Gene expression of GnRH, kisspeptin, neuropeptide Y and receptors for estrogen and leptin in the hypothalamus of suckled and weaned beef cows

(Ekspresi gen GnRH, kisspeptin, neuropeptida Y dan reseptor untuk estrogen dan leptin di dalam hipotalamus lembu pedaging yang menyusu dan dicerai susu)


Keywords: gene expression, hypothalamus, postpartum cows

Abstract

The aim of this study was to ascertain the expression of multiple genes in the hypothalamic sub-regions of postpartum beef cows that were suckled or weaned, and had different ovarian phenotypes. Genes studied were gonadotrophin-releasing hormone (GNRH1), kisspeptin (KISS1), estrogen receptor-α (ESR1), leptin receptor (LEPR) and neuropeptide Y (NPY) in two hypothalamic sub-regions, H1 (anterior) and H2 (ventral posterior). The expression of GNRH1 was around 7-fold greater \( (p < 0.01) \) within H1 than H2 while the expression of KISS1 was around 20-fold greater \( (p < 0.01) \) within H2 than H1. Both ESR1 and NPY showed greater \( (p < 0.05) \) expression within H2 than H1. Weaned cows had lower \( (p < 0.05) \) expression of ESR1, LEPR and NPY than suckled cows in H1. Cows with suppressed follicle growth (follicles 4 – 5 mm) had greater \( (p < 0.05) \) LEPR expression within H1 compared to cows with active follicle growth (follicles 10 mm) and cows that had ovulated. This is the first report of KISS1 expression in the bovine brain and also of regional differences in the expression of multiple genes within the bovine hypothalamus. The study on simultaneous gene expression within the hypothalamus provides a new approach in understanding the complex gene networks linked to the effects of nutrition, lactation, ovarian steroids, metabolic homeostasis and maternal bonding on central mechanisms associated with postpartum reproduction in bovine.

Introduction

The postpartum anoestrous period in lactating beef cows results from the negative influences on reproductive function imposed by the nutrient requirements of lactation (Lucy 2003; Roberts et al. 2005), suckling stimulus (Wettemann et al. 2003), maternal bond (Silveira et al. 1993; Montiel and Ahuja 2005), ovarian steroids (Savio et al. 1990) and negative energy balance (Montiel and Ahuja 2005; Roberts et al. 2005; Shrestha et al. 2005). It has been proposed

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that the various factors which contribute to postpartum anoestrus in cows are integrated centrally at the hypothalamus through common pathways that regulate the secretion of gonadotrophin-releasing hormone (GnRH) (Short et al. 1990). According to this concept, the resumption of ovulation after calving is dependent on the return of GnRH secretion, and hence the follicle secreting hormone (FSH) and luteinising hormone (LH) secretion, and the patterns required for full maturation and ovulation of the dominant ovarian follicle (Daftary and Gore 2004).

An alternate model for postpartum reproduction in cows proposes that the return to ovulation after calving is determined at the level of the ovaries by mechanisms that involve intra-ovarian molecules, inter-ovarian molecules and sensitivity to LH (Diskin et al. 2003). In the latter model, an increase in sensitivity of the dominant follicle to LH, independent of changes in LH secretion, results in an increase in estrogen-17β (estrogen) production by the dominant follicle to amounts required to induce the pre-ovulatory surge release of LH. The two models for resumption of ovulation in the postpartum cow need not be mutually exclusive and both central (hypothalamic) and somatic (ovarian) tissue mediated mechanisms could be involved.

The relative contribution of central and somatic mechanisms to the resumption of ovulation postpartum may differ for beef and dairy cattle due to marked differences in lactation demand and most likely differ considerably in general metabolic homeostasis (Lucy 2003). Alternatively, the primary mechanism could be related to the prevailing metabolic status based on lactation demand and other factors. The resumption of postpartum ovulation in beef cows can be induced by weaning, provided that the cows are in an appropriate body condition (Wettemann et al. 2003). The mechanisms whereby a calf suppresses reproduction could include, singularly or in combination, the nutrient requirements of milk production, suckling stimulus, maternal bond and metabolic homeostasis.

