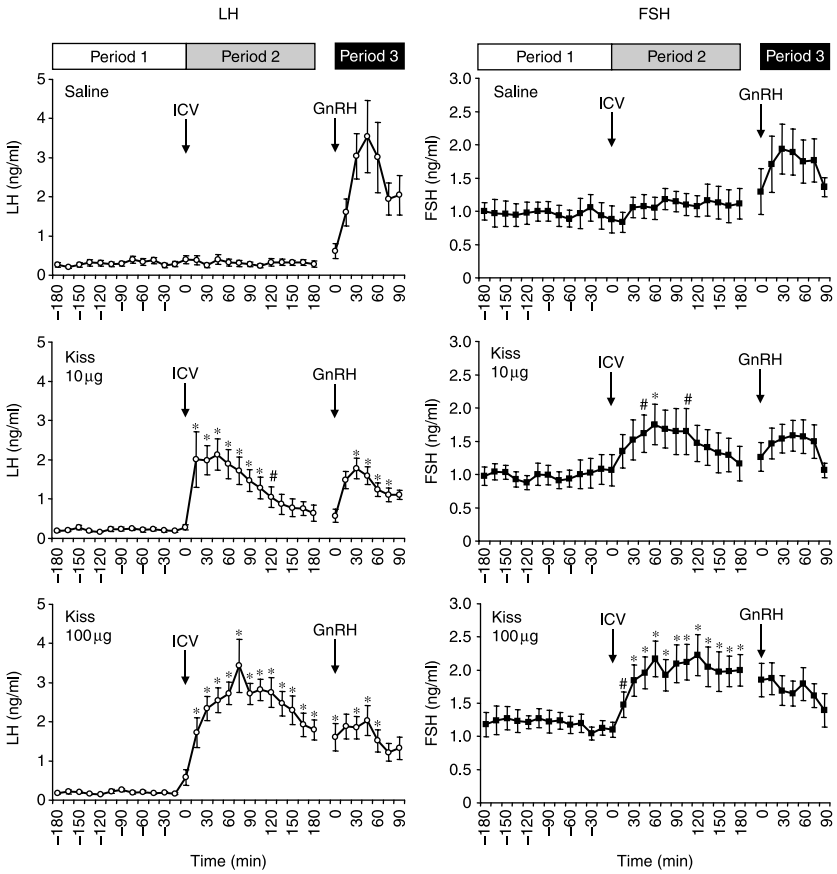


**Figure 1** Serum concentrations of LH and FSH (means±S.E.M.) for prepubertal gilts receiving i.c.v. infusion of saline or either 10 or 100 μg kisspeptin (Kiss). Period 1 is the 3 h prior to i.c.v. treatment; period 2 is the 3 h after i.c.v. treatment; period 3 is the 90 min following an i.v. injection of 100 μg GnRH. A treatment–time interaction was detected for LH ($P<0.001$) and FSH ($P<0.001$). *Times at which effects of treatment were different from saline-treated control animals ($P<0.05$). #Times at which effects of treatment tended to be different from saline-treated control animals ($P<0.10$).

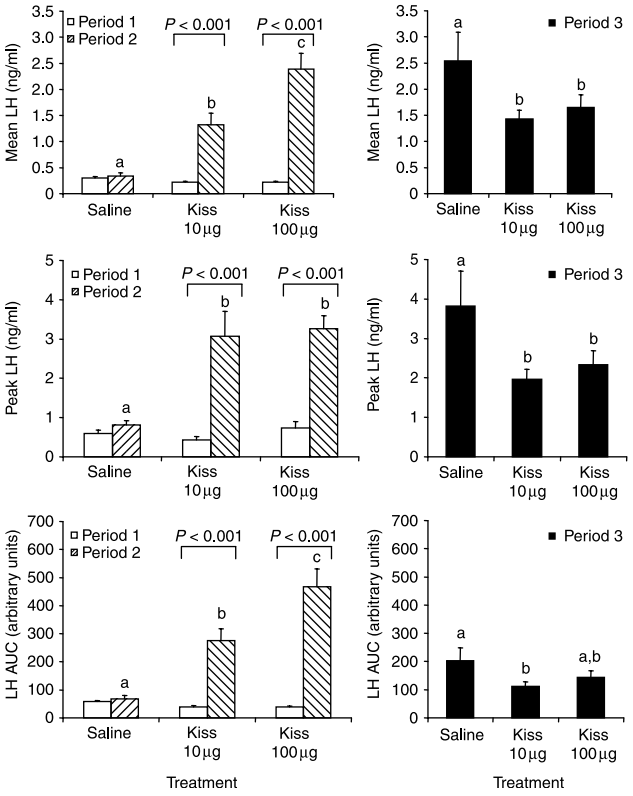


**Figure 2** Mean LH, peak LH, and area under the curve (AUC) of serum LH for prepubertal gilts receiving i.c.v. infusion of saline or either 10 or 100 µg kisspeptin (Kiss). Data in period 1 are from the 3 h prior to i.c.v. treatment; data in period 2 are from the 3 h after i.c.v. treatment; and data in period 3 are from the 90 min following an i.v. infusion of 100 µg GnRH. Data are presented as mean ± S.E.M. [a,b]Means within a period differ between treatments ($P<0.05$).

