Effects of Short Term Administration of Genistein on Hypothalamic and Anterior Pituitary Hormones in Ovariectomized Gilts

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Abstract

Administration of genistein to barrows increased anterior pituitary (AP) concentrations of IGF-I and LH and increased expression of AP IGF receptor. Whether similar changes occur in gilts remains to be determined. The objective of this experiment was to determine if short term administration of genistein increased expression of components of the AP IGF system and hypothalamic hormones and receptors involved in gonadotropin synthesis and/or release in the gilt. Sixteen crossbred gilts of similar weight (97.7 kg) were ovariectomized and assigned to either control (C; n = 8) or genistein (G; n = 8) groups. Genistein pigs received 800 mg of genistein in DMSO while C pigs received an equal volume of DMSO i.m. on day 0, 1, 2, and 3. Blood samples were obtained on day 0, 1, 2, and 3. Pigs were slaughtered on d 4 when blood, AP, and medial basal hypothalami (MBH) were collected. No difference was detected ($P > 0.05$) in AP concentrations of IGF-I or serum concentrations of IGF-I in C and G pigs. Anterior pituitary concentrations of LH were greater ($P < 0.05$) in G pigs compared with C pigs. Relative expression of AP IGF-I was greater ($P < 0.05$) in G pigs than C pigs. Relative expression of AP IGF-IR, IGFBP-2 and IGFBP-3 did not differ ($P > 0.05$) in C and G pigs. Relative expression of AP IGFBP-5 and GnRHR was increased ($P < 0.05$) in G pigs compared with C pigs. Relative expression of AP LHβ did not differ between C and G pigs. Relative expression of MBH kisspeptin was greater ($P < 0.01$) in G pigs than C pigs. These data provided evidence that short term administration of genistein increased expression of hypothalamic and hypophyseal hormones in gilts which could influence subsequent reproduction.

Keywords

Genistein, Gilt, LH, GnRH, Kisspeptin

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1. Introduction

Phytoestrogens are plant substances similar in chemical structure to estradiol. Phytoestrogens can be chemically divided into three categories, namely, flavonoids, coumestans and lignans [1]. Contained within the flavonoid group are isoflavones, such as genistein. Genistein is structurally similar to estradiol and has the ability to bind to estrogen receptors α and β causing an estrogenic effect [1]. Genistein binds to the estrogen receptor (ER)β with greater affinity than the ERα and can compete with other estrogens such as estrone, estradiol, and diethylstilbestrol for binding to the estrogen receptor [1]. Although the estrogenic potency of genistein is much less than that of estradiol-17β, it has been shown to be mitogenic in breast tumors [1] and to stimulate MCF-7 tumor cell growth [2] similar to that of estradiol-17β.

Genistein has been shown to have effects on different reproductive processes in pigs. When ovariectomized gilts were injected intramuscularly with genistein for 15 d, their uterine and cervical mass increased, and there was enhanced expression of proliferating cell nuclear antigen in the reproductive tract, suggesting increased cell division [3]. Other investigators demonstrated that IGF-I and estrogen output increased in cultured porcine granulosa cells treated with genistein [4].

Previous research in our laboratory has demonstrated genistein’s effects on the anterior pituitary gland in barrows. Intramuscular administration of 400 mg of genistein to barrows for 16 d resulted in an increase in anterior pituitary gland concentrations of IGF-I and LH, and increased expression of components of the AP IGF system [5], all of which could lead to increased secretion of LH [6] [7]. To our knowledge, investigations of genistein’s effects on the AP and hypothalamus in the prepubertal gilt are lacking.

Therefore, the objective of this experiment was to determine if administration of 800 mg genistein to ovariectomized gilts for 4 d increased expression of components of the AP IGF system and hypothalamic hormones and receptors known to be involved in gonadotropin synthesis and/or release.