Evidence at the hypothalamic level for interrelationships between metabolic homeostasis and reproduction includes the presence of leptin receptors on kisspeptin and neuropeptide Y (NPY) neurons (Messager et al. 2005; Gamba and Pralong 2006). Based on this information, the present study was undertaken to determine the expression of GNRH, kisspeptin and NPY genes and receptor genes for estrogen and leptin, in the hypothalamus of suckled and weaned beef cows. Weaning was used to induce increased ovarian follicular growth and ovulation, and hypothalamic gene expression was compared for cows of different ovarian phenotypes. This study was the first to report multiple gene expression within the bovine hypothalamus.

Materials and methods

Animals and experimental design

The study was approved by The University of Queensland Animal Ethics Committee (SAS/719/06/CRC). Cows used in the study were kept on pasture and received standard management except when required for experimentation.

Three-year-old first-parity Zebu cows (Bos indicus, Brahman breed) were chosen for the study as this breed typically has an extended period of anoestrus when lactating. The cows were managed so that they were in relatively good body condition as reflected by body condition score (BCS) between 3.5 and 4.0 on a scale of 1.0 to 5.0. Assessment of BCS is based on the animal’s weight for age and weight for height ratios, as well as its relative proportions of muscle and fat. The amount of tissue cover between the points of the hip, over the transverse processes of the lumbar vertebrae, the cover over the ribs and the pin bones below the tail are taken into consideration.

Cows were maintained in this body condition so that, to the extent possible, the presence of a calf (i.e. nutrient demand...
of lactation, suckling stimulus, maternal bonding) was the major (and possibly only) constraint to the resumption of ovulation postpartum. Six cows were weaned between day 20 and 37 of postpartum and slaughtered either 6 (n = 2) or 13 (n = 4) days after weaning. On each day of slaughter, supplementary cows that continued to suckle a calf, and matched by days of postpartum, were also slaughtered (n = 6). The latter cows were separated from their calves around 18 to 24 h before slaughter.

**Brain collection and sectioning**

Cows were slaughtered at a commercial abattoir using the non-penetrating captive bolt technique and brains were recovered within 45 to 90 min of slaughter. Brains were initially sectioned using a medial sagittal incision to reveal the hypothalamus in each hemisphere. The hypothalamic region on each side of the brain was dissected into 3 sub-regions classified as H1, H2 and H3 as shown in Figure 1. To ensure standardised sampling, the optic chiasma (OC), mammillary body (MB), thalamus (T) and the pituitary stalk were used as references. Each sub-section was cut to a depth of 3 – 4 mm.

**Ovarian phenotypes**

The cows were divided into three groups based on their ovarian phenotype at slaughter. Cows in Group 1 (ovarian phenotype 1, OP1) had suppressed ovarian follicular growth with follicles of maximum diameter of 4 – 5 mm, which in cattle is the size at which follicles are gonadotrophin dependent. Cows in Group 2 (OP2) had increased follicular growth to a diameter of around 10 mm but without ovulation, and cows in Group 3 (OP3) had a corpus haemorrhagicum and/or corpus luteum at slaughter. Ovarian phenotype 1 (n = 4) comprised only suckled cows; OP2 (n = 4) comprised two suckled cows and two 13-day weaned cows; and OP3 (n = 4) comprised only 13-day weaned cows.

**RNA extraction and reverse transcription**

Total RNA was extracted using the RNeasy extraction kit (Qiagen, Victoria, Australia) with the following modifications: (i) approximately 400 mg of hypothalamic tissue was homogenized in lysis buffer (RLT) and centrifuged at 3000 x g for 10 min and (ii) 1/10 volume of chloroform:isoamyl alcohol (24:1) was
added to the supernatant and centrifuged 12,000 \( \times \) g at 4 °C for 10 min. Extracted RNA was stored at –80 °C until required. RNA integrity number (RIN) was verified using the Bioanalyzer (Agilent Technologies, Victoria, Australia). Residual genomic DNA contamination was removed with the DNA free kit (Ambion, Texas, USA).

A mixture of 500 ng Oligo (dT)\(_{12-18}\) (Invitrogen, California, USA), 300 ng random primer oligonucleotides (Invitrogen) and 200U Superscript III Reverse Transcriptase (Invitrogen) were added to 1 \( \mu \)g of DNA-free RNA and reverse transcribed in storage buffer at 50 °C for 1 h. The cDNA was then subjected to a final purification using the QIAquick PCR Purification kit (Qiagen). The consistency of cDNA quality was verified in ethidium-bromide stained 1% agarose gel electrophoresis.

