THE EFFECTS OF FLUOXETINE ON LACTATION AND LAMB GROWTH IN SHEEP.


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ABSTRACT: Fluoxetine (a selective serotonin reuptake inhibitor; FLX) has been shown to delay in the onset of lactogenesis stage II when taken during pregnancy and/or lactation. A study was conducted to evaluate if ewes would be an appropriate model to determine the effects of FLX on milk production. Twenty-nine ewes (Avg BW 85 ± 12 kg; body condition score 2.6 ± 0.3) in late gestation were used in this study. Ewes allotted to treatments were stratified by fetal numbers and breeding date. Ewes were dosed orally daily with empty capsules as a control or capsules containing 40 mg of FLX. Dosing began on d 121 of gestation and continued until lambing. Ewes were dosed every morning at 0700 h. Following parturition and before nursing, milk and blood samples were collected from each ewe/lamb(s) pair. The first milk yield was measured 8 h after birth and subsequent milkings were conducted at 1500 and 1800 h every other day for 9 d. Milk letdown was induced by a 1 mL intravenous injection of oxytocin, 1 min prior to milking. Milk yields were measured over a 3 h period when lamb(s) were removed. We observed a treatment by parity interaction, as ewes with multiple lambs treated with FLX had greater (P = 0.01) milk yields than treated or control ewes giving birth to single lambs and control ewes giving birth to multiple lambs. Lambs were weighed at birth (d 0) and following the milk yield study (d 9). We observed no differences (P > 0.05) in birth weight or d 9 lamb weights. Lamb gain over the 9 d milking period was similar among treated and control ewes (P > 0.05). No interactions were observed between parity and treatment in lamb weights or gain. Fluoxetine treatment during late pregnancy resulted in greater milk production in ewes giving birth to multiple lambs. However, FLX had no effect on lamb weights or lamb weight gain.

Keywords: Fluoxetine, lactation, sheep

INTRODUCTION

Fluoxetine (FLX; Prozac, Eli Lilly & Co., Indianapolis, IN) and other selective serotonin reuptake inhibitors (SSRI) have become popular for the treatment of depression during pregnancy because of their safety, effectiveness, and lower occurrence of maternal side effects (Nonacs and Cohen, 2002; Simon et al., 2002). In 1987, FLX became the first SSRI introduced in North America (Catterson and Preshkorn, 1996; Hiemke and Härter, 2000). Selective serotonin reuptake inhibitors act to increase extracellular serotonin (5-HT) levels sharply over a short period of time, while acting on serotonergic neurotransmissions on a continual basis. These events could lead to the reported adverse effects on pregnancy outcome and postnatal development in humans (Laine et al., 2003; Casper et al., 2003; Morrison et al., 2002). Serotonin’s role in the mammary system and lactation has only recently been observed (Matsuda et al., 2004; Hernandez et al., 2008). It is likely that 5-HT is part of the autocrine-paracrine homeostatic feedback mechanism (feedback inhibitor of lactation), which resists endocrine stimulation of mammary development and milk secretion (Wilde et al., 1995; Matsuda et al., 2004). Thus, SSRI act to inhibit lactation by preventing reuptake of 5-HT, and its subsequent degradation into the its metabolite 5-hydroxy indole acetic acid. Another potential issue with SSRI is the passage of the drug across the placenta to the infant. Studies have shown that FLX can cross the placenta in the rat (Pohland et al., 1989), humans (Spencer, 1993; Mhanna et al., 1997) and sheep (Kim et al., 2004). Harding and Bocking (2001) noted that the fetal lamb and human are alike in physiologic functions. As a result, pregnant sheep are often utilized to evaluate maternal-fetal drug disposition and effects (Rurak et al., 1991). The objective of this study was to evaluate if ewes would be an appropriate model for studying the effects of FLX on lactation.

MATERIALS AND METHODS

Animals, Facilities, and Diet. Procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. Twenty-nine Suffolk ewes (Avg BW 85 ± 12 kg; body condition score 2.6 ± 0.3) in late gestation were used in this study. Ewes were penned according to fetal number until parturition and then individually penned for 5-7 d post-partum. Ewes received 2.7 kg chopped alfalfa and 0.45 kg ground corn once daily during their last trimester and through the data collection period.

