Abstract
Kisspeptin receptor (Kiss1r) agonists with increased half-life and similar efficacy to kisspeptin (KP) in vitro potentially may provide beneficial applications in breeding management of many species. However, many of these agonists have not been tested in vivo. This study was designed to test the effect of a Kiss1r agonist (FTM080) on luteinizing hormone (LH) in vivo. Sheep were treated with FTM080 (500 pmol/kg BW) or sterile water in a 2-ml bolus via the jugular cannula. Serial blood samples were collected every 15-min before (1 hr) and after (1 hr) treatment. Intravenous (IV) injection of FTM080 increased (P < 0.05) LH plasma concentrations through the 45-min sample following treatment. Moreover, the area under the curve of LH in the period from 0 to 60 min following IV treatment was also increased (P < 0.05). These data provide evidence to suggest that FTM080 stimulates the gonadotropin axis of ruminants in vivo.

Introduction
Kisspeptin and Kiss1r are integral to central regulation of the gonadotropin-axis [1]. The demonstration that IV infusion of KP can stimulate gonadotropin secretion and ovulation in seasonally anestrous female sheep offers a means of manipulating the reproductive axis [2]. However, KP may be of limited clinical use because of the short circulating half-life [3]. Rational modification of Kiss1r agonists were synthesized to be resistant to matrix metalloproteinase activity and found to have increased intravenous half-life in murine serum, and to have comparable binding affinity and efficacy in vitro to KP [4,5]. However, in vivo activities of these peptides have not yet been studied. Thus, the present experiment was designed to determine the effect of a novel Kiss1r agonist on plasma LH concentrations in seasonally anestrous female sheep.

Materials and Methods
All procedures were approved by the Berry College (Rock, GA) Institutional Animal Care and Use Committee. Eight adult parous Katahdin female sheep [41.6 ± (SEM) 1.3 kg] were used in this experiment. Sheep were exposed to ambient temperature (25°C average daily temperature) and photoperiod (14:10 [L:D] hr) throughout the experiment (June), and fed a maintenance diet calculated to meet daily requirements. The effect of a novel Kiss1r agonist (FTM080: 4-fluorobenzoyl-Phe-Gly-Lau-Apg-Tpr-His), Oklahoma School of Pharmaceutical Sciences, Kyodo University [4,5] on plasma LH concentrations in anestrous sheep was tested. The study was conducted during a long photoperiod to increase the likelihood of ewes being in the anestrous period, which was done to reduce variation in response(e) as the KP-Kiss1r system is clearly influenced by sex steroids [6].

Blood samples were collected before and after the experiment (7 days between samples) and assayed to determine plasma LH concentrations. Data from animals with circulating progesterone concentrations greater than 1 ng/mL was excluded from the analysis. Each animal was fitted with an indwelling intravenous jugular catheter the day before experimentation. Sheep were exposed to ambient temperature (25°C average daily temperature) and photoperiod (14:10 [L:D] hr) throughout the experiment (June), and fed a maintenance diet calculated to meet daily requirements.

Circulating concentrations of LH were tested for effect of treatment (FTM080 or sterile water), time, and treatment by time interaction using ANOVA procedures for repeated measures with JMP Software (version 7 SAS Inst. Inc., Cary, NC). Area under the LH concentration curve pre (-60 to 0 min) and post (0 to 60 min) treatment was calculated using the trapezoid method with MSExcel Software. Area under the LH curve was tested for effect of treatment (FTM080 or sterile water), period (pre- or post-treatment), and treatment by period interaction using ANOVA procedures for repeated measures with JMP Software (version 7 SAS Inst. Inc., Cary, NC). Means separation was performed using Student’s t-test when appropriate.

Results
Three ewes per treatment were reported; two ewes (one per treatment) were excluded from the analysis and results due to plasma progesterone concentrations >1 ng/mL (2.60 and 1.70 ng/mL).

Mean ± SEM plasma LH concentration was 0.31 ± 0.16 ng/mL and 0.14 ± 0.06 ng/mL before treatment with sterile water and FTM080, respectively. Plasma LH concentration following treatment with FTM080 was significantly greater than the controls (P < 0.05) through the 45-min sample (Figure 1) and there was no effect of treatment (P = 0.1590) on area under the LH curve. However, there was an effect of period (pre- and post-treatment) (P = 0.0464) and an interaction of period and treatment (P = 0.0150) on area under the LH curve. The area under the curve of LH in the period from 0 to 60 min following FTM080 treatment was greater than all other treatments and periods (Figure 2).

Summary
The half-life of FTM080 in murine serum (6.6 h) is greater than KP-10 (1 h) while maintaining bioactivity for Kiss1r in vitro [4]. However, the in vivo activity of FTM080 had not been studied.

In vitro and in vivo activity/potency of Kiss1r agonists are not always the same [9,10]. Some KP-10 analogs may act as Kiss1r superagonists in specific in vitro systems, but may not have greater activity than KP-10 in vivo.

This study of the effect of a Kiss1r agonist in anestrous sheep revealed that intravenous FTM080 stimulated plasma LH concentrations through the 45-min sample. There was approximately a 6-fold increase in plasma LH concentrations following intravenous treatment with FTM080 (from 0.142 to 0.884 ng/mL).

The magnitude and duration of the LH-response following treatment with FTM080 was similar to previous observations in ovariectomized sheep given comparable doses of KP-10 [2,11].

The reported stimulation of the gonadotropin axis by KP-10 in seasonally acyclic ewes [2] is greater than the effect of FTM080 reported in this study. Caraty et al., [2] reported that an intravenous bolus of KP-10 of approximately half the molar dose used here increased concentrations of LH in plasma of seasonally acyclic ewes from 0.2 ng/mL to 8.0 ng/mL. A similar response was observed by the authors following intravenous KP-10 treatment of seasonally acyclic ewes (unpublished observations).

These data provide evidence to suggest that FTM080, a Kiss1r agonist, stimulates the gonadotropic axis of ruminants in vivo. However, the increased half-life and comparable efficacy of FTM080 to KP-10 in vitro [4,5] does not appear to translate to longer duration of efficacy in vivo.

References