COMPARISON OF TRANSDERMAL FENTANYL AND INTRAMUSCULARLY ADMINISTERED BUPRENORPHINE FOR POSTOPERATIVE PAIN IN PREGNANT SHEEP

A Thesis

by

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ABSTRACT

Designing perioperative analgesic regimen for ruminants is problematic as pain assessment is difficult and pregnancy adds additional considerations. The aim of this study was to assess the nociceptive properties of intramuscularly administered buprenorphine and transdermally administered fentanyl utilizing a composite pain score system. To better confirm that the observed abnormal behavior was related to pain, the current study attempted to characterize the nociceptive properties of the analgesic agents at a given plasma drug concentration, which has not previously been done. Additionally, the study characterized transplacental movement of analgesic agents via fetal plasma drug concentrations.

In this study, we compared intramuscularly administered buprenorphine at a dose of 0.01 mg/kg every 8 hours for 48 hours starting at induction for surgery (n=6) to transdermal fentanyl patches (n=6) applied in the dorsal thorax region 24 hours before surgery at a dose of 2μg/kg/hr for postoperative pain. Ewe blood samples were collected and signs of pain and sedation were measured 24 hours before surgery (time -24), induction to surgery (time 0), and 2, 4, 6, 8, 12, 24, 36, 48 hours after. Using an indwelling fetal arterial catheter that was placed during the surgery, fetal blood pressure was recorded and blood samples were collected. Drug concentrations were measured in maternal and fetal plasma and amniotic fluid. The buprenorphine treated ewes exhibited more pain consistent behaviors than those treated with fentanyl, and their postoperative pain scores were significantly higher than the preoperative value. There were also significant differences in cardiovascular variables from the anesthesia records between

the two groups. Overall, transdermal administration of fentanyl provided adequate analgesia with little adverse effects, making it a candidate for optimal postoperative pain management in sheep.

DEDICATION

To my nephew and nieces, Kelby, Weston, and Avery Hargrove. Never lose your sense of wonder and know that I am always there to support and encourage you.

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Part 1. Faculty committee recognition

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Part 2. Student/collaborator contributions

The project was conceived and designed by Dr. Shannon Washburn and Ashley Padgett of the Department of Veterinary Physiology and Pharmacology and Dr. Mauricio Lepiz of the Department of Small Animal Clinical Sciences.

Experiments were performed by Dr. Shannon Washburn, Ashley Padgett, and Austin Driskill of the Department of Veterinary Physiology and Pharmacology, Dr. Mauricio Lepiz of the Department of Small Animal Clinical Sciences, and Dr. Evelyn Mackay of the Department of Large Animal Clinical Sciences.

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NOMENCLATURE

AVB	Atrioventricular block
BUP	Buprenorphine treatment
CI	Cardiac index
CO	Cardiac output
CRI	Constant rate infusion
CVP	Central venous pressure
DEX	Dexmedetomidine
FENT	Fentanyl treatment
HR	Heart rate
ICU	Intensive care unit
IM	Intramuscular
IV	Intravenous
LD	Loading dose
MAC_{iso}	Minimum alveolar concentration of isoflurane
MAC_{ISO}	Minimum alveolar concentration of isoflurane
MAP	Mean arterial pressure
MED	Medetomidine
OMT	Oral transmucosal route
Pa_{CO2}	Partial pressure of carbon dioxide
PAP	Pulmonary artery pressure
PVR	Pulmonary vascular resistance

SAP	Systolic arterial pressure
SVR	Systemic vascular resistance
TFP	Transdermal fentanyl patch

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1. INTRODUCTION AND LITERATURE REVIEW

1.1 Overview

Dexmedetomidine (DEX) is an alpha$_2$ agonist that was introduced into clinical practice as a short-term medication for sedation and analgesia. It has a wide application in veterinary and human medicine: as a premedication, an anesthetic adjunct, and as a perioperative sedative and analgesic. Alpha$_2$ agonists elicit their effects by binding to their corresponding receptor subtypes located throughout the central nervous system and periphery. However, there are considerable interspecies differences in the diversity and distribution of alpha$_2$ adrenoreceptor subtypes.

1.1.1 Alpha-2 Adrenoceptor

Alpha adrenoreceptors are located throughout the central nervous system and peripheral tissues, and they are divided into two subtypes; alpha$_1$ which exerts excitatory functions and alpha$_2$ that mainly produces inhibitory functions such as sedation, analgesia, and muscle relaxation. The magnitude of these effects is dependent on the affinity and selectivity of the alpha$_2$ agonists between alpha$_1$ and alpha$_2$. DEX has a dose-dependent alpha$_2$-adrenocreceptor selectivity. When administered at low to medium doses at slow infusion rates, DEX is a highly selective alpha$_2$ agonist, almost 8 times more specific than clonidine, making it a much more effective sedative and analgesic agent (Gertler, Brown, Mitchell, & Silvius, 2001). At higher doses or in rapid infusions, both alpha$_1$- and alpha$_2$-adrenoreceptor activates are observed. Numerous alpha$_2$ adrenoreceptor subtypes have been found, alpha$_{2A}$, alpha$_{2B}$, alpha$_{2C}$, and alpha$_{2D}$, and there are interspecies differences in receptor subtypes, distributions, and densities leading to differences in DEX dosing regimens and clinically relevant effects.

1.1.2 Aim

The goal of this review was to provide an understanding of the current role and the side effects associated with DEX in different species. Rather than focusing on the physiologic and pharmacologic bases of DEX, this article describes the clinical application of this agent and its relevant adverse effects.

1.2 Humans

1.2.1. Uses

DEX is commonly used as a sedative in human practice, especially in the intensive care unit (ICU) setting, because it is analgesic and anxiolytic, has a rapid onset, procures sedation with hemodynamic stability, and allows postoperative patients to remain sedated but easily aroused with stimulation (Giovannitti, Thoms, & Crawford, 2015; Venn & Grounds, 2001). It has been used as a sole sedative agent and as an adjunct for noninvasive and invasive procedures, respectively (Rao, Sudhakar, & Subramanyam, 2012; Tobias, 2008). DEX infusion dosing, described as Loading Dose/Maintenance, as per manufacturer recommendations is as follows: 1μg/kg over 10 minutes/ 0.2-0.7μg/kg/hr for adult ICU sedation, 1μg/kg over 10 minutes/ 0.6μg/kg/hr and titrated with doses from 0.2-1.0μg/kg/hr for adult procedural sedation, 0.5μg/kg over 10 minutes/consider dose reduction for patients over 65 years of age, and 0.25-0.5μg/kg in slow divided doses/consider dose reduction in patients with impaired hepatic or renal function (PRECEDEX, 2013). In conscious patients, increasing concentrations of DEX results in progressive increases in sedation (Ebert, Hall, Barney, Uhrich, & Colinco, 2000). Administering DEX as a bolus injection of 0.25-0.5μg/kg in slow divided doses produces a noticeable mellowing effect within 15- 30 minutes without a transient increase in blood pressure

(Giovannitti et al., 2015). When administered at a higher dose, clinically effective onset of sedation generally occurs within 10-15 minutes. At higher plasma concentrations, and thus a greater sedation state, DEX appears to compromise the ability to recall and recognize events, so lower concentrations may be useful in providing sedation while preserving memory (Ebert et al., 2000). The use of low dose constant rate infusion (CRI), without a loading dose (LD), for sedation in intubated patients who were already sedated with opiates and benzodiazepines, demonstrated cardiovascular safety, with minimal cardiovascular effects observed (Cummings et al., 2015; Tobias, 2008). When co-administered with agents such as opioids, benzodiazepines, and propofol, DEX induced sedation is enhanced (Giovannitti et al., 2015).