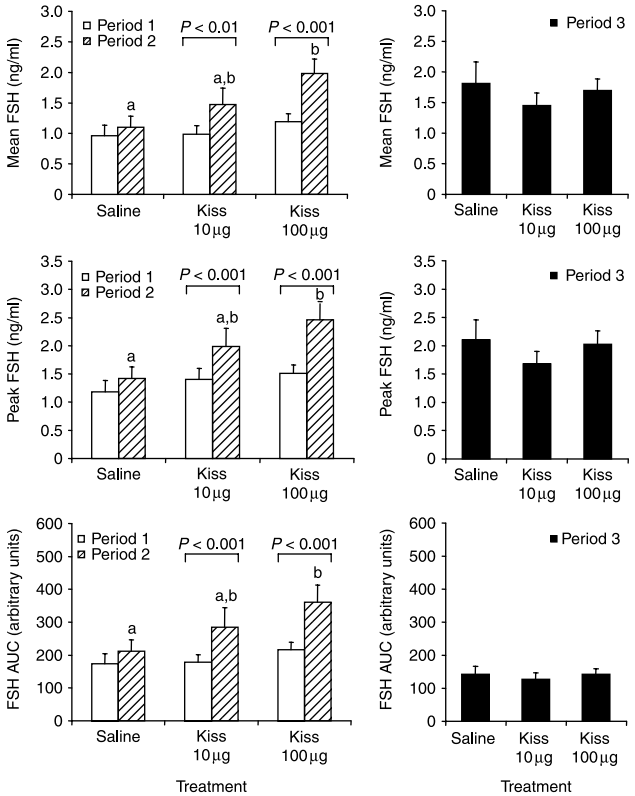


**Figure 3** Mean FSH, peak FSH, and area under the curve (AUC) of serum FSH for prepubertal gilts receiving i.c.v. infusion of saline or either 10 or 100 µg kisspeptin (Kiss). Data in period 1 are from the 3 h prior to i.c.v. treatment; data in period 2 are from the 3 h after i.c.v. treatment; and data in period 3 are from the 90 min following an i.v. infusion of 100 µg GnRH. Data are presented as mean ± S.E.M. [a,b]Means within a period differ between treatments ($P<0.05$).

following GnRH when compared with control animals treated with GnRH (Fig. 2). Area under the LH curve following GnRH was similar for control animals and those treated with the 100 µg dose of kisspeptin (Fig. 2), since serum concentrations of LH in the kisspeptin-treated animals were still above baseline at the beginning of period 3 (Fig. 1).

Similar to LH, there was a treatment–time interaction ($P<0.001$) for the effect of i.c.v. kisspeptin on FSH secretion (Fig. 1). Mean concentration, peak concentration, and AUC of serum FSH were increased ($P<0.001$) in period 2 for kisspeptin-treated animals in a dose-dependent manner when compared with control animals (Fig. 3). Serum FSH concentrations were increased ($P<0.05$) in control animals following GnRH treatment in period 3 (Fig. 1). Serum concentrations of FSH had not returned to baseline levels in the kisspeptin-treated animals at the beginning of period 3 (Fig. 1), thus there was no difference in the parameters of FSH secretion between control and kisspeptin-treated animals following GnRH infusion (Fig. 3).

An expected variation in secretory patterns of GH between animals was observed; however, serum concentrations of GH were not affected by i.c.v. treatment (Fig. 4). Mean concentrations of GH in serum following i.c.v. infusion were 4.15 ± 1.9, 2.33 ± 0.5, and 2.96 ± 1.1 ng/ml for controls, Kiss 10 µg, and Kiss 100 µg treated animals respectively.

### *Experiment 2*

There was a treatment–time interaction ($P<0.001$) for serum concentrations of LH. Serum LH concentrations increased following i.v. infusion in all kisspeptin-treated animals (Fig. 5). Compared with period 1, mean concentration, peak concentration, and AUC of serum LH of control animals were unchanged in period 2 but were greater ($P<0.05$) for all kisspeptin-treated animals (Fig. 7). Mean LH for animals treated with 5 mg kisspeptin tended ($P<0.1$) to be greater than for animals treated with 1 mg kisspeptin (Fig. 7). Area under the LH curve increased ($P<0.05$) in a linear fashion with increasing dose of kisspeptin (Fig. 7), which resulted from more sustained release of LH following the higher doses (Fig. 5).

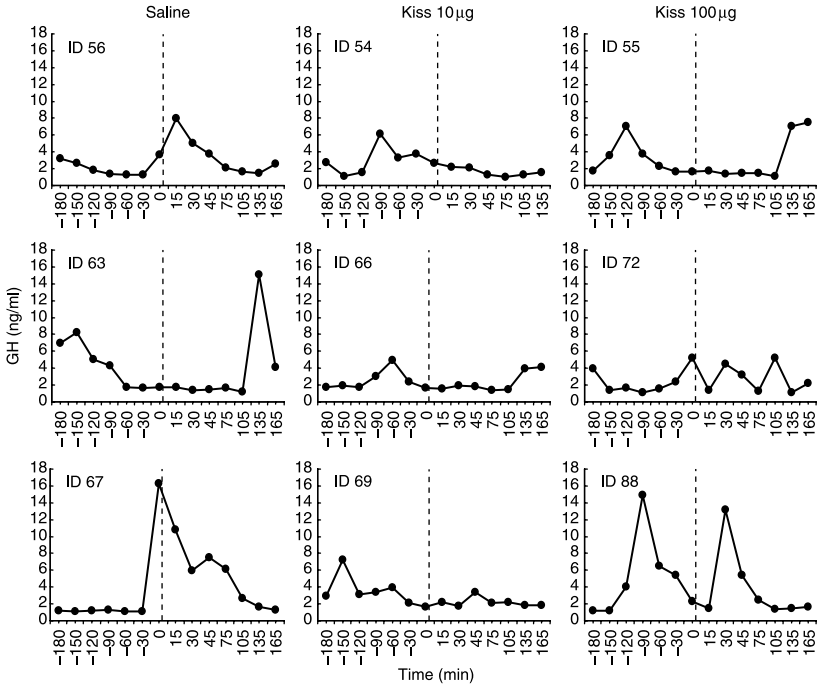


**Figure 4** Serum GH concentrations for prepubertal gilts receiving i.c.v. infusion of saline or either 10 or 100 µg kisspeptin (Kiss). Data of three representative animals from each treatment are shown. The dashed vertical line indicates the time at which i.c.v. treatments were administered. There was no treatment–time interaction detected.