Design and Treatments. The experiment was a completely randomized design with a 2X2 factorial (FLX and fetal number) arrangement of treatments. Fetal numbers were determined by external flank ultrasound at d 70 of gestation. Approximately 21 d prior to lambing, ewes were sorted and penned according to fetal number to facilitate management decisions and treatment administration commenced. Treatments consisted of no FLX (control), or
samples were centrifuged (1,500 × g at 4°C for 15 min.) and serum was harvested and stored frozen. Initial milk samples were taken before lamb(s) nursed and subsequent milk yield data were collected 8 h after birth (d 1), as well as 3, 5, 7, and 9 d postpartum at 1500 and 1800, following procedures reported by Reynolds and Brown (1991). Milk yields were measured over a 3 h period after lamb(s) were removed. At birth, lambs were individually identified by unique premise ear tag and scrapie tag; their navals dipped with iodine and birth weights recorded. Following the end of the 9 d milking period, lamb BW were recorded to measure weight gain.

Statistical Analysis. The MIXED model of SAS (SAS Inst. Inc., Cary, NC) was used to analyze milk yield, lamb birth weight, and lamb gain. Individual ewe was the experimental unit, whereas, lamb was used as the experimental unit for weight data. Milk production was analyzed as a split plot design with treatment and parity in the whole plot and day and appropriate interactions in the sub plot.

RESULTS

We observed no behavioral responses during the dosing period, parturition or lactation. Milk yield averages, as well as milk production per day are reported in Table 1. A treatment × parity interaction (P = 0.01) was observed. Therefore, treatment means were compared within parity. Ewes with multiple lambs receiving FLX had greater (P < 0.05) milk yields than treated or control ewes giving birth to single lambs and control ewes giving birth to multiple lambs. All other treatment groups were similar (P > 0.05) in milk yields. We observed no interactions of treatment × day, day × parity, or treatment × day × parity, (P > 0.10). Daily milk yield was similar (P > 0.10) from d 1 through d 9 (Table 1).

Lambs were weighed at birth (d 0) and following the milk yield collection period (d 9; Table 2). Treatment by parity interactions were not evident (P > 0.10) for birth weight, d-9 weight, and gain. Birth weight and d-9 weights were similar (P > 0.10) between control vs. FLX and singles vs. multiples. Birth weights, d-9 weights, and gain were similar (P > 0.10) between lambs born to control and FLX ewes. However, as expected, single born lambs were heavier (P < 0.05) at birth and d 9 than controls and had higher (P < 0.05) weight gain (Table2).

DISCUSSION

Serotonin has been previously reported to depress lactation in mouse, bovine, and human models (Matsuda et al., 2004; Hernandez et al., 2008; Marshall et al., 2010). Matsuda et al. (2004) reported that 5-HT plays a role in mouse mammary gland development and homeostasis. When mouse mammary epithelial cells were treated with different levels of 5-HT, PRL-induced β-casein gene expression was repressed in a level-dependent manner. Also, upon inhibition of 5-HT synthesis through blockade of tryptophan hydroxylase I, milk protein gene expression was increased (Matsuda et al., 2004. Hernandez et al. (2008) reported similar findings, as they observed that 5-HT restricted milk protein mRNA expression in dairy cattle and suggested that 5-HT acts as a negative regulator of lactation.

Selective 5-HT reuptake inhibitors act to increase the bioavailability of 5-HT by preventing its reuptake into the cell, and subsequent degradation into its metabolite. In lactating mice, a local treatment of the lactating mammary gland with FLX resulted in involution of the mammary gland (Marshall et al. 2010). Additionally, a delay was noted in the onset of lactogenesis stage II in humans who had taken SSRI during pregnancy and lactation. While we did not observe a depression in lactation in ewes receiving FLX treatments during the last trimester of pregnancy it is possible that a dose of 40 mg FLX daily may have been too low to elicit a measurable decrease in lactation. In a previous study, (unpublished data), we observed that passage through the rumen reduced the level of FLX absorbed into the circulatory system more than half (15 mg) of the 40 mg daily dose of FLX. Serotonin has been shown to elicit a biphasic effect on tight junctions, the junctional complexes that close at lactation and open at involution, with lower concentrations resulting in a decrease in tight junction permeability, and higher concentrations increasing tight junction permeability (Pai and Horseman, 2008). Additionally, we did not administer FLX treatments while ewes were lactating.