It has been demonstrated that DEX provides a moderate analgesic and analgesic-sparing effects in humans (Cortinez et al., 2004; Jaakola, Salonen, Lehtinen, & Scheinin, 1991). Determined by cold-pressor testing in volunteers, the analgesic effects elicited from DEX appear to have a linear relationship with dose, as increasing the dose leads to a decrease in pain sensation (Ebert et al., 2000). When administered as a 0.5μg/kg/h infusion, DEX has specific analgesic effects and provides visceral pain relief (Cortinez et al., 2004). Single administration of DEX also elicits an analgesic effect on ischemic pain; however, an apparent ceiling effect has been observed at a dose of 0.5μg/kg (Jaakola et al., 1991). The analgesic-sparing effects of DEX for post-surgical pain have been well documented (M. S. Aho, Erkola, Scheinin, Lehtinen, & Korttila, 1991; Arain, Ruehlow, Uhrich, & Ebert, 2004; Rao et al., 2012). Patients who were scheduled for major surgical procedures and received a LD of 1μg/kg over 10 minutes followed by a CRI of 0.4μg/kg/hr of DEX required 66% less morphine postoperatively, when compared to patients without DEX treatment (Arain et al., 2004). Similarly, a single premedication injection of 1μg/kg of DEX in patients undergoing abdominal surgery, resulted in a significant reduction

in postoperative morphine consumption for up to 24 hours (Unlugenc, Gunduz, Guler, Yagmur, & Isik, 2005).

DEX is widely used as a premedication agent because of its perioperative benefits. It has been shown to blunt responses to intubation and extubation, provide a stable hemodynamic profile, exert anesthetic-sparing effects, and to potentiate the anesthetic effects of intraoperative anesthetics (M. Aho, Lehtinen, Erkola, Kallio, & Korttila, 1991; Khan et al., 1999; Patel, Engineer, Shah, & Madhu, 2013; Rao et al., 2012; Scheinin, Lindgren, Randell, Scheinin, & Scheinin, 1992) Endotracheal intubation induces hemodynamic responses; however, anesthetic premedication of 0.6μg/kg IV DEX has been shown to attenuate, but did not completely blunt, the sypathoadrenal stimulation that occurs during tracheal intubation (Jaakola et al., 1991; Scheinin et al., 1992). The potent sympatholytic properties of DEX also aid in promoting hemodynamic stability and protect against radical cardiovascular fluctuations intraoperatively (Hogue et al., 2002; Rao et al., 2012). Induction and inhalant anesthetic requirements are reduced in patients receiving DEX (M. S. Aho et al., 1991; Scheinin et al., 1992). As a single intravenously (IV) premedication bolus, DEX treatment has been reported to reduce the isoflurane requirements by 25% (M. Aho et al., 1991). An intraoperative infusion of DEX has also been shown to reduce the isoflurane requirement by 25%-50% in a dose-related manner (M. S. Aho et al., 1991; Khan et al., 1999). Similarly, a 20% reduction in sevoflurane requirement has been reported in patients receiving DEX as an adjuvant in general anesthesia (Patel et al., 2013). DEX also facilitates a smooth recovery, and this in combination with the other benefits mentioned, makes it a favorable perioperative agent (Rao et al., 2012).

There is a growing interest in the use of DEX for sedation of patients with traumatic brain injuries because of its role as a neuroprotective agent, (Erdman et al., 2014; Handlogten, Sharpe,

Brost, Parney, & Pasternak, 2015; Tobias, 2008). Beneficial effects of DEX administration on the central nervous system include a protective effect during ischemia, no effect on intracranial pressure, and a reduction of cerebral blood flow. Various animal models have been used in attempt to characterize these effects, and while blunting of endogenous catecholamine release during ischemia appears to play a role, further investigation on the underlying mechanisms is needed (Chalikonda, 2009; Sugita, Okabe, & Sakamoto, 2013). Typical hemodynamic effects, such as bradycardia and a biphasic change in blood pressure have been observed with DEX administration to neuropathic patients; however, they were generally well tolerated (Tobias, 2008)

1.2.2 Adverse Effects

Common adverse effects of DEX include hypotension, hypertension, bradycardia, atrial fibrillation, and hypoxia (Arain et al., 2004; Gertler et al., 2001). Typically, these are mild and appear in a dose-related manner. Increasing concentrations of DEX through continuous IV infusion resulted in progressive cardiovascular changes in healthy volunteers (Ebert et al., 2000). At lower plasma concentrations, <2.0ng/ml, a progressive decrease in heart rate (HR) and cardiac output (CO) was observed, as well as a significant decrease in mean arterial pressure (MAP). Higher concentrations of DEX, >2.0ng/ml, caused a significant decrease in HR and CO while increasing central venous pressure (CVP), pulmonary capillary wedge pressure (PCWP), pulmonary artery pressure (PAP), pulmonary vascular resistance (PVR) and systemic vascular resistance (SVR) (Ebert et al., 2000). Additionally, the potential for systemic and pulmonary hypertension is a concern related to high DEX concentrations.

Minimal effects of DEX on the respiratory system have been observed in humans, but when they do, it appears to be dose-related (Belleville, Ward, Bloor, & Maze, 1992; Ebert et al.,

2000). A rapid infusion of high dose DEX in healthy volunteers has shown to produce a slight increase in Pa_{CO2} and a decrease in minute ventilation, with little change in ventilatory frequency (Ebert et al., 2000; Khan et al., 1999). The respiratory effects of DEX are thought to be more of a reflection of its sedative properties rather than direct respiratory depression.

1.3 Dogs

1.3.1 Uses

Sedation induced by DEX appears to be suitable for diagnostic and therapeutic procedures in dogs for which moderate to deep sedation is required, and the degree of sedation is dose-dependent (Kuusela et al., 2001). Dogs receiving 0.2μg/kg of DEX IV appeared to only be lightly sedated while those receiving 20μg/kg were deeply sedated, as determined by a sedation score system (Kuusela et al., 2001). The IV administration of 15μg/kg of DEX provides peak sedation within 15 minutes that lasts at a clinically acceptable level for approximately 120 minutes (M. Granholm, McKusick, Westerholm, & Aspegren, 2007). When administered intramuscularly (IM) at 30μk/kg, peak sedation occurs within 30 minutes and is within clinical range for 180 minutes (M. Granholm et al., 2007).

DEX has been shown to provide analgesia in a dose-dependent manner when administered to dogs as a single IV or IM injection. The degree of analgesia, determined by a score system, in dogs receiving DEX IV at a dose of 20μg/kg was found to be very prominent; however, no analgesic effect was apparent in dogs receiving 0.2μg/kg (Kuusela et al., 2001). Clinically relevant analgesic effects in dogs are noticed 5-15 minutes after IV and 15-30 minutes after DEX IM injection, and the duration of analgesia is approximately 45 minutes (M. Granholm et al., 2007). The pedal withdrawal test has been used to compare the analgesic effects

of IM administered DEX to medetomidine (MED) at equipotent doses and found that DEX initially had lower scores until after the observed peak; then, the analgesic scores tended to be higher in the DEX treatment group (M. Granholm et al., 2007; Kuusela et al., 2000). This was not observed when the agents were administered IV, and thus could possibly be explained by differences in pharmacokinetics.