**Quantitative real time polymerase chain reaction (qRT-PCR)**

Specific primers were designed to *Bos taurus* sequences using the Primer3 software (Rozen and Skaletsky 2000). The nucleotide sequences of each primer pair are shown in Table 1. All reactions were performed with 2X SYBR Green I Master Mix Buffer (Applied Biosystems, CA, USA) and 400 nM of primers. Approximately 10 ng of cDNA was used for each reaction which was run on the ABI PRISM 7900HT Sequence Detector (Applied Biosystems) and each sample was assayed in triplicate. Replicates were dispensed with the Biomek 2000 Lab Automation Workstation (Beckman Coulter, New South Wales, Australia).

The following cycling conditions were applied for amplification: Step 1 = 50 °C for 2 min; Step 2 = 95 °C for 10 min; Step 3 = 95 °C for 15 s and 60 °C for 1 min. Step 3 was repeated for 40 cycles. A melt curve analysis step was included (95 °C for 15 s, 60 °C for 15 s and 95 °C for 15 s) to confirm the specificity of the reaction. Furthermore, PCR products were separated on agarose gel

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primers (5’-3’)</th>
<th>Reverse Primers (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNRH1</td>
<td>GGGGACTCTCTGCTCTCAAA</td>
<td>TGCCCAGTTCCTCCITAAT</td>
</tr>
<tr>
<td>KISS1</td>
<td>ATGAACGTGCTGCTTTCCTG</td>
<td>CGAGCTGTTGTGTCAGGATT</td>
</tr>
<tr>
<td>ESR1</td>
<td>ATGATGAAAGGCGGAATACG</td>
<td>AAGGTTGGCAGCTCTCATGT</td>
</tr>
<tr>
<td>NPY</td>
<td>CAGGCAGAGATACGGGAAAC</td>
<td>GGGAGGACTGGCAGACTCTA</td>
</tr>
<tr>
<td>LEPR</td>
<td>GGCCTGGATGAACTTTTGAA</td>
<td>TGTGAGCAACTGTCCTGGAG</td>
</tr>
<tr>
<td>YWHAZ</td>
<td>ACCTACTCCGGACACAGAACAT</td>
<td>GGAGATTCCTCTCCATGGGA</td>
</tr>
</tbody>
</table>

Table 1. Primers used for real time qRT-PCR
Normalisation of PCR data
All genes were normalised against tyrosine3-monooxygenase/tryptophan monooxygenase activation protein, zeta polypeptide (YWHAZ) which was stably expressed across all animals and hypothalamic tissues by the web-based software GeNorm (Vandesompele et al. 2002). PCR efficiencies (E) for each gene were calculated using the LinReg PCR analysis program (Ramakers et al. 2003). The average of the triplicate readings was normalised to obtain relative expression of each gene (Pfaffl 2001).

Statistical analyses
All statistical analyses were carried out using log10 transformed data to achieve normal distribution with the R software (Team 2006). In all gene expression experiments, analyses for treatment and ovarian phenotype effects were tested separately. In addition, hypothalamic sub-regions, treatment, ovarian phenotype, day of collection and days postpartum, were fitted as fixed variables in a linear model before factorial analysis of variance (ANOVA). Significance was set at \( p < 0.05 \). The differences between hypothalamic sub-regions were determined using the Bonferroni Multiple Test. The differences between means for the factorial levels i.e. ovarian phenotype and treatment were tested by one-way ANOVA.

Correlation tests were carried out using Spearman’s product moment correlation. Before the correlation tests, data distribution were visualised to identify any confounding effects. The following correlations were tested: (i) correlation between the relative expression values of two candidate genes for 12 cows separately for sub-region H1 and H2 and (ii) correlation between the relative gene expression values and RNA integrity number (RIN) values for all genes mentioned above. Spearman’s rank correlation coefficient (\( \rho \)) was established.

An H2 sample of a weaned cow in OP2 and an H3 sample of a suckled cow in OP1 were excluded from the analysis due to technical variability.