While the direct effects of SSRI treatments are to increase the amount of extracellular serotonin, additional side effects are possible. Weight loss is a common side effect when adults take FLX; researchers suggested that FLX may directly decrease weight gain in infants who receive FLX through breast milk (Chambers et al., 1999). Chambers et al. (1996) suggested that weight loss during pregnancy of FLX treated women could be linked directly to decreased birth weights due to lower maternal weight gain which would limit fetal growth. In humans, reduced birth weights and postnatal weight gain were observed when women were exposed to fluoxetine during their third trimester (Chambers et al., 1996, 1999; Cohen et al., 2000; Nordeng et al., 2001). A study conducted with rats, showed that pregnant rats receiving FLX had poorer weight gain and delivered smaller pups (Vorhees et al., 1994). No differences were observed likely due to a potentially low concentration of FLX actually reaching the circulatory system.

We expected to see decreases in milk yield and lamb weight. However, we actually observed increased milk yield in FLX treated animals that had twinned, while lamb weight gain remained similar. We believe that this may be the result of a low dose of FLX actually reaching the circulatory system, resulting in a transient increase in milk yield, as previously reported. Serotonin has been noted...
to have biphasic effects on mammary epithelial resistance. Thus, at lower concentrations and earlier in time, 5-HT increased mammary function, however, at higher concentrations and later in time, 5-HT decreased mammary function (Pai and Horseman, 2008). The biphasic response of 5-HT may be the reason for the increase in lactation of FLX ewes with multiple lambs. It is our hypothesis that a larger dosage may provide a measurable depression on lactation.

**IMPLICATIONS**

Fluoxetine when dosed orally to pregnant ewes increased milk yields in ewes that gave birth to multiple lambs. However, lamb birth weight and gain were not affected by treatment. More research is needed to evaluate different dosage levels, the role of the rumen in degradation of FLX, and consequences on lamb growth and development.

**ACKNOWLEDGEMENTS**

Research was supported by New Mexico Agriculture Experiment Station and New Mexico State University Department of Animal and Range Sciences.

**LITERATURE CITED**


Table 1. Average milk yields over a 9 d lactation period in Suffolk ewes treated without (Control) and with 40 mg fluoxetine (FLX) during the last 21 to 28 days of pregnancy.1

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>FLX</th>
<th>Control</th>
<th>FLX</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield, g</td>
<td>317.2a</td>
<td>299b</td>
<td>308.9a</td>
<td>404.9b</td>
<td>23.5</td>
</tr>
<tr>
<td>Daily milk yield, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 1</td>
<td>333.53</td>
<td>294.99</td>
<td>263.34</td>
<td>418.05</td>
<td>54.86</td>
</tr>
<tr>
<td>d 3</td>
<td>343.26</td>
<td>298.61</td>
<td>311.17</td>
<td>383.70</td>
<td>55.68</td>
</tr>
<tr>
<td>d 5</td>
<td>271.72</td>
<td>320.95</td>
<td>327.88</td>
<td>401.22</td>
<td>44.83</td>
</tr>
<tr>
<td>d 7</td>
<td>326.35</td>
<td>258.98</td>
<td>312.52</td>
<td>421.96</td>
<td>56.46</td>
</tr>
<tr>
<td>d 9</td>
<td>311.27</td>
<td>321.31</td>
<td>329.71</td>
<td>399.68</td>
<td>44.83</td>
</tr>
</tbody>
</table>

1Milk yields were measured over a 3 h period after lamb removal. A treatment x parity interaction was observed ($P < 0.05$). Therefore, treatment means were compared within parity. Daily milk yield is presented for reference only.

Table 2. Lamb weights and gain during 9 d lactation period from Suffolk ewes treated without (Control) and with 40 mg fluoxetine (FLX) during the last 21 to 28 days of pregnancy.1

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>FLX</th>
<th>SE</th>
<th>Single</th>
<th>Multiple</th>
<th>SE</th>
<th>P-value4</th>
<th>Treatment</th>
<th>Parity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight, kg</td>
<td>5.85</td>
<td>6.26</td>
<td>0.69</td>
<td>6.79</td>
<td>5.32</td>
<td>0.73</td>
<td>0.90</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>9 d weight, kg</td>
<td>8.79</td>
<td>9.10</td>
<td>0.69</td>
<td>10.07</td>
<td>7.82</td>
<td>0.74</td>
<td>0.90</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Gain, kg</td>
<td>2.36</td>
<td>3.31</td>
<td>0.69</td>
<td>3.48</td>
<td>2.18</td>
<td>0.73</td>
<td>0.11</td>
<td>0.46</td>
<td></td>
</tr>
</tbody>
</table>

1No interaction of treatment x parity was observed ($P > 0.05$). Therefore, only levels within main effects were compared.

2Most conservative standard error (N = 23)

3Most conservative standard error (N = 8)

4Probability value