Anesthetic-sparing effects of DEX are observed in dogs and appear to be dose-dependent. The propofol requirement for induction in dogs that received a premedication dose of 20μg/kg of DEX IV was reduced by almost 87% when compared to dogs that had only received 0.2μg/kg (Kuusela et al., 2001). Moreover, the same study reported a high premedication dose of DEX reduced the isoflurane requirement by 85% when compared to the low dose. The influence of DEX on cardiovascular-stability in anesthetized dogs also appears to be dose-dependent, with a moderate level dose, 2μk/kg, resulting in greater stability (Kuusela et al., 2001).

1.3.2 Adverse Effects

Typical DEX cardiovascular effects have been reported in conscious dogs following single IV or IM administration of DEX. A significant decrease in the HR has been shown to occur within 15 minutes after administration in both IM and IV routes and persisted for approximately 180 minutes (M. Granholm et al., 2007). When used as a premedicant for propofol-isoflurane/propofol anesthesia, dogs were bradycardic after receiving DEX for approximately three hours, and it was not influenced by inhalant anesthetic (Kuusela, Raekallio, Hietanen, Huttula, & Vainio, 2002). The use of propofol as an induction agent has shown to attenuate DEX induced bradycardia; however, prominent bradycardia still persisted in dogs premedicated with a high dose of DEX (Kuusela et al., 2001). The effect of DEX on HR appears to last longer than its sedative and analgesic effects and should be kept in mind during

postoperative monitoring. The development of second-degree atrioventricular-blocks (AVB) during the premedication period have been recorded in dogs receiving 10μk/kg IM, but the blocks decreased or vanished after the induction of anesthesia (Kuusela et al., 2002). DEX exhibits a biphasic blood pressure response in a dose-dependent manner in propofol-isoflurane/propofol anesthetized dogs (Kuusela et al., 2001). Additionally, induction with propofol on DEX premedicated dogs has shown to significantly decrease MAP, regardless of the DEX dose used (Kuusela et al., 2001). Administration of atipamezole has shown to partially reverse the cardiorespiratory effects of DEX (M. Granholm et al., 2007).

Respiratory depression from DEX is considered to be minimal; however, a study using client-owned dogs observed a significant decrease, below baseline, in respiratory rate following a single IV or IM administration of 15μk/kg and 30μk/kg, respectively (M. Granholm et al., 2007). This finding may be associated with the excitement and stress of preprocedural handling in a veterinary hospital rather than a direct effect of DEX, but nevertheless it was observed.

A decrease in rectal temperature has been associated with DEX administration in dogs. Although their temperature remained within clinically acceptable limits, dogs should be kept warm during the procedure and recovery (M. Granholm et al., 2007).

1.4 Cats

1.4.1 Uses

The effects of DEX on sedation in cats has been described, and a single IM injection at 0.04mg/kg has been shown to be effective and safe in clinically healthy cats requiring sedation and analgesia for minor procedures (Mikael Granholm, McKusick, Westerholm, & Aspegrén, 2006). A single dose of 0.04mg/kg administered via oral transmucosally (OTM) has also been

shown to produce sedative effects with similar time of onset, intensity, and duration as the IM route (Slingsby, Taylor, & Monroe, 2009). A single intravenously administered bolus has also shown to induce sedation, and while the sedative effect, determined by a sedation score system, did not depend on the dose, the duration of sedation was dose dependent (Pypendop & Ilkiw, 2014). Studies using low doses of DEX, 0.005-0.05mg/kg, have shown an increase in sedation with an increase in dose; however, Ansah et al. described the dose-dependent sedative effect to be limited (Ansah, Raekallio, & Vainio, 2000). The authors compared sedative effects elicited during a 50-minute CRI dosed at 0.25, 1.0, and 4.0μg/kg/min, and it appeared that an increase in serum concentration beyond a certain level leads to the reversal of sedation (Ansah et al., 2000). The reversal was noticed in the highest dose group and could be the result of drug interaction with alpha-1 adrenoceptors or desensitization of alpha-2-adrenergic receptors following continuous exposure. A co-administration of DEX with buprenorphine has shown to enhance the sedative effects (Slingsby, Murrell, & Taylor, 2010).

In a clinical trial, it has been demonstrated that administration of a single IM dose of DEX (0.04mg/kg) resulted in clinically effective analgesia (Mikael Granholm et al., 2006). However, its use a single agent may not be ideal for more invasive procedures, such as castrations. Evaluation of the analgesic effects of DEX via score systems suggests DEX produces dose-dependent analgesic effects and duration (Ansah et al., 2000; Mikael Granholm et al., 2006). Thermal threshold testing has shown DEX to provide adequate antinociceptive effects after a bolus administration of 0.04mg/kg IM or OTM, as well as doses ranging from 0.005-0.05mg/kg via IV (Pypendop & Ilkiw, 2014; Slingsby et al., 2009). In those studies, a thermal antinociceptive effect was found 35, 30, and ~2 minutes following IM, OTM, and IV administration, respectively. The average duration of analgesia following IM and OTM

administration was 61.3 and 98.8 minutes, which is similar to the duration observed, ~60 minutes, following IV bolus of 0.005mg/kg and 0.02mg/kg. However, the thermal antinociceptive effect of 0.05mg/kg via IV bolus lasted nearly two hours (Pypendop & Ilkiw, 2014). In a study evaluating the antinociceptive effects of DEX co-administered with buprenorphine in IV bolus, dosed at 0.02mg/kg and 0.01mg/kg, respectively, found that the combination produced greater analgesia with earlier onset and a longer duration than either agent alone (Slingsby et al., 2010).

Anesthetic sparing effects of DEX in cats appears to be dependent on regimen (CRI or as premedication) and/or dose. When administered as a low dose CRI in propofol-isoflurane anesthetized cats, DEX did not significantly affect the dose or propofol required for induction (Simon, Scallan, Coursey, Kiehl, & Moore, 2018; Souza et al., 2010). However, when utilized as a premedication, administered as a high, single dose (IM, IV, and OTM), DEX reduced the induction requirement of propofol by approximately 44%-58% (McSweeney, Martin, Ramsey, & McKusick, 2012; Mendes, Selmi, Barbudo-Selmi, Lins, & Figueiredo, 2003). Moreover, preanesthetic administration of DEX has been shown to significantly reduce the dose of ketamine necessary to intubate cats by approximately 77%-85% the current FDA approved dose (McSweeney et al., 2012). That study also reported an increase in intubation success rate in DEX treated cats (89%) compared with the placebo group (11%).

The relationship between plasma DEX concentration and the minimum alveolar concentration of isoflurane (MAC_{ISO}) has been examined using target-controlled IV infusions and reported a plasma-concentration dependent decrease in MAC_{ISO} (Escobar, Pypendop, Siao, Stanley, & Ilkiw, 2012). A low dose LD followed by CRI, 0.5µk/kg and 0.5µk/kg/hr, respectively, on propofol-isoflurane anesthetized cats found an overall reduction in end-tidal

isoflurane requirement by 21%(Simon et al., 2018). A similar reduction was also noticed in cats undergoing propofol-isoflurane anesthesia with epidural lidocaine, who received DEX epidural and CRI (Souza et al., 2010).