2. Materials and Methods

2.1. Animals and Procedures

Sixteen crossbred gilts of similar age (150 d) and weight (97.7 kg) were ovariectomized and stratified into one of two treatment groups (n = 8/group): control (C) and 800 mg genistein (G). Ovariectomy was performed two weeks before the beginning of the experiment and all gilts were considered prepubertal due to the absence of corpora lutea and or corpora albicantia. Treatments were prepared by dissolving genistein (G-6055; LC Laboratories, Wobrun, MA) in dimethyl sulfoxide (DMSO). Treatments were administered by intramuscular (i.m.) injection daily at 1100 on day 0 and continuing through day 3. Each pig received its assigned treatment: genistein treated pigs were administered 800 mg of 99% pure form genistein dissolved in DMSO while the control group received equal amounts of DMSO. Pigs were fed 2 kg of a corn/soybean meal diet containing 17.3% crude protein, 1.1% lysine, and 3.1 Mcal ME/kg. Blood was collected via jugular venipuncture on day 0, 1, 2, and 3. Blood samples were allowed to clot overnight at 4°C, then serum was collected by centrifugation (1500 x g for 30 min at 4°C) and stored at −20°C. Pigs were slaughtered on day 4, when blood, anterior pituitary glands, and medial basal hypothalami (MBH) were collected. The MBH consisted of a block of tissue limited rostrally by the optic chiasm, caudally by the mammillary bodies, laterally 3 mm from the midline and dorsally 4 mm from the base of the hypothalamus. Anterior pituitary glands and MBH were sectioned mid-sagittally, wrapped in foil, frozen in liquid nitrogen, then stored at −80°C. All experimental procedures were approved by the South Dakota State University Animal Care and Use Committee.

2.2. Hormone Assays

Serum and anterior pituitary concentrations of IGF-I were determined in duplicate by RIA [8] [9] for all blood and anterior pituitary glands. One half of each anterior pituitary gland was homogenized in a 15 mL polypropylene tube with 1 mL of homogenization buffer (1% cholic acid, 0.1% SDS, 200 μM phenylmethylsulfonyl fluoride, 100 μM EDTA, 1 μM leupeptin, 1 μM pepstatin). Anterior pituitaries were homogenized on ice with a T25 Ultra-Turrax tissue disperser (IKA Works, Wilmington, NC, USA) for 30 s at 20,500 rpm. Anterior pituitary homogenates were then diluted to 100 mg of AP tissue/ml with homogenization buffer. Homogenates were centrifuged at 12,000 x g for 10 min at 4°C and the supernatant was removed and stored at −20°C. Protein content
of the AP homogenates (1:10 dilution) was determined by the Bradford method using reagents provided by Bio-Rad (Hercules, CA, USA). Insulin-like growth factor binding proteins (IGFBP) were removed from sera and anterior pituitary gland samples with a 1:17 ratio of sample to acidified ethanol (12.5% 2 N HCL: 87.5% absolute ethanol) [10]. Extracted samples were centrifuged (12,000 x g at 4°C) to separate IGFBP. A portion of the supernatant from the extracted sample was removed and neutralized with 0.855 M Tris base, incubated an additional 4 h at 4°C then centrifuged at 12,000 x g at 4°C to remove any additional IGFBP. When samples of this extract, equivalent to the original serum or AP homogenate sample were subjected to Western ligand blot analysis and subsequent phosphorimagergy, no detected binding of [125I] IGF-I to IGFBP was observed. Recombinant human IGF-I (GF-050; Austral Biological, San Ramon, CA) was used as the standard and radioiodinated antigen. Antisera UB2-495 (National Hormone and Peptide Program, NIDDK) was used at a dilution of 1:100,000. Sensitivity of the assay was 16.3 pg/tube. Inter-assay coefficient of variation was 16.7%. Intra-assay coefficient of variation was 7.0%.

Anterior pituitary concentrations of LH were determined in triplicate by RIA [11]. Porcine LH (AFP3881A; National Hormone and Peptide Program, NIDDK) was used as the radioiodinated antigen and standard. Luteinizing hormone antiserum (AFP15103194; National Hormone and Peptide Program) was used at a dilution of 1:200,000. Anterior pituitary homogenates were diluted 1:25,000 in 0.01 M PBS-0.1% gelatin prior to assay. Sensitivity of the assay was 0.13 ng/tube. Intra-assay coefficient of variation was 13.3%.

2.3. Reverse Transcriptase Polymerase Chain Reaction (PCR) and Real-Time PCR

Total RNA was isolated from one half of each AP gland and MBH using TRIzol (TR118, Molecular Research Company, Cincinnati, OH). Purity of RNA was determined by measuring the A260/A280 ratio. The ratio of all samples ranged from 1.8 to 2.0. The integrity of RNA was confirmed by agarose gel electrophoresis. Samples were treated with DNase according to the manufacturer’s protocol (TurboDNA-free kit, Applied Biosystems, Foster City, CA, USA).