Intravenous infusion of kisspeptin at the highest dose tended ($P=0.08$) to increase serum concentrations of FSH at 45–90 min after treatment (Fig. 6). There was neither a treatment nor treatment–period interaction on parameters of FSH secretion (Fig. 7). However, the overall mean in period 2 was greater ($P<0.05$) for peak FSH ($2.29\pm0.18$ vs $2.56\pm0.18$ ng/ml for periods 1 and 2 respectively) and AUC of FSH ($343\pm33$ vs $395\pm33$ ng/ml for periods 1 and 2 respectively) than in period 1. Concentrations of GH in serum were similar for all animals and were unchanged by i.v. infusion of 1, 2.5, or 5 mg kisspeptin (Table 1).

## Discussion

We report here for the first time that central infusion of kisspeptin to prepubertal gilts rapidly induces LH secretion. This strongly supports the concept that the role kisspeptin has in regulating the onset of puberty in the rodent (Navarro *et al*. 2004*a*, 2004*b*, Castellano *et al*. 2006) and primate (Plant *et al*. 2006) may be conserved in the domestic pig, which is an important biomedical model (Tumbleson 1986, Lunney 2007). The AUC, which reflects the integrated pattern of LH secretion, was increased by kisspeptin in a dose-dependent manner. The 10 µg dose (7.5 nmol) of kisspeptin resulted

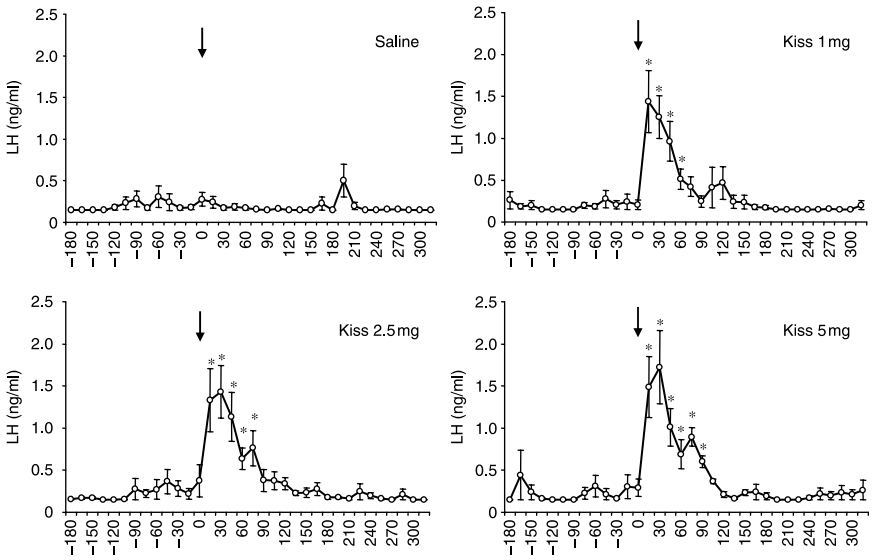


**Figure 5** Serum LH concentrations (mean ± S.E.M.) for prepubertal gilts receiving i.v. infusions of saline or either 1, 2.5, or 5 mg kisspeptin (Kiss). A treatment–time interaction was detected ($P<0.01$). *Times at which effects of treatment were different from saline-treated control animals ($P<0.05$).

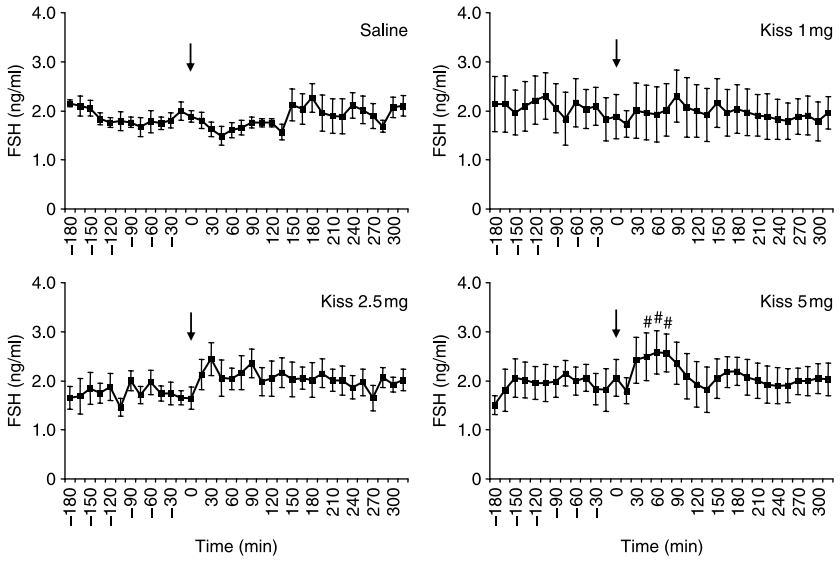


**Figure 6** Serum FSH concentrations (mean ± S.E.M.) for prepubertal gilts receiving i.v. infusions of saline or either 1, 2.5, or 5 mg kisspeptin (Kiss). A tendency for a treatment–time interaction was detected ($P=0.08$). #Times at which effects of treatment tended to be different from saline-treated control animals ($P<0.10$).