Results and discussion
Effect of sampling on RNA integrity
RNA integrity number (RIN) values ranged from 6.4 – 7.5 and there was no correlation between RIN value and relative gene expression (data not shown). The RIN information indicated that the hypothalamic sampling and processing procedures did not compromise the integrity of mRNA.

Regional gene expression
The normalised expression of all genes was low within hypothalamic sub-region H3 (Table 2). Given the low gene expression within sub-region H3, this region was excluded from further analyses and consideration. GNRH1, KISS1, ESR1 and NPY showed differential expression between sub-regions H1 and H2. The expression of GNRH1 within H1 was around 7-fold greater \( (p < 0.01) \) than expression within H2 whilst KISS1 expression within H2 was around 20-fold greater \( (p < 0.01) \) than the expression within H1 (Table 2). ESR1 and NPY both had greater \( (p < 0.05) \) expression within H2 than H1, although absolute expression for the two genes differed (Table 2).

Effect of weaning on ovarian activity
Cows that continued to suckle a calf had ovaries with suppressed ovarian follicular growth (follicles 4 – 5 mm) except for two suckled cows that showed evidence of increased follicular growth (follicles 10 mm). The latter two cows were at postpartum day 51 and day 57 at the time of slaughter. The ovaries of two cows weaned 6 days before slaughter showed increased follicular growth (follicle sizes up to 10 mm). These cows were at day 26 and day 28 postpartum at the time of slaughter. The ovaries of four cows weaned 13 days before slaughter had either a corpus haemorrhagicum or corpus luteum. These cows were at days
33, 35, 46 and 50 postpartum at the time of slaughter. Hence, cows weaned between day 20 and 37 postpartum had increased ovarian follicular growth within several days of weaning and ovulation occurred between 7 and 13 days after weaning.

**Weaning and gene expression**

Weaning was associated with lower (\(p < 0.05\)) expression of ESR1, LEPR and NPY within sub-region H1, and there was no significant effect (\(p > 0.05\)) of weaning on the respective expression of these genes within sub-region H2 (Table 3). There was also no significant effect (\(p > 0.05\)) of weaning on the expression of GNRH1 and KISS1 within either sub-region H1 or H2. A feature of gene expression was the relatively large variation between samples within weaned and suckled cows (e.g. GNRH1 for sub-region H1 and KISS1 for sub-region H2).

**Ovarian phenotype and gene expression**

The expression of LEPR within sub-region H1 was greater (\(p < 0.05\)) for cows with ovarian phenotype OP1 (99.3 ± 10.6) than in cows with ovarian phenotype OP2 (59.2 ± 10.7) and OP3 (42.6 ± 16.4) (Table 4). There was a similar trend for LEPR expression within sub-region H2 but this was not significant. There were no significant differences in the expression of GNRH1, KISS1, ESR1 and NPY between ovarian phenotypes within sub-regions H1 and H2. As noted above, a feature of gene expression was the relatively large variation between samples within the different categories of ovarian phenotypes (e.g. GNRH1 for sub-region H1 and KISS1 for sub-region H2).

**Correlation of gene expression within hypothalamic sub-regions**

Relative expression values for GNRH1, KISS1, NPY, ESR1, and LEPR were tested for correlations within sub-regions H1 and H2. Within sub-region H1, the highest correlation was between the relative expression of ESR1 and NPY (Spearman’s rank correlation coefficient value \(r = 0.99\), \(p < 0.01\)) (Table 5). Also within sub-region H1, ESR1 and NPY expression were positively correlated with expression of KISS1 (\(r = 0.87\), \(p < 0.01\) and \(r = 0.86; p < 0.01\) respectively), GNRH1 (\(r = 0.81; p < 0.01\)) and LEPR (\(r = 0.60; p < 0.05\) and \(r = 0.64; p < 0.05\) respectively). The expression of GNRH1 was positively correlated with KISS1, ESR1, and NPY.