The effects of DEX on anesthetic recovery is variable. When administered at a LD of 0.5μk/kg followed by a 0.5μk/kg /hr CRI during propofol-isoflurane anesthesia no significant effects on recovery were found (Simon et al., 2018). In contrast, a separate study using a higher LD, 4μk/kg, found DEX to increase the duration of recovery and improve its quality (Mendes et al., 2003; Souza et al., 2010). The preanesthetic single IV administration of DEX at a high dose was shown to provide a better quality of recovery yet did not increase the duration (Mendes et al., 2003).

Through the use of score systems, DEX has shown to produced good muscle relaxation when administered as a single IM injection or as a CRI via IV (Ansah et al., 2000; Scrollavezza, Tambella, Vullo, & Piccionello, 2009). The effects of DEX on seizure threshold in anesthetized cats has also been investigated and found that high-dose DEX reduced seizure threshold (Miyazaki et al., 1999).

1.4.2 Adverse Effects

The cardiovascular effects of DEX in cats have been reported in the literature; decrease in HR, cardiac index (CI), and stroke index (SI) with an increase in SVR and CVP (Pypendop, Honkavaara, & Ilkiw, 2017). The DEX induced decrease in HR in cats is thought to be little influenced by dose, as the observed magnitudes of this effect appear similar through a range of doses; however, the higher doses appear to result in a longer duration of the effect (Ansah et al., 2000; M. Granholm et al., 2007; Monteiro, Campagnol, Parrilha, & Furlan, 2009). Cardiorespiratory studies in cats treated with DEX show atypical effects on mean arterial

pressure compared to the typical biphasic effect reported in other species. A study evaluating blood pressure response to DEX in cats receiving either 15μk/kg or 30μk/kg IM observed a slight decrease in systolic arterial pressure (SAP) after administration followed by a continual gradual decrease, regardless of the dose (Monteiro et al., 2009). In other studies, cats receiving 25μk/kg (either IV bolus or IM) resulted in a significant increase in SAP, which is in agreement with DEX vasoconstriction properties at alpa2-adrenoceptors on vascular smooth muscle (Mikael Granholm et al., 2006; Pypendop et al., 2017). Premedication with atropine or co-administration with MK-467, an alpha$_2$ adrenoreecptor antagonist, has been shown to improve the hemodynamics when compared to DEX alone; however, further studies are needed to evaluate the cardiovascular effects (Monteiro et al., 2009; Pypendop et al., 2017). Hemodynamic effects related to IV target-controlled CRI administration of DEX in isoflurane-anesthetized cats was found to decrease HR and CI and increase SVR, which is in agreement with the findings in the conscious state (Pypendop & Verstegen, 2001).

It has been reported that DEX decreases respiratory rate similar to that of medetomidine, whether its administered as a single IM injection or an IV CRI (Ansah et al., 2000; Mikael Granholm et al., 2006; Monteiro et al., 2009). However, there is discrepancy about the relationship between drug concentration and magnitude of effect and further research is warranted.

Emesis is observed in the majority of studies evaluating DEX in cats, with some studies documenting 67%-83% of those receiving DEX vomiting (Monteiro et al., 2009; Slingsby et al., 2009). In contrast, another study has reported a smaller incidence of 8% (Mikael Granholm et al., 2006). While DEX administration in cats has shown to cause vomiting, the fasting state of the cats prior to injection may impact the prevalence.

1.5 Equine

1.5.1 Uses

In equine practice, standing protocols are preferred over general anesthesia, and so the beneficial profile of DEX, with a short half-life and rapid distribution, makes it a potential option. Using head height above the ground as a quantitative measure of the degree of sedation in horses, DEX provided clinically relevant sedation within 5 minutes after the initiation of a CRI, dosed at 5μk/kg/hr, with the maximum effect reached within 15 minutes (Medeiros et al., 2017). A study comparing the sedative effects of DEX to MED found that more than 50% of the horses administered DEX, 3.5μk/kg IV, required one or more supplemental doses to meet sedation criteria for induction (Sacks, Ringer, Bischofberger, Berchtold, & Bettschart-Wolfensberger, 2017). They further reported the dose necessary to fulfill sedation criteria prior to anesthesia induction was approximately 4μk/kg (range, 4-9μk/kg). Similar studies have reported the same findings, suggesting a slightly higher dose might be preferable in order to obtain acceptable sedation prior to intubation (Miguel G. Marcilla, Schauvliege, Segaert, Duchateau, & Gasthuys, 2012). The administration of 5μk/kg of DEX via epidural provided a moderate degree of sedation in donkeys undergoing surgical procedures in the perinea region, and the DEX induced sedation had a longer duration compared to xylazine (Hamed, Abouelnasr, Ibrahim, & El-khodery, 2017).

Mechanical nociceptive threshold testing has shown DEX to induce analgesic effects in horses for 30 minutes following a 5μk/kg IV bolus (Rezende, Grimsrud, Stanley, Steffey, & Mama, 2015). When administered as three escalating IV CRIs, 2, 4, and 6μk/kg/hr, DEX increased the nociceptive threshold to single and repeated stimulation at the two higher doses and increased nociceptive tolerance at all CRI levels (Risberg, Spadavecchia, Ranheim, Krontveit, &

Haga, 2014). Furthermore, the antinociceptive effects observed in that study persisted for 30 minutes after the CRI. A study on the effects of epidural DEX, 5μk/kg, in donkeys found that DEX induced more potent antinociceptive effect with a faster onset and longer duration than xylazine (Hamed et al., 2017).

Although DEX has been shown to reduce anesthetic requirements in several other species, a study administering an IV bolus of DEX followed by a CRI, 1.75μk/kg/hr, to isoflurane-anesthetized horses failed to reduce the dose of isoflurane required for maintenance (Miguel G. Marcilla et al., 2012). A similar study comparing a CRI of morphine to DEX, at the same dose previously reported, found that inhalant anesthetic requirement between the two regimens was only significantly different 60 minutes after start of infusion (Gozalo-Marcilla, Steblaj, Schauvliege, Duchateau, & Gasthuys, 2013). However, the authors did report that horses receiving DEX appeared to maintain a more stable surgical depth of anesthesia, required lower doses of ketamine, and presented better quality recoveries, which suggest potential benefits of DEX as an anesthetic adjuvant. An improvement in recovery quality has also been observed in diazepam+ketamine/isoflurane anesthetized horses that received a LD and CRI of DEX, when compared to those receiving MED or saline (Sacks et al., 2017). Furthermore, horses receiving DEX as a bolus premedication, CRI during anesthesia, and an IV bolus at the end of the surgical procedure had significantly higher recovery scores and longer times to sternal recumbency and first attempt to stand, when compared to horses that did not receive DEX (Miguel G. Marcilla et al., 2012).