in a rapid pulse of LH that gradually declined over the next 3 h, whereas the pulse of LH induced by 100 µg (75 nmol) kisspeptin persisted throughout the sampling period. Presumably, this reflects the kisspeptin-induced pattern of GnRH secretion. Kisspeptin has been shown to stimulate the *in vitro* release of GnRH from hypothalamic explants of rats (Thompson *et al.* 2004), and central infusion of ovariectomized ewes with 50 nmol kisspeptin over a 4-h period caused the sustained secretion of GnRH into the cerebrospinal fluid

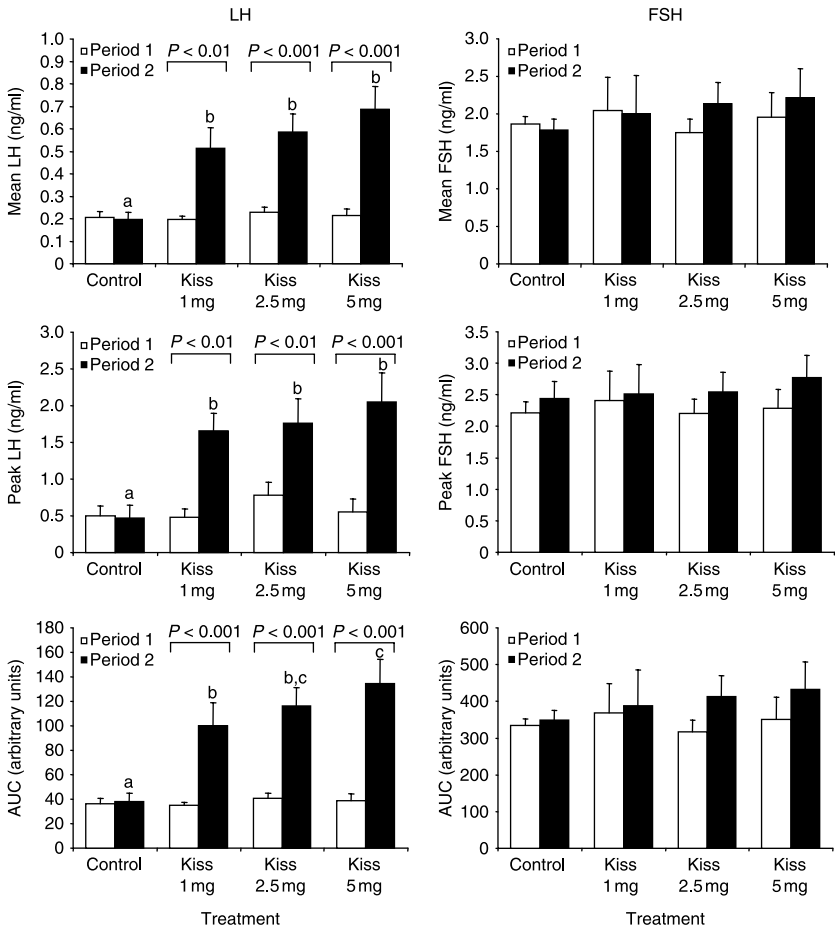


**Figure 7** Mean concentration, peak concentration, and area under the curve (AUC) of serum LH and FSH for prepubertal gilts receiving i.v. infusion of saline or either 1, 2.5, or 5 mg kisspeptin (Kiss). Data in period 1 are from of the 3 h prior to treatment and data in period 2 are from the 3 h after treatment. Data are presented as mean ± S.E.M. [a,b,c]Means within a period differ between treatments ($P<0.05$).

**Table 1** Effect of i.v. administration of kisspeptin (Kiss) on serum concentrations of growth hormone (GH; ng/ml) in prepubertal gilts.

| | Treatment | | | | | *P* value | | |
|---|---|---|---|---|---|---|---|---|
| Period[a] | Control | Kiss 1 mg | Kiss 2.5 mg | Kiss 5 mg | S.E.M.[b] | Treatment | Period | Treatment × period |
| 1 | 3.11 | 3.17 | 2.92 | 3.90 | 0.39 | 0.16 | 0.56 | 0.70 |
| 2 | 2.56 | 3.23 | 2.74 | 4.03 | 0.47 | | | |

[a]Period 1, before treatment; period 2, after treatment. [b]S.E.M., overall, S.E.M. within treatment.

concomitantly with elevated serum concentrations of LH (Messager *et al*. 2005). We cannot preclude, however, that the pattern of LH secretion observed here might be caused by alterations in GnRH pulse frequency or amplitude. Whether or not kisspeptin regulates the GnRH pulse generator is not presently known. Regardless, pituitary function was intact in these animals as indicated by the increased LH secretion in control pigs following an i.v. infusion of GnRH in period 3. The GnRH-induced release of LH in animals that had received i.c.v. infusions of kisspeptin was reduced when compared with that of the control animals that had received i.c.v. infusion of saline. In view of the prolonged stimulation of LH release following kisspeptin treatment, this reduced response to GnRH likely reflects a reduction in the releasable pool of LH.

In order to determine whether peripheral administration of kisspeptin could induce LH secretion in prepubertal pigs, we administered an i.v. bolus of kisspeptin at three different doses. All three doses tested were able to elevate secretion of LH in these animals. This is substantiated by reports in rodents and monkeys which demonstrate kisspeptin stimulates LH secretion when given either centrally or peripherally (Matsui *et al*. 2004, Navarro *et al*. 2005*b*, Shahab *et al*. 2005). At the time our study was conducted, it was reported that i.v. administration of $<3$ mg kisspeptin to ovariectomized ewes resulted in somewhat variable LH release (Arreguin-Arevalo *et al*. 2007). Since the completion of our study, it has now been shown that i.v. administration of kisspeptin at doses of $<1$ mg can reliably elicit LH secretion, at least in the ovariectomized estradiol-treated ewe (Caraty *et al*. 2007). In the present study, the magnitude of LH release was similar for all doses of kisspeptin but the duration was longer with increasing dose. Whether or not i.v. infusion of kisspeptin at $<1$ mg can reliably induce LH secretion in the pig warrants further investigation.