Reverse transcriptase PCR was used to measure the abundance of each specific mRNA relative to the abundance of porcine β-actin in the total RNA isolated from AP and MBH tissue. Expression of β-actin did not differ between treatments. Two micrograms of total RNA was reverse transcribed using random hexamer primers (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems, Foster City, CA) to produce cDNA. One hundred micrograms of cDNA was used in each reaction. Twenty-five-microliter PCR reactions were performed using RT2 SYBR Green/ROX qPCR Master Mix (SuperArray Bioscience Corp., Foster City, CA). Primer pairs used for specific amplification of porcine IGF-I, IGF-I-type-I receptor (IGF-IR), IGFBP-2, -3, and -5, LHβ-subunit, GnRH receptor (GnRHR), kisspeptin, kisspeptin receptor and β-actin are listed in Table 1.

Reactions were measured using the Stratagene MX3000P quantitative real-time PCR instrument (Agilent Technologies, Foster City, CA) using thermal cycling conditions recommended by the manufacturer (40 cycles of 30 sec at 95°C, 1 min at 55°C and 1 min at 72°C). Concentrations of forward and reverse primers used for the genes of interest were 300 nM, except KISS1R, in which concentrations of forward and reverse primers were used at 800 nM due to its low abundance. A linear response was obtained when these concentrations of primer pairs were used with increasing amounts of cDNA. Dissociation curve analysis was performed after each real time PCR run and confirmed that a single amplicon of appropriate melting temperature was present. Additionally, all amplicons were electrophoresed through a 2% agarose gel and stained with ethidium bromide to visualize that only amplicons of the appropriate size were present in each sample.

2.4. Statistical Analysis

To determine the effect of genistein on serum and AP concentrations of IGF-I and LH, statistical analyses were performed using the Proc Mixed procedure of JMP 8.0 model of SAS. The model for determining differences in serum concentrations of IGF-I was $Y_{ijkl} = \mu + Pig_i + Treatment_j + Pig_i \times (Treatment_j) + Day_k + Treatment_j \times Day_k + Pig_i \times (Treatment_j) \times Day_k$. Pig within treatment by day was the subplot error term used to test pig, day, and treatment by day effects. The model to determine the effect of genistein on AP concentrations of IGF-I and LH was $Y_l = \mu + Treatment_l$. The residual error term was used to test the effect of treatment.

Fold differences in expression of AP IGF-I, IGF-type-I receptor, IGFBP-2, IGFBP-3, IGFBP-5, GnRH receptor, LHβ, and MBH KISS1, and KISS1R between treatments were determined using the relative expression software tool [12].
Table 1. Primer sequences and corresponding cDNA fragments.

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<th>Gene and Accession Number</th>
<th>Primer</th>
<th>Amplicon Size</th>
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<tr>
<td>IGF-I (NM_214256.1)</td>
<td>Forward: 5’-ATGCCCAAGGCTCAGAAGGAAGTA-3’</td>
<td>146 bp</td>
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<td>Forward: 5’-CCAGGCCTCCAAACGACATATGA-3’</td>
<td>105 bp</td>
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<td></td>
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<tr>
<td>pIGFBP2 (AF120326.1)</td>
<td>Forward: 5’-CGAGCAGGTTGCAGACAATG-3’</td>
<td>142 bp</td>
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<tr>
<td></td>
<td>Reverse: 5’-GAACACAGCAGCTCCTCAT-3’</td>
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<td>pIGFBP3 (AF085482.1)</td>
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<td>Reverse: 5’-TCCACGCACAGCAGATG-3’</td>
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<tr>
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1porcine IGF-I; 2porcine IGF-1 receptor; 3porcine IGFBP-2; 4porcine IGFBP-3; 5porcine IGFBP-5; 6porcine GnRH receptor; 7porcine LHβ; 8porcine ERα; 9porcine KISS1; 10porcine KISS1 receptor; 11porcine b-actin.
3. Results

Serum, AP Hormone, and Gene Expression

Serum concentrations of IGF-I were determined on each of the four days of genistein administration. Mean serum concentrations of IGF-I did not differ ($P > 0.05$) between G pigs and C pigs from days 0 through 4 (Figure 1). Likewise, mean AP concentrations of IGF-I did not differ ($P > 0.05$) between G pigs and C pigs (Figure 2(a)). However, mean AP concentrations of LH were greater ($P < 0.05$) in G pigs than C pigs (Figure 2(b)).