### Table 2. Gene expression levels (mean ± SEM) in hypothalamic sub-regions H1, H2 and H3 for primiparous Brahman cows

<table>
<thead>
<tr>
<th>Gene</th>
<th>Hypothalamic sub-region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H1 (n = 12)</td>
</tr>
<tr>
<td>GNRH1</td>
<td>350 ± 105a</td>
</tr>
<tr>
<td>KISS1</td>
<td>44.4 ± 14.8a</td>
</tr>
<tr>
<td>ESR1</td>
<td>8.1 ± 1.2a</td>
</tr>
<tr>
<td>NPY</td>
<td>13.5 ± 2.1a</td>
</tr>
<tr>
<td>LEPR</td>
<td>67.0 ± 9.8a</td>
</tr>
</tbody>
</table>

Mean values with similar letters within rows are not significantly different (\(p < 0.05\)).

### Table 3. Gene expression levels (mean ± SEM) in hypothalamic sub-regions H1 and H2 for suckled and weaned primiparous Brahman cows

<table>
<thead>
<tr>
<th>Gene</th>
<th>Hypothalamic sub-region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H1</td>
</tr>
<tr>
<td></td>
<td>Suckled (n = 6)</td>
</tr>
<tr>
<td>GNRH1</td>
<td>502 ± 186a</td>
</tr>
<tr>
<td>KISS1</td>
<td>71.2 ± 24.1a</td>
</tr>
<tr>
<td>ESR1</td>
<td>9.9 ± 1.9a</td>
</tr>
<tr>
<td>NPY</td>
<td>17.8 ± 3.3a</td>
</tr>
<tr>
<td>LEPR</td>
<td>90.2 ± 10.0a</td>
</tr>
</tbody>
</table>

Mean values with similar letters within rows and hypothalamic sub-region are not significantly different (\(p < 0.05\)).

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Table 4. Gene expression levels (mean ± SEM) in hypothalamic sub-regions H1 and H2 for primiparous Brahman cows with different ovarian phenotypes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Hypothalamic sub-region</th>
<th>Ovarian phenotype</th>
<th>OP1 (n = 4)</th>
<th>OP2 (n = 4)</th>
<th>OP3 (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNRH1</td>
<td>H1</td>
<td>OP1 (n = 3)</td>
<td>247 ± 102a</td>
<td>127 ± 81a</td>
<td>1068 ± 82a</td>
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<tr>
<td></td>
<td></td>
<td>OP2 (n = 4)</td>
<td>562 ± 284a</td>
<td>577 ± 290a</td>
<td>1668 ± 10.7a</td>
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<tr>
<td></td>
<td></td>
<td>OP3 (n = 4)</td>
<td>241 ± 89a</td>
<td>577 ± 10.7a</td>
<td>16.6 ± 3.94a</td>
</tr>
<tr>
<td>KISS1</td>
<td>H1</td>
<td>OP1 (n = 3)</td>
<td>67.3 ± 37.9a</td>
<td>26.0 ± 12.7a</td>
<td>29.4 ± 10.8a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OP2 (n = 4)</td>
<td>40.1 ± 22.6a</td>
<td>7.6 ± 1.6a</td>
<td>29.8 ± 6.84a</td>
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<td>OP3 (n = 4)</td>
<td>26.4 ± 20.8a</td>
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<tr>
<td>ESR1</td>
<td>H1</td>
<td>OP1 (n = 3)</td>
<td>8.9 ± 1.9a</td>
<td>14.4 ± 20.6a</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>OP2 (n = 4)</td>
<td>8.8 ± 3.2a</td>
<td>13.4 ± 4.7a</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>OP3 (n = 4)</td>
<td>7.9 ± 1.9a</td>
<td>13.4 ± 4.7a</td>
<td></td>
</tr>
<tr>
<td>NPY</td>
<td>H1</td>
<td>OP1 (n = 3)</td>
<td>16.7 ± 4.3a</td>
<td>13.4 ± 4.7a</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>OP2 (n = 4)</td>
<td>99 ± 10.6a</td>
<td>13.4 ± 4.7a</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>OP3 (n = 4)</td>
<td>99 ± 10.6a</td>
<td>13.4 ± 4.7a</td>
<td></td>
</tr>
<tr>
<td>LEPR</td>
<td>H1</td>
<td>OP1 (n = 3)</td>
<td>99.3 ± 10.6a</td>
<td>13.4 ± 4.7a</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>OP2 (n = 4)</td>
<td>102 ± 28.4a</td>
<td>13.4 ± 4.7a</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>OP3 (n = 4)</td>
<td>102 ± 28.4a</td>
<td>13.4 ± 4.7a</td>
<td></td>
</tr>
</tbody>
</table>