Information regarding the effects of kisspeptin on FSH secretion is limited. We demonstrate that the central administration of kisspeptin to prepubertal gilts increased serum concentrations of FSH. This effect of kisspeptin on FSH secretion in the pig is in agreement with reports in rodents (Matsui *et al*. 2004, Thompson *et al*. 2004, Navarro *et al*. 2005*a*) and primates (Plant *et al*. 2006). The increase in FSH induced by kisspeptin showed more gradual onset and the magnitude was less than that of LH. The ED50 of kisspeptin to release FSH has been estimated to be ~100 times higher than that for LH in rats (Navarro *et al*. 2005*a*). This may reflect the fact that FSH is not wholly under the control of GnRH (Kile & Nett 1994, Phillips 2005). Despite a clear effect of i.c.v.-infused kisspeptin on FSH secretion, i.v. infusion only tended to alter FSH secretory patterns in prepubertal gilts infused with the highest dose of kisspeptin. This is similar to observations in the ovariectomized ewe (Arreguin-Arevalo *et al*. 2007). However, it was recently reported that doses of kisspeptin up to tenfold less than used in our experiment were able to stimulate FSH secretion in ovariectomized estradiol-treated ewes (Caraty *et al*. 2007). Differences in species and physiological status of the animals may account for differing results when kisspeptin is infused intravenously.

Concentrations of FSH in serum remained elevated at the end of the 3 h sampling period in the animals receiving i.c.v. infusion of 100 μg kisspeptin, despite the fact that LH in those animals was declining. Interestingly, a similar observation has recently been reported in ovariectomized estradiol-treated ewes (Caraty *et al*. 2007). In the present experiment, we were unable to determine whether this is due to a true change in secretion, or to the longer half-life of FSH (Macdonald *et al*. 2007). In the pig, circulating concentrations of LH are reduced more rapidly than those of FSH after a bolus GnRH infusion (Wise *et al*. 1996). Nonetheless, the initial pattern of FSH secretion induced by i.c.v. infusion of kisspeptin is similar to that caused by i.v. infusion of GnRH in the control animals. This supports the concept that the primary mechanism of kisspeptin-induced FSH secretion is mediated through the GnRH neuronal network (Thompson *et al*. 2004, Irwig *et al*. 2005, Messager *et al*. 2005).

During preparation of this manuscript, it was reported that i.v. administration of kisspeptin stimulated GH secretion in prepubertal heifer calves (Kadokawa *et al*. 2008). Indeed, age-related changes in serum concentrations of GH occur in the pig but typically decline as the animal is approaching puberty (Machlin *et al*. 1968, Klindt & Stone 1984, Dubreuil *et al*. 1987). We found no effect of either central or peripheral administration of kisspeptin on secretion of GH in the present study. Divergence with regard to kisspeptin's actions on GH secretion may be related to species differences. However, our experiment included a true control group, which may have allowed us to more

accurately delineate the effect of kisspeptin on GH secretion in the pig. The inclusion of saline-treated controls clearly illustrate that, in our hands, kisspeptin does not modulate the secretion of GH in the prepubertal pig, and agrees with observations in monkeys (Plant personal communication).

In conclusion, here we report that kisspeptin can activate the gonadotropic axis in the prepubertal pig. The ability of kisspeptin to stimulate hormone secretion from the anterior pituitary gland of the pig seems to be specific for LH and FSH and did extend to GH in this study. We presently do not know whether other anterior pituitary hormones in the pig are affected by kisspeptin. Our data are consistent with the idea that kisspeptin's actions on gonadotropin secretion are mediated primarily through modulating secretion of GnRH and support the concept that kisspeptin plays an important role in regulating puberty.

## Materials and Methods

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of The University of Georgia. Prepubertal gilts (sow line C42, boar line 280; PIC, Franklin, KY, USA) weighing 67.6±7.2 kg (mean±S.D.) and 130-day age were used. Animals were born and reared at the Swine Research Center at The University of Georgia. During experiments, animals were moved to a Large Animal Research Unit, where they were kept in individual pens under controlled temperature (22 °C) and had access to feed and water *ad libitum* unless otherwise noted. The diet was formulated to meet National Research Council guidelines (NRC 1988) for growing swine. Kisspeptin consisted of the ten C-terminal amino acids of the murine peptide and was obtained from GenScript Corp. (Scotch Plains, NJ, USA). GnRH was obtained from Sigma or Merial Limited (Cystorelin; Iselin, NJ, USA).

### *Experiment 1*

Animals ($n$=14) were surgically fitted with a lateral i.c.v. cannula using a steriotaxic procedure described previously (Estienne *et al*. 1990, Barb *et al*. 1993). Placement of the i.c.v. cannula of each animal was verified by X-ray. At least 1 week after the placement of i.c.v. cannula, and 24 h prior to treatment, all animals were fitted with indwelling jugular catheters (Barb *et al*. 1982). Animals were randomly assigned to one of three groups. Control animals received 150 µl PBS. The other groups received either 10 or 100 µg kisspeptin in 150 µl PBS. Doses of kisspeptin were based on our previous experience with i.c.v. administration of hormones to the pigs (Barb *et al*. 2004, Barb & Barrett 2005). Serial blood samples were drawn every 15 min for 3 h before (period 1) and 3 h after (period 2) i.c.v. treatment, and for 90 min (period 3) following an i.v. bolus infusion of 100 µg GnRH. The time from the end of period 2 to the beginning of period 3 was 15–45 min. The inclusion of the GnRH treatment was to verify that pituitary function was intact and that LH secretion could in fact be induced in these prepubertal gilts. One week later, the experiment was replicated with animals reassigned to treatment so that no animal received the same treatment a second time, resulting in eight pigs for the control treatment and ten pigs for each dose of kisspeptin. Gilts were killed and the ovaries were subjected to gross inspection to confirm the absence of luteal structures.