1.5.2 Adverse Effects

DEX administered as a LD of 3.5μk/kg followed by a CRI of 5μk/kg/hr in conscious horses has been shown to decrease HR, CI, SAP, and MPAP while increasing SVRI, yet all

values remained within clinically acceptable limits (Medeiros et al., 2017). In the same study, all horses receiving DEX developed AVBs, and this was thought to be attributed to the IV bolus of 3.5μk/kg. Typical DEX associated cardiovascular effects in anesthetized horses have been recorded but appear to remain within clinically acceptable limits and the magnitude of change does not appear to be dose-dependent (Miguel Gozalo Marcilla, Schauvliege, Duchateau, & Gasthuys, 2010; Miguel G. Marcilla et al., 2012).

Respiratory depression has been observed in horses receiving a CRI of 5μk/kg/hr, and respiratory rate continued to be lower for 30 minutes after infusion (Medeiros et al., 2017). The co-administration of butorphanol does not appear to have a significant influence on DEX induced ventilatory effects (Medeiros et al., 2017).

Decreased gastrointestinal motility following DEX administration has been described in the literature (Koenig, Martin, Nykamp, & Mintchev, 2008; C. Zullian, Menozzi, Pozzoli, Poli, & Bertini, 2011). In horses receiving 5μk/kg IV bolus, significant decreases in borborygmic values were observed following administration; however, no clinically significant effects, such as colic, were reported (Rezende et al., 2015).

1.6 Small Ruminants

1.6.1 Uses

DEX has been used as a premedication prior to general anesthesia in sheep (Carroll & Hartsfield, 1996; Kastner, 2006; Kutter, Kastner, Bettschart-Wolfensberger, & Huhtinen, 2006). Through the use of a sedation score system, 0.005mg/kg of DEX administered IV has been shown to provide a clinically significant degree of sedation within 15 minutes, lasting an average of 45 minutes (Borges et al., 2016). When administered IM, at a dose of 15μg/kg, DEX provided

sheep with moderate to severe degree of sedation within 20 minutes after injection (Kastner, Boller, Kutter, Akens, & Bettschart-Wolfensberger, 2001). The concurrent administration of DEX, 0.005mg/kg IV, with methadone, morphine, or butorphanol in sheep does not appear to enhance sedation; however, the combinations did prolong the duration of sedation compared to sole administration of DEX (Borges et al., 2016).

Pain management is difficult in this cohort because of their atypical anatomy and the lack of literature investigating the efficacy of analgesic agents. Results from small ruminant clinical studies indicate alpha$_2$ agonist elicit analgesic effects; however, these agents alone are not sufficient for painful and major surgical procedures (Shah, Ding, & Hu, 2014). A dose as low of MED, 5μg/kg, has been shown to produce a significant analgesic effect to mechanical stimulus for 60 minutes (Muge, Chambers, Livingston, & Waterman, 1994). In a comparison study between DEX and MED, an equipotent dose of 1:2 was proposed for sheep, and so, a dose of 2.5μg/kg could potentially provide clinically relevant analgesia; however, further research in ruminant pain management is needed (Kastner, Von Rechenberg, Keller, & Bettschart-Wolfensberger, 2001).

DEX produced anesthetic-sparing effects in sheep are similar to those caused by MED (Kastner, Von Rechenberg, et al., 2001). A single pre-medication dose of 5μg/kg DEX IV has been reported to reduce the isoflurane requirement by approximately 30% in ketamine-isoflurane anesthetized sheep (Kastner, Von Rechenberg, et al., 2001). A similar reduction was observed in sheep premedicated with 15μk/kg IM 30 minutes before induction with ketamine (Kastner, Boller, et al., 2001).

The use of DEX as a sedative prior to euthanasia has been debated because it has been thought that DEX induced decrease in cardiac output slows the circulation of euthanasia solution,

thus leading to a slower euthanasia compared to those unpremeditated. This has been demonstrated in other species; however, euthanasia was not affected by the administration of 5μk/kg of DEX to sheep five minutes prior to an injection of euthanasia solution, compared to those not treated with DEX (Barletta et al., 2018). In the same study, vocalization during euthanasia was only observed in sheep receiving DEX, and thus it is advised to warn the owner and other personal of the possibility of vocalization.

1.6.2 Adverse Effects

A significant decrease in heart rate has been associated with DEX administration in conscious sheep (Borges et al., 2016). Low dose DEX administration IM or IV to conscious sheep does not significantly alter blood pressure, contradicting the typical biphasic effect noticed in other species (Borges et al., 2016; Kastner, Boller, et al., 2001). This could be attributed to the low dose administered rather than the species, and so further investigation is needed. In sevoflurane-anesthetized goats and sheep, DEX administration has been associated with a decrease in HR and CO and an increase in CVP (Kutter et al., 2006). Studies have shown the magnitude and duration of cardiopulmonary changes in response to DEX to be different between sheep and goats, suggesting a species-specific difference in sensitivity to the centrally mediated cardiovascular effects of DEX (Kästner et al., 2007; Kutter et al., 2006).

Respiratory depression and hypoxemia are possible adverse effects of alpha-2 agonists administration in ruminants (A., A., & A., 1986; Kutter et al., 2006). The pathological mechanisms behind these adverse effects are thought to be caused by consecutive alveolar hypoventilation and impairment of gas exchange due to pulmonary edema (Kastner, Von Rechenberg, et al., 2001). Impaired oxygenation appears to be mediated by peripherally located alpha-2 adrenoreceptors that respond in a dose-dependent manner (A. et al., 1986). Studies in

sheep suggests hydrostatic stress may be the underlying cause of pulmonary edema formation caused by DEX (Kastner, Ohlerth, Pospischil, Boller, & Huhtinen, 2007). Specifically, to this cohort, ventilation and Pa_{O2} are additionally compromised because of the abnormal position these animals are typically placed in while anesthetized. This position promotes ventilation-perfusion mismatching and restricts diaphragmatic excursion by abdominal contents (Carroll & Hartsfield, 1996). The prevalence of clinically obvious pulmonary changes in goats is sparse and highly variable; however similar changes in pulmonary hemodynamics of both sheep and goats have been observed (Kutter et al., 2006).

1.7 Conclusion

DEX is commonly used in veterinary and human practice for sedation, analgesia, and as an anesthetic adjuvant. The pharmacological activities of DEX in different species are almost similar, but some differences exist due to species specific differences in $alpha_2$-adrenoreceptor subtypes, distributions, and densities.

2. INTRODUCTION

Sheep have become a common animal model, particularly in surgery and fetal-development research (Pape & Madry, 2013; Washburn, Tress, Lunde, Chen, & Cudd, 2013; Wilson & Cudd, 2011). In such research, intra and postoperative pain management are of utmost importance; not just to ensure animal welfare, but also to avoid the consequences of untreated pain. Early pain recognition contributes to optimum recovery by allowing analgesics to maximize their anti-nociceptive potential before the initial afferent pain barrage, thus preventing all the consequences of untreated pain and the establishment of chronic pain. The utilization of a pain scoring system facilitates early detection of pain. Although there is not a validated score system in sheep, multidimensional scoring systems are superior to a simple visual analog scale (Musk, Catanchin, Usuda, Woodward, & Kemp, 2017; Musk et al., 2014; Stasiak, Maul, French, Hellyer, & VandeWoude, 2003).