OP1 = suppressed ovarian follicular growth; OP2 = active follicular growth without ovulation; OP3 = follicular growth with ovulation

Mean values with similar letters within rows, and hypothalamic sub-region, are not significantly different at $p < 0.05$

Discussion

The present study demonstrates marked regional differences in the expression of GNRH1 and KISS1 within the hypothalamus of the bovine brain. The expression of KISS1 in the bovine brain is reported for the first time with the greatest expression observed in sub-region H2. This sub-region contains the posterior medial basal hypothalamus-median eminence, ventromedial hypothalamus, posterior portion of the arcuate nucleus, and the anterior portion of the mamillary body. The expression of KISS1 in this region of the hypothalamus is consistent with predominant expression in the arcuate nucleus in sheep (Estrada et al. 2006; Smith et al. 2007; Smith 2009) and the anteroventral periventricular nucleus in other species (Han et al. 2005; Smith et al. 2006; Popa et al. 2008).

Previous GnRH localisation studies in bovine using immunocytochemistry (Leshin et al. 1988) and in situ hybridisation (Weesner et al. 1993) reported a concentration of GnRH neurons in the preoptic area. The finding in the present study of predominant GNRH1 expression in the same area confirms the importance of this region of the hypothalamus for GnRH synthesis in bovine. The same region of the hypothalamus also has the highest GNRH1 expression in sheep (Harris et al. 1998)) and rats (Jennes and Conn 1994).

Less marked regional expression was observed for ESR1, LEPR and NPY, and all three of these genes had greater expression in sub-region H2 than sub-region H1. The similar regional pattern of ESR1, LEPR and NPY expression is consistent with reports of estrogen and leptin receptors on NPY neurons (Williams et al. 1999; Tena-Sempere 2006; Acosta-Martinez et al. 2007; Smith 2009). It is also consistent with the coordinated actions of estrogen and leptin within the hypothalamus (Gao and Horvath 2008).

Receptors for estrogen and leptin have been reported on KISS1 neurons (Williams...
Gene expression in the hypothalamus of beef cows

et al. 1999; Tena-Sempere 2006; Acosta-Martinez et al. 2007; Smith 2009) and, given that the greatest expression of \textit{KISS1} was also in sub-region H2, the present findings strongly support morphological and functional relationships between \textit{ESR1}, \textit{LEPR} and \textit{KISS1} in the bovine hypothalamus. The localisation of regional expression for \textit{GNRH1}, \textit{KISS1}, \textit{ESR1}, \textit{LEPR} and \textit{NPY} in the bovine hypothalamus contributes important information for future studies on the regulation of these important reproductive genes in bovine.

The expression of \textit{ESR1}, \textit{LEPR} and \textit{NPY} was lower in weaned cows compared with suckled cows for both hypothalamic sub-regions H1 and H2, although differences in expression for the three genes were only significant for sub-region H1. While our data do not explain the molecular or cellular basis for this coordinated change in gene expression, the fact that \textit{ESR1}, \textit{LEPR} and \textit{NPY} were all expressed at a lower level in weaned cows is consistent with their above-mentioned interrelationships within the hypothalamus. There was a suggestion that the expression of \textit{KISS1} in sub-region H2 was greater for weaned cows compared with suckled cows but this was not significant.

A feature of gene expression in the present study was the relatively large variation in expression for hypothalamic tissues in the same lactation and ovarian phenotype categories. For \textit{GNRH1}, \textit{KISS1} and \textit{NPY}, the variation in expression within treatment categories might be explained, in part, by the pulsatile nature of their secretion but this would be considered less likely to explain the variation for \textit{ESR1} and \textit{LEPR}. Notwithstanding the individual variation in gene expression, it could be speculated that the apparent decrease in \textit{ESR1} expression in weaned cows could be associated with a decrease in estrogen negative feedback on GnRH, and increased secretion of FSH and LH required for the resumption of increased follicular growth and ovulation which indeed occurred in weaned cows.