### *Experiment 2*

Experiment 2 was conducted with six prepubertal gilts in each treatment group to determine the response to increasing doses of kisspeptin administered as a single bolus i.v. infusion. Animals were fitted with indwelling jugular catheters the day prior to the experiment. Animals received 1, 2.5, or 5 mg kisspeptin in 3 ml PBS or 3 ml PBS alone for the control animals. Doses were chosen in part based on the reported effective dose in sheep (Arreguin-Arevalo *et al*. 2007). At 0730 h, feeders were removed from pens and blood sampling started at 0800 h. Serial blood samples were drawn every 15 min for 3 h before (period 1) and 5 h after (period 2) treatment. Feeders were returned to all pens after the last blood sample was drawn.

### *Hormone analysis*

Blood was allowed to clot for 1 h at room temperature and then 4 °C overnight. Serum was separated by centrifugation and stored at −20 °C for subsequent analysis of LH (Kesner *et al*. 1987) and FSH (Trout *et al*. 1992) by RIA. The reference standard for LH (AFP-10506A) and FSH (AFP-10640B) was provided by Dr A F Parlow, Scientific Director of the NIH, NIDDK, National Hormone and Peptide Program. Sensitivity of the assays was 0.15 and 0.2 ng/ml for LH and FSH respectively. Intra- and inter-assay coefficient of variation (CV) of LH assays were 7.9 and 9.8% respectively. Four pools of porcine serum with FSH concentrations that ranged from 1 to 3 ng/ml were included in each assay; these had intra-assay CV that ranged from 4 to 16%. Serum concentrations of GH were determined using a porcine RIA kit (Millipore Corp., Billerica, MA, USA). Sensitivity of the assay was 1 ng/ml and intra- and inter-assay CV were 8.6 and 13.4% respectively.

### *Statistical analysis*

To determine the effect of kisspeptin on serum concentrations of LH, FSH, and GH, data were subjected to generalized least squares ANOVA with repeated measures using the MIXED procedure of SAS (1999). The model included replicate, treatment, time, and all first- and second-order interactions, with a compound symmetric function used to model the covariance structure for the repeated measures. If a significant ($P$<0.05) treatment–time interaction was detected, the simple effects of treatment within a time were compared using the SLICE option of the LSMEANS statement of SAS. Mean concentration, peak concentration, and AUC of serum LH and FSH at fixed periods were subjected to generalized least squares ANOVA with repeated measures. In experiment 1,

periods 1 and 2 were defined as the 3 h before and 3 h after i.c.v. treatment respectively. Period 3 was defined as the 90 min following i.v. infusion of GnRH. In experiment 2, periods 1 and 2 were defined the same as in experiment 1. The model included replicate, treatment, period, and all first- and second-order interactions, with a compound symmetric function used to model the covariance structure for the repeated measures.

## Acknowledgements

The authors wish to thank B Jackson, L Lee-Rutherford, C Rogers, G McKinney, A Kruger, and J Segerf for technical assistance as well as M Daniels for expert animal husbandry. The authors thank Drs A Nelson, J K Bertrand, and R Rekaya for their statistical consultation. The authors wish to acknowledge Dr A F Parlow, Scientific Director of the NIH, NIDDK, National Hormone and Peptide Program, for providing porcine LH and FSH for radioiodination and standard preparation as well as FSH antibody (AFP-C5288113Rb) for RIA. This research was supported by USDA funds and State funds allocated to the Georgia Agricultural Experiment Station and in part by a National Research Initiative Competitive Grant (no. 2005-35023-15949 and 2005-35203-16852) from the USDA Cooperative State Research, Education, and Extension Service. Mention of trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the US Department of Agriculture or The University of Georgia and does not imply its approval to the exclusion of other products which may be suitable. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

## References

**Arreguin-Arevalo JA, Lents CA, Farmerie TA, Nett TM & Clay CM** 2007 KiSS-1 peptide induces release of LH by a direct effect on the hypothalamus of ovariectomized ewes. *Animal Reproduction Science* **101** 265–275.

**Barb CR & Barrett JB** 2005 Neuropeptide Y modulates growth hormone but not luteinizing hormone secretion from prepuberal gilt anterior pituitary cells in culture. *Domestic Animal Endocrinology* **29** 548–555.

**Barb CR, Kraeling RR, Rampacek GB, Fonda ES & Kiser TE** 1982 Inhibition of ovulation and LH secretion in the gilt after treatment with ACTH or hydrocortisone. *Journal of Reproduction and Fertility* **64** 85–92.

**Barb CR, Kraeling RR, Estienne MJ & Rampacek GB** 1993 Technique for cannulation of the lateral ventricle of the brain in swine. *Kopf Carrier* **35** 1–5.

**Barb CR, Kraeling RR, Rampacek GB & Estienne MJ** 1999 Current concepts of the onset of puberty in the gilt. *3rd Conference of the European Society for Domestic Animal Reproduction Supplement* **6** 82–88.

**Barb CR, Barrett JB & Kraeling RR** 2004 Role of leptin in modulating the hypothalamic–pituitary axis and luteinizing hormone secretion in the prepuberal gilt. *Domestic Animal Endocrinology* **26** 201–214.