Regimens containing combinations of non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are most commonly used for perioperative analgesia. Buprenorphine is a partial mu agonist, with a potency at least 30 times that of morphine and a long duration of action, which allows for extended dosing intervals. Fentanyl is a short acting full mu agonist 80 to 100 times as potent as morphine. The transdermal fentanyl patch (TFP) is a promising alternative because it is designed to release fentanyl in a sustained rate to produce stable plasma concentrations for a prolonged period of time, decreasing the chances of variable concentrations often found with periodic intravenous and intramuscular injections of opioids. Potential non-analgesic effects elicited by these agents must also be considered.

Analgesics are often administered to sheep, and other species, on the basis of data derived from human studies, and the proposed doses may not provide adequate analgesia in sheep. Variability in drug disposition and bioavailability from route of administration further complicates evaluation of efficacy of the agents at the suggested dose. Although the pharmacokinetics of TFP in a sheep model have previously been described, factors such as patch location, ewe body composition, and pregnancy vary widely among studies and warrant further investigation to better characterize the maternal and fetal plasma fentanyl concentrations from TFP use (Heikkinen et al., 2015; Jen, Dyson, Lester, & Nemzek, 2017); the literature regarding the efficacy of TFP in pregnant sheep is limited. There is also a need to assess the effectiveness of the TFP in sheep to a more established analgesic drug, such as buprenorphine.

In humans, the TFP is increasingly being used to treat chronic pain. There are few reports of its use during pregnancy, and its safety has not been established in this population of patients. Buprenorphine's effects on the fetus are not completely understood, yet it is frequently utilized for medication-assisted treatment in cases of opioid use disorder in pregnant women (Chavan, Ashford, Wiggins, Lofwall, & Critchfield, 2017). This creates the need to characterize and understand both the maternal and fetal effects of fentanyl and buprenorphine. This study will not only provide much needed information on analgesia in veterinary patients, but since sheep are a highly translational model for human pregnancy (Pape & Madry, 2013; Washburn et al., 2013; Wilson & Cudd, 2011), results can also offer some clues about its use in pregnant women and potential fetal fetus.

The goal of this study was to compare intraoperative and postoperative analgesic effects of TFP, applied in a novel location, to the effects of buprenorphine in pregnant ewes. In addition,

to examine the influence of those analgesics on anesthesia and to characterize plasma drug concentrations and placental transfer.

3. MATERIALS AND METHODS

3.1 Animals

Twelve pregnant Suffolk-cross ewes (ages 2-4 years) with time-dated pregnancies underwent anesthesia and surgery, which was approved by Texas A&M University Institutional Animal Care and Use Committee. All ewes were examined and weighed upon arrival, and then acclimatized and housed in individual indoor pens and fed a commercially prepared pelleted feed and Bermuda grass hay throughout the study.

Twenty-four hours before the planned surgery, each sheep was randomly assigned into one of two treatment groups. The buprenorphine-treated ewes (n=6) received buprenorphine hydrochloride (Par Pharmaceuticals Companies, Spring Valley, NY) intramuscularly at a dose of 0.01 mg/kg every 8 hours for 48 hours starting at induction for surgery (time 0), and a placebo patch, containing no drugs, was applied to each ewe. Each sheep in the fentanyl group (n=6) received a combination of fentanyl patches (Mylan Pharmaceuticals, Morgantown, WV) 24 hours before surgery (time -24) to achieve a dose of 2μg/kg/hr. All patches were applied at the dorsal thorax area of the ewes. Before application, the area was clipped, cleaned with isopropyl alcohol, and allowed to air dry. It was covered with an adhesive bandage (Elastikon, Johnson & Johnson, New Brunswick, NJ) and vet wrap (Tape Pet Flex, Andover, Salisbury, MA) and further secured with tubular netting (Stretch Net N84, Nich Marketers, Gulf Breeze, FL).

3.2 Surgical Instrumentation

On gestation day 115 (+/- 2 days), calculated from an observed breeding date and later confirmed with ultrasound, the pregnant sheep underwent anesthesia and surgery for fetal

catheterization. Ewes were fasted for 24 hours prior to surgery. Anesthesia was induced intravenously with a combination of midazolam (0.35 mg/kg, Akorn, Lake Forest, IL) and ketamine hydrochloride (4mg/kg, Zetamine™, VetOne, Boise, ID), supplemented as needed in order to intubate the trachea of the ewes. The sheep were positioned in dorsal recumbency, and anesthesia was maintained with isoflurane (Fluriso, VetOne, Boise, ID) delivered in oxygen by a small animal anesthesia machine (Matrx™ Model 3000, Midmark, Orchard Park, NY). Ringer's lactate solution was administered through a jugular catheter throughout anesthesia at 5mL/kg/hr.

Standard surgical techniques previously described (Cudd, Chen, Parnell, & West, 2001) were used to catheterize the fetal cranial tibial arteries and saphenous veins and advance to the abdominal aorta and inferior vena cava, respectively. To collect amniotic fluid, a catheter was designed and constructed from 3/32" inner diameter, 5/32" outer diameter polyvinyl chloride tubing (Tygon® #ADF00004) with added perforations. The tubing was sutured to the inside of a fenestrated golf ball (Wilson Ultra® whiffle golf balls) to prevent the fetal membranes from collapsing over and obstructing the holes in the tubing (Figure B-1). During surgery, the amniotic catheter was sutured to the skin over the fetal tarsus, with the golf ball end floating inside the amniotic cavity. Using a trocar, all catheters were exteriorized in the right flank of the ewe, where they were retained in a cloth pouch. All ewes received flunixin meglumine (1mg/kg, Banamine®, Merck Animal Health, Summit, NJ) intravenously and tulathromycin (2.5mg/kg, Draxxin®, Zoetis US, Parsippany, New Jersey) subcutaneously prior to surgery. After surgery, anesthesia was discontinued, ewes were placed in sternal position and allowed to recover. Once the sheep began to swallow and lift their heads, they were extubated.

3.3 Anesthetic Monitoring

An individual blinded to treatment and independent of the person adjusting the isoflurane settings recorded anesthetic variables. Heart rate, ventilation rate, hemoglobin saturation (SpO2), and body (esophageal) temperature were monitored during surgery using a Datascope Passport® 2 monitor (Mindray, Mahwah, NJ). Blood pressure was measured non-invasively with an inflatable cuff (Cardell Veterinary Monitor, 9401 BP., Midmark, Dayton, OH) placed on the metacarpal region and systolic, diastolic, and mean blood pressures were recorded. The end-tidal isoflurane concentration (Et_{ISO}) and end-tidal carbon dioxide concentration (Et_{CO2}) were measured using a Datex Capnomac Ultima monitor (Instrumentarium Corp., Helsinki, Finland), calibrated before each experiment. A separate individual, blinded to treatment, adjusted the isoflurane vaporizer settings to maintain an adequate depth of anesthesia throughout surgery. Times were recorded for the following markers: induction, intubation, start of surgery, end of surgery, extubation, and standing; induction was set as time zero.