Relative expression of components of the AP IGF system changed with genistein treatment. Mean relative expression of AP IGF-I was 1.2 fold greater ($P < 0.01$) in G pigs than C pigs (Figure 3(a)). Mean relative expression of AP IGF-I receptor did not differ ($P > 0.05$) between G pig and C pigs (Figure 3(b)). Mean relative expression of AP IGFBP-2 and -3 were each not different ($P > 0.05$) between G pigs and C pigs (Figure 4(a) and Figure 4(b)), however, mean relative expression of AP IGFBP-5 was 1.6 fold greater ($P < 0.01$) in G pigs than in C pigs (Figure 4(c)).

Relative expression of GnRH receptor, but not LH$\beta$ was influenced by genistein. Mean relative expression of AP GnRH receptor was 1.4 fold greater ($P < 0.01$) in G pigs compared to C pigs (Figure 5(a)). No difference was detected ($P > 0.05$) in mean relative expression of AP LH$\beta$ subunit between G pigs and C pigs (Figure 5(b)).

![Figure 1. Mean serum concentrations of IGF-I on days 0 through 4 in control pigs (C; n = 8), and genistein treated pigs (G; n = 8; 800 mg genistein) administered i.m. treatments daily from day 0 through day 4. Means are expressed as least-square means ± SEM.](image1)

![Figure 2. Mean anterior pituitary concentrations of IGF-I (a) and LH (b) on d 4 in control pigs (C; n = 8) and genistein treated pigs (G; n = 8; 800 mg genistein) administered i.m. treatments daily from day 0 through day 4. Means are expressed as least-square means ± SEM. *Mean differs from control pigs ($P < 0.05$).](image2)
Figure 3. Mean relative expression of anterior pituitary IGF-I (a) and IGF-I receptor (b) on d 4 in control pigs (C; n = 8) and genistein treated pigs (G; n = 8; 800 mg genistein) administered i.m. treatments daily from day 0 through day 4. Total RNA was DNase treated and analyzed for the level of porcine IGF-I and IGF-I receptor by quantitative RT-PCR with normalization for the corresponding level of porcine β-actin. *Mean differs (P < 0.01) from controls as determined by the relative expression software tool.

Figure 4. Mean relative expression of anterior pituitary IGFBP-2 (a) IGFBP-3 (b) and IGFBP-5 (c) on d 4 in control pigs (C; n = 8) and genistein treated pigs (G; n = 8; 800 mg genistein) administered i.m. treatments daily from day 0 through day 4. Total RNA was DNase treated and analyzed for the level of porcine IGFBP-2, IGFBP-3, and IGFBP-5 by quantitative RT-PCR with normalization for the corresponding level of porcine β-actin. *Mean differs (P < 0.01) from controls as determined by the relative expression software tool.

Figure 5. Mean relative expression of anterior pituitary GnRHR (a) and LHβ subunit (b) on d 4 in control pigs (C; n = 8) and genistein treated pigs (G; n = 8; 800 mg genistein) administered i.m. treatments daily from day 0 through day 4. Total RNA was DNase treated and analyzed for the level of porcine GnRHR and LHβ subunit by quantitative RT-PCR with normalization for the corresponding level of porcine β-actin. *Mean differs (P < 0.01) from controls as determined by the relative expression software tool.
Relative expression of kisspeptin, but not the kisspeptin receptor, was decreased by the administration of genistein. Mean relative expression of KISS1 in the MBH was reduced \((P < 0.001)\) in G pigs compared to C pigs (Figure 6(a)). Mean relative expression of KISS1R was not different \((P > 0.05)\) between G pigs and C pigs (Figure 6(b)).

4. Discussion

Genistein, a bioactive isoflavone, is structurally similar to estradiol [13] and has been shown to have estrogenic activities in numerous species [3]-[16]. Genistein has the ability to bind to estrogen receptors and compete with estradiol for binding to the estrogen receptors [13]. Chen and Wong [17] administered genistein to MCF-7 breast cancer cells, and determined that genistein caused an induction of the IGF-I signaling pathway, similar to estradiol. Genistein has the ability to affect numerous reproductive processes, including stimulation of IGF-I release from bovine and porcine granulosa cells, and estradiol release from porcine ovarian follicles [4]. It has been demonstrated that serum concentrations of IGF-I increase with circulating levels of estradiol-17β in pigs [18], sheep [11], and cattle [19]. Rempel and Clapper [18] also determined that administration of estradiol to barrows increased anterior pituitary concentrations of IGF-I.