**Brinkley HJ** 1981 Endocrine signaling and female reproduction. *Biology of Reproduction* **24** 22–43.

**Caraty A, Smith JT, Lomet D, Said SB, Morrissey A, Cognie J, Doughton B, Baril G, Briant C & Clarke IJ** 2007 Kisspeptin synchronizes preovulatory surges in cyclic ewes and causes ovulation in seasonally acyclic ewes. *Endocrinology* **148** 5258–5267.

**Castellano JM, Navarro VM, Fernandez-Fernandez R, Castano JP, Malagon MM, Aguilar E, Dieguez C, Magni P, Pinilla L & Tena-Sempere M** 2006 Ontogeny and mechanisms of action for the stimulatory effect of kisspeptin on gonadotropin-releasing hormone system of the rat. *Molecular and Cellular Endocrinology* **257–258** 75–83.

**Clarke IJ, Cummins JT & de Kretser DM** 1983 Pituitary gland function after disconnection from direct hypothalamic influences in the sheep. *Neuroendocrinology* **36** 376–384.

**Desjardins C** 1981 Endocrine signaling and male reproduction. *Biology of Reproduction* **24** 1–21.

**Dhillo WS, Chaudhri OB, Patterson M, Thompson EL, Murphy KG, Badman MK, McGowan BM, Amber V, Patel S, Ghatei MA *et al.*** 2005 Kisspeptin-54 stimulates the hypothalamic–pituitary–gonadal axis in human males. *Journal of Clinical Endocrinology and Metabolism* **90** 6609–6615.

**Dubreuil P, Pelletier G, Petitclerc D, Lapierre H, Couture Y, Brazeau P, Gaudreau P & Morisset J** 1987 Influence of age and sex on basal secretion of growth hormone (GH) and on GH-induced release by porcine GH-releasing factor pGRF(1-29$NH_2$) in growing pigs. *Domestic Animal Endocrinology* **4** 299–307.

**Estienne MJ, Kesner JS, Barb CR, Kraeling RR, Rampacek GB & Estienne CE** 1990 Gonadotropin and prolactin secretion following intraventricular administration of morphine in gilts. *Proceedings of the Society for Experimental Biology and Medicine* **193** 92–97.

**Foster DL, Yellon SM & Olster DH** 1985 Internal and external determinants of the timing of puberty in the female. *Journal of Reproduction and Fertility* **75** 327–344.

**Funes S, Hedrick JA, Vassileva G, Markowitz L, Abbondanzo S, Golovko A, Yang S, Monsma FJ & Gustafson EL** 2003 The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system. *Biochemical and Biophysical Research Communications* **312** 1357–1363.

**Gharib SD, Wierman ME, Shupnik MA & Chin WW** 1990 Molecular biology of the pituitary gonadotropins. *Endocrine Reviews* **11** 177–199.

**Gottsch ML, Cunningham MJ, Smith JT, Popa SM, Acohido BV, Crowley WF, Seminara S, Clifton DK & Steiner RA** 2004 A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. *Endocrinology* **145** 4073–4077.

**Irwig MS, Fraley GS, Smith JT, Acohido BV, Popa SM, Cunningham MJ, Gottsch ML, Clifton DK & Steiner RA** 2005 Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. *Neuroendocrinology* **80** 264–272.

**Kadokawa H, Matsui M, Hayashi K, Matsunaga N, Kawashima C, Shimizu T & Miyamoto A** 2008 Peripheral administration of kisspeptin-10 increases plasma concentrations of GH as well as LH in prepubertal holstein heifers. *Journal of Endocrinology* **196** 331–334.

**Kesner JS, Kraeling RR, Rampacek GB & Johnson B** 1987 Absence of an estradiol-induced surge of luteinizing hormone in pigs receiving unvarying pulsatile gonadotropin-releasing hormone stimulation. *Endocrinology* **121** 1862–1869.

**Kile JP & Nett TM** 1994 Differential secretion of follicle-stimulating hormone and luteinizing hormone from ovine pituitary cells following activation of protein kinase A, protein kinase C, or increased intracellular calcium. *Biology of Reproduction* **50** 49–54.

**Klindt J & Stone RT** 1984 Porcine growth hormone and prolactin: concentrations in the fetus and secretory patterns in the growing pig. *Growth* **48** 1–15.

**Kotani M, Detheux M, Vandenbogaerde A, Communi D, Vanderwinden JM, Le Poul E, Brezillon S, Tyldesley R, Suarez-Huerta N, Vandeput F *et al.*** 2001 The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *Journal of Biological Chemistry* **276** 34631–34636.

**Lunney JK** 2007 Advances in swine biomedical model genomics. *International Journal of Biological Sciences* **3** 179–184.

**Lutz JB, Rampacek GB, Kraeling RR & Pinkert CA** 1984 Serum luteinizing hormone and estrogen profiles before puberty in the gilt. *Journal of Animal Science* **58** 686–691.

**Macdonald GJ, Wise TH, Sluss PM & Ford JJ** 2007 Breed differences in clearance of porcine FSH in hypophysectomized rats. *Animal Reproduction Science* **102** 328–334.

**Machlin LV, Horino M, Hertelendy F & Kipnis DM** 1968 Plasma growth hormone and insulin levels in the pig. *Endocrinology* **82** 369–376.

**Matsui H, Takatsu Y, Kumano S, Matsumoto H & Ohtaki T** 2004 Peripheral administration of metastin induces marked gonadotropin release and ovulation in the rat. *Biochemical and Biophysical Research Communications* **320** 383–388.

**Messager S, Chatzidaki EE, Ma D, Hendrick AG, Zahn D, Dixon J, Thresher RR, Malinge I, Lomet D, Carlton MB *et al.*** 2005 Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. *PNAS* **102** 1761–1766.