3.4 Analgesic Measures and Maternal Sample Collection

An individual blinded to treatment group assessed each sheep for pain. Ewe heart rate, respiratory rate, and rectal temperature were measured and behavioral indicators of pain and sedation were recorded using a modification of the Stasiak et al. (2003) composite scoring system (Table A-1 & A-2). Each of the five criterion, comfort, movement, appetite, posture, and response to palpation of surgical incision, was scored on a scale of 0-3, with a score of one or greater indicating abnormal behavior. These scores were added together for a total score, with a maximum possible total of 15; rescue analgesia was planned if scores exceeded a value of 8. Sedation was scored based on posture and demeanor, using a scale of 1-10, with 10 being most

sedated. The first assessment was made 24 hours before surgery (t=-24), before the fentanyl or placebo patch was placed. Assessments were repeated immediately before induction of anesthesia (t=0) and at 4, 6, 8, 12, 24, 36, and 48 hours after induction. Consumption was determined by subtracting the remaining amounts of feed and water from the standard ration each ewe received. Blood samples from the ewe jugular vein (5 mL) were collected into lithium heparin tubes before the patches were applied (t= -24) and at the following time points: 0, 2, 4, 6, 8, 12, 24, 36, 48, 72, 122, and 288 hours. The blood samples were immediately centrifuged, and then plasma samples were stored at -80°C until analysis.

3.5 Fetal Sample Collection

At time points 4, 8, 12, 24, 36, and 48 hours, the indwelling fetal arterial catheter and amniotic catheter were accessed to measure blood pressure and collect samples. Before sampling, all catheters were cleaned with iodine scrub and isopropyl alcohol, and waste fluid was removed from the line. Fetal arterial blood samples (1.0mL) were collected into lithium heparin tubes, and amniotic fluid (1.5mL) was collected into polystyrene tubes without additives. Blood samples were immediately separated via centrifugation, and all samples were stored at -80C until analysis. An additional fetal arterial blood sample (~0.5mL) was collected to analyze blood gas chemistries using an i-Stat portable clinical analyzer (model 300A, Abbott, Inc., Princeton, NJ). The fetal blood pressure was recorded using a PowerLab® data acquisition system (PowerLab 8/30, model ML870), and from this, fetal heart rate, diastolic pressure, systolic pressure, and mean arterial pressure were determined using LabChart® software (ADInstruments, Inc., Colorado Springs, CO). After collection, the amniotic catheter was flushed with saline, and the arterial catheter was flushed with heparinized saline.

3.6 Sample Analysis

3.6.1 Standards, Materials, and Reagents

Negative sheep plasma and amniotic fluid were obtained from untreated sheep used in this study. Fentanyl (#F-013) and buprenorphine (#B-902) reference materials were obtained from Cerilliant (Round Rock, Texas USA). The internal standard, mepivacaine, was obtained from Sigma (St. Louis, MO, USA). A 0.05M potassium phosphate (K_2HPO_4) solution was made by adding 4.36 g potassium phosphate monobasic to 500 mL RO-DI water and agitating until dissolved. The enzyme used for hydrolysis was β-glucuronidase obtained from limpets (*Patella vulgata,* G8132-1MU) from Sigma. A solution was made by adding 1 bottle to 200 mL reverse-osmosis deionized water (RO-DI) water, sourced in-house, and stirring to mix. An acetate buffer, pH 5.0 ± 0.2 was made by adding 272 g sodium acetate trihydrate (CH_3CONa) to 1800 mL RO-DI water. The pH of the acetate buffer was adjusted with 66 mL glacial acetic acid. All chemicals and reagents were ACS grade and obtained from VWR Scientific, Randor, PA USA.

3.6.2 Sample Preparation: Plasma

Using 1 mL negative sheep plasma and 1 mL RO-DI water, the calibration curve was made accordingly, with calibration points at 0.05, 0.1, 0.5, 1, 10, 50, and 100 ng/mL for fentanyl and buprenorphine. To each sample, 50 µL of each internal standard was added. Mepivacaine was used as the internal standard for buprenorphine and fentanyl. All buprenorphine, fentanyl, and mepivacaine working solutions were made in RO-DI water.

Samples were extracted by solid phase extraction (SPE) using the SPEWare CEREX48 Processor (SPEWare Corp., Baldwin Park, CA, USA). Water wettable polymer (WWP) solid phase extraction cartridges, 3 mL, (SPEWare #12-170418) were used. The SPE cartridges were

conditioned with 1 mL methanol and 1 mL RO-DI water. Samples were added to cartridges and allowed to filter through at 1-2 mL/min. Cartridges were washed with 1 mL RO-DI water and dried at full pressure (80 psi) for approximately 10 minutes. Samples were eluted with 1 mL methanol and dried to a residue under nitrogen at 45°C. Sample residues were reconstituted in 100 µL of mobile phase A (0.1% formic acid in water) prior to analysis by LC/MS (liquid chromatography/mass spectrometry). Ten microliters were injected.

3.6.3 Sample Preparation: Amniotic Fluid

All samples were centrifuged at 13,800 x g for 3 minutes before aliquoting 1 mL for extraction in order to pellet particulate matter for a cleaner extraction. The calibration curve was made using the same solutions and internal standards used in the plasma assay but using 1 mL negative amniotic fluid. The calibration curve for buprenorphine and fentanyl used the same concentrations as the plasma assay. Because amniotic fluid may contain some fetal urine, and thus phase II metabolism (glucuronidation) products, enzyme hydrolysis of the amniotic fluid samples was used to increase the recovery of the parent drug. To each sample, 0.4 mL acetate buffer (pH 5.0 ± 0.2) and 0.2 mL β-glucuronidase were added and samples vortexed to mix. The samples were heated at 60°C for 2.5 hours and allowed to cool to room temperature before proceeding to SPE.

Because of the viscous nature of amniotic fluid, UCT XTracT DAU 3 mL SPE cartridges were used (XRDAH203, United Chemical Company, Bristol, PA USA). All samples and solvents were allowed to filter through the cartridge by gravity. Using the SPEWare CEREX48 Processor, the cartridges were conditioned with 1 mL methanol, 1 mL RO-DI water, and 1 mL acetate buffer (pH 5.0 ± 0.2). Samples were applied to the cartridge and allowed to filter through. Then the cartridges were washed with 1 mL RO-DI water and 2 mL acetate buffer (pH

5.0 ± 0.2) and dried for 10 minutes at 45°C and pressure at 80 psi. Samples were eluted with 1.5 mL dichloromethane:isopropanol:ammonium hydroxide (78:20:12) and dried at 45°C. Residues were reconstituted in 100 µL of mobile phase A prior to analysis by LC/MS. Ten microliters were injected.

3.6.4 Instrumental Parameters

Samples were analyzed with a Thermo Q Exactive Plus Orbitrap LC/MS system (Thermo Instruments, San Jose, CA USA). The analytes were separated using an Agilent Eclipse Plus C18 2.1 x 50 mm, 1.8 µm column (#959757-902, Agilent Technologies, Santa Clara, CA USA). The mobile phases consisted of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). The gradient began at 5%B and increased to 95%B to 8.0 minutes with a flow of 250 µL/min, held ratio from 8.0 to 8.6 minutes, then resumed 5%B until reaching 9.3 minutes. The LC/MS used a HESI ion source in positive ion mode and a mass resolution of 17,500. Buprenorphine and fentanyl used a collision energy of 15 eV. This assay used high resolution accurate mass spectrometry (HRAMS) with 4 decimal places; buprenorphine 468.3106m/z, fentanyl 337.2271m/z, and 247.1802m/z. Fragment values for buprenorphine, fentanyl, and mepivacaine were none, 188.1430, and 98.0967, respectively.