In the current study, genistein was administered to ovariectomized gilts at 800 mg/day for a short period of time (4 days), but serum and AP concentrations of IGF-I did not increase. The amount of genistein used in the present experiment was two-fold as much as that used by Clapper and Tomlin [5], but was administered for a shorter length of time. Clapper and Tomlin [5] injected barrows with 400 mg genistein daily for 16 days and found it increased anterior pituitary concentrations of IGF-I, however, serum concentrations of IGF-I were not different than controls. Farmer et al. [20] injected sows with an equivalent amount of genistein and found no change in serum concentrations of IGF-I. Thus, the gilts in the present study may not have been treated with genistein long enough to increase AP or serum IGF-I or the effects of genistein on the IGF system in the pig are tissue specific.

In the present study, genistein increased expression of IGF-I in the anterior pituitary. Low concentrations of genistein also increased mRNA expression of IGF-I in human uterine leiomyoma cells [21]. Kalbe et al. [22] found contradicting results in porcine skeletal muscle cells cultures. In proliferating and differentiating porcine skeletal muscle cultures low concentrations of genistein caused no increase in expression of IGF-I, while high doses of genistein caused reduced expression of IGF-I [22]. Even though AP expression of IGF-I increased there was not a corresponding increase in the protein. Perhaps the short duration of the experiment did not provide sufficient time for the translation of the mRNA into protein.

Anterior pituitary expression of IGF-I-type-1 receptor did not increase in the present study in response to short term administration of genistein. Genistein at high concentrations reduced expression of the IGF-I type 1 receptor in cultured porcine skeletal muscle fibers [22]. High concentrations of genistein also inhibited MCF-7 cell proliferation, while low concentrations of genistein stimulated MCF-7 cell proliferation [17].

![Figure 6](image.png)

Figure 6. Mean relative expression of medial basal hypothalamic KISS1 (a) KISS1 receptor (b) and ERα (C) on d 4 in control pigs (C; \(n = 8\)) and genistein treated pigs (G; \(n = 8\); 800 mg genistein) administered i.m. treatments daily from day 0 through day 4. Total RNA was DNase treated and analyzed for the level of porcine KISS1, KISS1R, and ERα by quantitative RT-PCR with normalization for the corresponding level of porcine β-actin. *Mean differs \((P < 0.001)\) from controls as determined by the relative expression software tool.
J. Clapper, C. Paulson

concluded that low doses of genistein increased both protein and mRNA expression of the IGF-I-type-1 receptor and increased the IGF-I receptor promoter activity in MCF-7 cells. Chen et al. [23] also established that the increase in expression of IGF-I type 1 receptor by genistein was dependent upon association of the estrogen receptor and the IGF-I type 1 receptor. Genistein has greater affinity for ERβ compared to ERα [1] and the anterior pituitary gland has greater amounts of ERα than ERβ [1]. Because genistein has higher affinity for the ERβ, genistein may not have interacted with the estrogen receptor in the anterior pituitary to the extent required to increase expression of AP IGF-I-type-1 receptor in the present study.

Relative amounts and expression of IGFBP have been shown to be modulated by estrogens in a number of species, including the pig. Insulin-like growth factor binding protein-5 is known as a potentiator of IGF-I activity, allowing IGF-I to be more available to its receptor [24]. Clapper and Taylor [25] determined that on day 19 of the porcine estrous cycle, relative amounts of IGFBP-5 increased with a concomitant increase in estradiol, implying IGFBP-5 maybe regulated by estradiol. In the bovine, it has also been determined that changes in anterior pituitary IGFBP-5 were correlated with changes in serum levels of estradiol during the estrous cycle [9]. Others have demonstrated that expression of IGFBP-5 to estrogens may be tissue specific. Insulin-like growth factor binding protein-5 expression was down-regulated when estrogen positive ovarian cancer cells were treated with estradiol [26]. However, bovine granulosa and theca cell expression of IGFBP-5 did not change with estradiol treatment [27]. A study comparing estrogen response genes in ovariectomized adult mice determined IGFBP-5 expression was increased in the vagina and not in the uterus or mammary gland after estrogen exposure [28]. In the current study, anterior pituitary expression of IGFBP-5 increased with short term administration of genistein. Genistein appears to have enough estrogenic activity to increase expression of AP IGFBP-5 in ovariectomized gilts which may make IGF-I more readily available to its receptor. The fact that expression of AP IGFBP-2 and -3 did not change with administration of genistein may be due to its estrogenic potency in comparison with estradiol, or the length of time it was administered. When expression or relative amounts of these two IGFBP changed in the AP as observed in the rat [29], ewe [11] or pig [18] [20] [30] it was in response to physiological levels of estradiol or administration of estradiol.