**Navarro VM, Castellano JM, Fernandez-Fernandez R, Barreiro ML, Roa J, Sanchez-Criado JE, Aguilar E, Dieguez C, Pinilla L & Tena-Sempere M** 2004*a* Developmental and hormonally regulated messenger ribonucleic acid expression of KiSS-1 and its putative receptor, GPR54, in rat hypothalamus and potent luteinizing hormone-releasing activity of KiSS-1 peptide. *Endocrinology* **145** 4565–4574.

**Navarro VM, Fernandez-Fernandez R, Castellano JM, Roa J, Mayen A, Barreiro ML, Gaytan F, Aguilar E, Pinilla L, Dieguez C *et al.*** 2004*b* Advanced vaginal opening and precocious activation of the reproductive axis by KiSS-1 peptide, the endogenous ligand of GPR54. *Journal of Physiology* **561** 379–386.

**Navarro VM, Castellano JM, Fernandez-Fernandez R, Tovar S, Roa J, Mayen A, Barreiro ML, Casanueva FF, Aguilar E, Dieguez C *et al.*** 2005*a* Effects of KiSS-1 peptide, the natural ligand of GPR54, on follicle-stimulating hormone secretion in the rat. *Endocrinology* **146** 1689–1697.

**Navarro VM, Castellano JM, Fernandez-Fernandez R, Tovar S, Roa J, Mayen A, Nogueiras R, Vazquez MJ, Barreiro ML, Magni P *et al.*** 2005*b* Characterization of the potent luteinizing hormone-releasing activity of KiSS-1 peptide, the natural ligand of GPR54. *Endocrinology* **146** 156–163.

**NRC** 1988 *Nutrient Requirements of Pigs,* Washington, DC: National Academy Press.

**Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, Terao Y, Kumano S, Takatsu Y, Masuda Y *et al.*** 2001 Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature* **411** 613–617.

**Ojeda SR & Urbanski HF** 1994 In *Puberty in the Rat,* Eds E Knobil & JD Neill. New York: Raven Press.

**Pelletier J, Carrez-Camous S & Thiery JC** 1981 Basic neuroendocrine events before puberty in cattle, sheep and pigs. *Journal of Reproduction and Fertility* **30** 91–102.

**Phillips DJ** 2005 Activins, inhibins and follistatins in the large domestic species. *Domestic Animal Endocrinology* **28** 1–16.

**Plant TM, Gay VL, Marshall GR & Arslan M** 1989 Puberty in monkeys is triggered by chemical stimulation of the hypothalamus. *PNAS* **86** 2506–2510.

**Plant TM, Ramaswamy S & Dipietro MJ** 2006 Repetitive activation of hypothalamic G protein-coupled receptor 54 with intravenous pulses of kisspeptin in the juvenile monkey (*Macaca mulatta*) elicits a sustained train of gonadotropin-releasing hormone discharges. *Endocrinology* **147** 1007–1013.

**de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL & Milgrom E** 2003 Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *PNAS* **100** 10972–10976.

**SAS** 1999 *SAS User's Guide (Resease 8.0),* Cary, NC: Statistical Analysis Systems Institute, Inc.

**Schally AV, Arimura A, Baba Y, Nair RM, Matsuo H, Redding TW & Debeljuk L** 1971 Isolation and properties of the FSH and LH-releasing hormone. *Biochemical and Biophysical Research Communications* **43** 393–399.

**Seminara SB & Kaiser UB** 2005 New gatekeepers of reproduction: GPR54 and its cognate ligand, KiSS-1. *Endocrinology* **146** 1686–1688.

**Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG *et al.*** 2003 The GPR54 gene as a regulator of puberty. *New England Journal of Medicine* **349** 1614–1627.

**Semple RK, Achermann JC, Ellery J, Farooqi IS, Karet FE, Stanhope RG, O'rahilly S & Aparicio SA** 2005 Two novel missense mutations in GPR54 in a patient with hypogonadotropic hypogonadism. *Journal of Clinical Endocrinology and Metabolism* **90** 1849–1855.

**Shahab M, Mastronardi C, Seminara SB, Crowley WF, Ojeda SR & Plant TM** 2005 Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates. *PNAS* **102** 2129–2134.

**Takino T, Koshikawa N, Miyamori H, Tanaka M, Sasaki T, Okada Y, Seiki M & Sato H** 2003 Cleavage of metastasis suppressor gene product KiSS-1 protein/metastin by matrix metalloproteinases. *Oncogene* **22** 4617–4626.

**Thompson EL, Patterson M, Murphy KG, Smith KL, Dhillo WS, Todd JF, Ghatei MA & Bloom SR** 2004 Central and peripheral administration of kisspeptin-10 stimulates the hypothalamic–pituitary–gonadal axis. *Journal of Neuroendocrinology* **16** 850–858.

**Trout WE, Killen JH, Christenson RK, Schanbacher BD & Ford JJ** 1992 Effects of weaning on concentrations of inhibin in follicular fluid and plasma of sows. *Journal of Reproduction and Fertility* **94** 107–114.

**Tumbleson ME** 1986 In *Swine in Biomedial Research,* Ed. ME Tumbleson. New York: Plenum Press.

**Urbanski HF & Ojeda SR** 1987 Activation of luteinizing hormone-releasing hormone release advances the onset of female puberty. *Neuroendocrinology* **46** 273–276.

**Wise T, Lunstra DD & Ford JJ** 1996 Differential pituitary and gonadal function of Chinese Meishan and European white composite boars: effects of gonadotropin-releasing hormone stimulation, castration, and steroidal feedback. *Biology of Reproduction* **54** 146–153.

Received 7 November 2007
First decision 4 December 2007
Revised manuscript received 25 January 2008
Accepted 26 February 2008