There were no apparent relationships between ovarian phenotype and gene expression, except for greater \textit{LEPR} expression associated with ovarian phenotype 1 (follicles 4 – 5 mm) compared with ovarian phenotype 2 (follicles 10 mm) and ovarian phenotype 3 (ovulation), which did not differ. A relatively small number of cows were represented for the different ovarian phenotypes and, given the variation in gene expression highlighted above; further studies are required in postpartum cows to define relationships between phases of ovarian follicular transition and hypothalamic gene expression.

**Conclusion**

The present study provides fundamental information on regional expression of \textit{GNRH1}, \textit{KISS1}, \textit{ESR1}, \textit{LEPR} and \textit{NPY} within the bovine hypothalamus. Preliminary information on interrelationships between the expressions of these genes is also provided. The ability to simultaneously

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### Table 5. Spearman’s rank correlation coefficients (\( r \)) for the expression of \textit{GNRH1}, \textit{ESR1}, \textit{NPY}, \textit{LEPR} and \textit{KISS1} within hypothalamic sub-regions H1 and H2 for primiparous Brahman cows

<table>
<thead>
<tr>
<th>Gene</th>
<th>Hypothalamic sub-region (H1)</th>
<th>Hypothalamic sub-region (H2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textit{GNRH1}</td>
<td>\textit{ESR1}</td>
</tr>
<tr>
<td>\textit{GNRH1}</td>
<td>0.81**</td>
<td>0.81**</td>
</tr>
<tr>
<td>\textit{ESR1}</td>
<td>0.81**</td>
<td>0.99**</td>
</tr>
<tr>
<td>\textit{NPY}</td>
<td>0.81**</td>
<td>0.99**</td>
</tr>
<tr>
<td>\textit{LEPR}</td>
<td>0.45</td>
<td>0.60*</td>
</tr>
<tr>
<td>\textit{KISS1}</td>
<td>0.67*</td>
<td>0.87**</td>
</tr>
</tbody>
</table>

*(\( p <0.05 \)); ** (\( p <0.01 \))
determine region specific expression of gene groupings within the hypothalamus provides a powerful approach to understanding complex gene networks linked to the effects of nutrition, lactation, ovarian steroids, metabolic homeostasis and maternal bonding on central mechanisms associated with postpartum reproduction in bovine.

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References
Gene expression in the hypothalamus of beef cows


Abstrak
Tujuan kajian ini adalah untuk memastikan ekspresi berbilang gen dalam hipotalamus lembu pedaging selepas beranak (postpartum) yang masih menyusukan anak atau mempunyai anak yang telah dicera susu serta yang mempunyai fenotip ovari yang berlainan. Gen yang dikaji ialah gen hormon pelepas gonadotrofin (GNRH1), kisspeptin (KISS1), reseptor estrogen alpha (ESR1), reseptor leptin (LEPR) dan neuropeptida Y (NPY) dalam dua bahagian hipotalamus; H1 (anterior) dan H2 (ventral posterior). Ekspresi gen GNRH1 dalam H1 adalah 7 kali ganda lebih besar (p <0.01) daripada H2, manakala ekspresi gen KISS1 dalam H2 adalah sekitar 20 kali ganda lebih besar (p <0.01) daripada H1. Kedua-dua gen ESR1 dan NPY menunjukkan ekspresi yang lebih besar (p <0.05) dalam H2 daripada H1. Lembu yang dicerai susu mempunyai ekspresi gen ESR1, LEPR dan NPY lebih rendah (p <0.05) pada bahagian H1 daripada lembu yang menyusu. Lembu dengan pertumbuhan folikel yang disekat (folikel 4 – 5 mm) mempunyai ekspresi gen LEPR yang lebih besar (p <0.05) dalam H1 berbanding lembu dengan pertumbuhan folikel yang aktif (folikel >10 mm) dan lembu yang telah ovulasi. Ini merupakan laporan pertama ekspresi gen KISS1 dalam otak lembu dan juga perbezaan bahagian dalam ekspresi berbilang gen dalam hipotalamus lembu. Kajian ekspresi gen secara serentak dalam hipotalamus menyediakan satu pendekatan baru dalam memahami rangkaian gen yang kompleks yang dikaitkan dengan kesan pemakanan, laktasi, steroid ovari, homeostasis metabolik dan hubungan ibu-anak ke atas mekanisma berpusat yang berkaitan dengan pembiakan lembu selepas beranak.

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