Anterior pituitary expression of GnRH receptor increased in ovariectomized gilts following short term administration of genistein. Genistein’s ability to increase expression of GnRH receptors may have been directly due to its estrogenic effect [1]. Genistein may have also been working indirectly by influencing the release of GnRH. In the absence of progesterone, estradiol can increase the secretion of GnRH in the ewe [31] and GnRH can stimulate expression of its own receptor [32]-[34]. If genistein, through its estrogenic activity, was capable of increasing GnRH, this may have resulted in greater expression of GnRH receptors. Genistein’s ability to increase the expression of GnRH receptors may play an important role during various phases of the estrous cycle by increasing sensitivity of the anterior pituitary to GnRH in much the same fashion as estradiol [35].

Anterior pituitary concentrations of LH increased with short term administration of genistein in this study. Intracerebroventricular infusion of genistein to ovariectomized ewes caused an increase in LH-immunoreactive cells in the pituitary, and also an increase in serum concentrations of LH [36]. In this latter study, it was speculated that genistein inhibited the tonic secretion of LH and caused a large release of LH like that of the preovulatory surge. However, in the present study, blood samples were not taken at frequent enough intervals to adequately characterize serum concentrations of LH. When Norrby et al. [37] treated gilts with approximately 300 mg of genistein daily for three days, serum concentrations of LH were reduced in genistein treated gilts versus controls. The ability of genistein to increase anterior pituitary LH was presumably due to areduction in LH release. This is further supported by the fact that expression of LHβ subunit was not increased by administration of genistein. Genistein may also have increased AP LH levels by increasing GnRH receptors and inhibiting the tonic release of LH [38].

Anterior pituitary expression of the LHβ subunit did not increase with short term administration of genistein in this study. However, ovariectomized ewes treated with an intracerebroventricular infusion of genistein experienced increased mRNA expression of LHβ in pituitary cells [36] [39]. In the current study, short term administration of genistein did not increase the LHβ subunit level in ovariectomized gilts. The administration technique may account for the lack of change in expression of LHβ in the present study. Intracerebroventricular infusion of genistein allows direct contact with the brain, which may account for the increased expression of LHβ subunit in the AP. Previous studies indicated an increase in LHβ expression after genistein treatment, which may be interpreted as a rapid synthesis and release of LH [39]. However, in this study, the lack of an increase in expression of LHβ may indicate genistein had no affect or was not delivered in a manner to cause a dramatic increase in
synthesis and release of LH.

Kisspeptin, a peptide product of the KISS1 gene, increases peripheral gonadotropin secretion when administered centrally or peripherally in a number of species, including the pig [40]. Kisspeptin neurons express steroid receptors [41] and it has been shown that gonadectomy increases kisspeptin expression in the arcuate nucleus of mice and rats while steroid replacement reduces this expression [42] [43]. The release of hypothalamic and hypophysial hormones is controlled by negative and positive feedback of gonadal steroids, with estradiol playing a key role in both kinds of feedback. Kisspeptin-expressing neurons have been found to be distributed in the periventricular nucleus and arcuate nucleus of the pig [44]. Tomikawa et al. [44] also reported that KISS1-expressing cells were decreased in the arcuate nucleus of ovariectomized pigs treated with estradiol benzoate. In the present experiment expression of KISS1 was decreased in the MBH of genistein treated gilts, an area containing both the periventricular and arcuate nuclei. Thus, the findings presented here suggest genistein influences KISS1 in the hypothalamus, however, detection of precise hypothalamic nuclei which may be involved was beyond the scope of our experiment. Whether genistein can modulate GnRH/LH secretion in the pig through kisspeptin neurons requires further investigation.

5. Conclusion

In conclusion, short term administration of genistein to ovariectomized gilts increased expression of components of the AP IGF-I system and GnRH receptors which could modulate gonadotropin synthesis or release. However, genistein may be acting at the level of the hypothalamus to decrease GnRH release by reducing kisspeptin synthesis. Whether genistein increases or decreases gonadotropin secretion or synthesis remains to be determined and will depend upon the sum total of the positive and negative influences.

References