3.7 Pharmacokinetics

Non-compartmental analysis was performed using industry standard software (Phoenix WinNonLin 8.0.0.3176, Certara, Princeton, NJ) to estimate various pharmacokinetic parameters of fentanyl or buprenorphine in plasma of each ewe and fetus. The following parameters were estimated: time of observed peak plasma drug concentration (T_{max}), observed peak drug concentration (C_{max}), apparent elimination half-life ($t_{1/2}$, calculated as $\ln(2)/\lambda_z$, λ_z being the first-

order rate constant associated with the terminal portion of the time-concentration curve as estimated by linear regression of time vs. log concentration ($AUC_{0\text{-last}}$, calculated by the linear trapezoidal rule), area under the time-concentration curve from time zero extrapolated to infinity ($AUC_{0\text{-inf}}$, calculated by adding the last observed concentration divided by λ_z to the $AUC_{0\text{-last}}$), area under the moment curve from time zero to last observed concentration ($AUMC_{0\text{-last}}$), area under the moment curve from time zero extrapolated to infinity ($AUMC_{0\text{-inf}}$), mean resident time estimated using time zero to last observed concentrations ($MRT_{0\text{-last}}$, calculated as $AUMC_{0\text{-last}}$ /$AUC_{0\text{-last}}$), and mean residence time estimated using time zero to infinity ($MRT_{0\text{-inf}}$, calculated as $AUMC_{0\text{-inf}}$ /$AUC_{0\text{-inf}}$).

3.8 Data Analysis

Data was compared using a two-way ANOVA with a Fisher's LSD test and trends were observed using linear regression (Prism 7, GraphPad Software Inc., La Jolla, CA). Significance was set at $p < 0.05$. Pharmacokinetic parameters are presented as median (range) while other data is presented as mean ± SD.

4. RESULTS

4.1 Analgesic Effects

There was no significant difference in the overall postoperative total pain scores averaged across time points between FENT (1.6 ± 0.4) and BUP groups (2.3 ± 1.3) (Figure B-2). However, pain scores from BUP tended to be higher than FENT (p=0.06) and were significantly higher than their preoperative value (t=-24) at 4, 12, 24, 36, and 48 hours. Specifically, when the ewes had returned to their pen following recovery (t=4), those in BUP group had significantly higher pain scores (BUP = 4 ± 3.2 and FENT 1 ± 1.3) (p=0.01) and heart rates (BUP= 129 ± 22.2bpm and FENT = 101.4 ± 22.4bpm) (p=0.04) than FENT ewes. FENT ewes consumed more feed (73.3 ± 24.4%) on the day of surgery compared to BUP ewes (42.0 ± 35.9%) (p=0.0396); however, both treatment groups showed significant decreases in feed consumption postoperatively compared to preoperative consumption (Figure B-3). All sheep completed the study without requiring administration of rescue analgesia. Sedation scores did not differ between treatment groups (FENT score = 0.57 ± 0.5 and BUP score = 1.0 ± 1.0), and neither group had a significant change in sedation after analgesic administration.

4.2 Intra-Anesthetic Effects

BUP ewes exhibited significantly higher intraoperative heart rate (BUP = 113 ± 5.4bpm and FENT = 91.3 ± 4.7bpm) and mean arterial pressure (BUP= 85 ± 4.8mmHg and FENT = 72.7 ± 8.3mmHg) than FENT ewes ($p<0.0001$ and $p<0.001$ respectively) (Table A-3). End-tidal isoflurane concentration also was significantly higher in BUP (1.4 ± 0.3) than FENT (1.1 ± 0.2) (p=0.0001). Treatment group did not influence average surgery duration (96.5 ± 4.24 minutes)

and all surgeries proceeded without complications. On average, BUP ewes took an additional 24.33 ± 10.62 minutes to be extubated (p=0.0269). The time from extubation to standing was also 30.6 ± 11.63 minutes longer in the BUP group (p=0.0118) (Figure B-3). It was noted that four of the six fentanyl-treated ewes exhibited excitatory behavior during recovery, but light manual restraint was sufficient to control them.

4.3 Pharmacokinetic/ Plasma Sample Analysis

Median estimated pharmacokinetic parameters in ewes and fetuses for buprenorphine (n=6) and for fentanyl (n=5) are presented in Table A-4 & A-5. Parameters could not be calculated for three of the fetuses for buprenorphine because of the relatively unchanging concentrations of drug over time, and thus were excluded from the pharmacokinetic values reported. One ewe in the fentanyl group showed atypical time-concentration curve and thus was not included in the median estimated parameters, but the values are reported separately (Table A-5). Mean concentrations of both drugs from ewe and fetal plasma are outlined in Figure B-4 & B-5.

4.4 Fetal Effects

The heart rate, mean arterial pressure, and blood gas chemistries did not differ significantly between treatment groups and values represented fetal viability. In both groups, higher fentanyl or buprenorphine concentration moderately correlated with lower heart rate values (p=0.0188 and p=0.0209, respectively) (Figure B-6). The average fetal plasma buprenorphine concentration was 0.06 ± 0.04ng/ml, and it was significantly lower than the maternal concentrations at 4 and 8 hours (p=<0.0001 and p=0.0022) and remained lower

throughout the study but of no significance. The fetal plasma fentanyl concentration, average 0.34 ± 0.11ng/ml, was significantly lower than the maternal concentration throughout the postoperative period (Figure B-4 & B-5). Regarding the amniotic samples, buprenorphine was detected in all ewe amniotic samples, with average concentration 8.48 ± 9.31ng/ml (Figure B-7). Fentanyl was only detected in three ewes with an average concentration of 1.03 ± 0.82ng/ml (Figure B-8).

5. CONCLUSION

Results of this study indicated the TFP provided equivalent to superior analgesia compared to buprenorphine, without noticeable adverse effects, suggesting fentanyl via transdermal administration could be considered as a viable option for perioperative pain management in pregnant sheep. Furthermore, we were able to evaluate how these drugs impacted anesthesia and measure the maternal and fetal drug concentrations.

5.1 Analgesia

Pain is defined as an unpleasant sensory and emotional experience, associated with actual or potential tissue damage (Merskey, 1979). Antinociceptive properties of the analgesic agents were assessed utilizing a composite pain score system previously utilized in other sheep studies (Musk et al., 2017; Musk et al., 2014; Stasiak et al., 2003). This score system recognizes pain as a multidimensional experience; incorporating behavior, physiology, and productivity to detect and capture the intensity of pain. The use of pain scores, or any scaled assessment, is inevitably imperfect due to atypical animal housing, interobserver variability, and subjective scoring. Because it is difficult to provide an environment that allows normal flock behavior, an evaluation of "normal" was attempted by obtaining baseline scores 24 hours before surgery in the same environment where they would be kept for the duration of the study. Furthermore, in an effort to increase consistency, the same observer scored all the animals through the study, and objective parameters, such as ewe vital signs, feed intake, and response to palpation of the wound, were also measured.

In the current study, both analgesic agents' regimens exceeded the minimum effective concentration that has been extrapolated from human studies for its use in sheep (Jen et al., 2017;

Chiara Zullian et al., 2016). However, the TFP appeared to have a more profound analgesic effect, which is consistent with the findings of a study using nonpregnant sheep (Ahern, Soma, Boston, & Schaer, 2009). Ewes in the buprenorphine group may have had a ceiling analgesic effect related to the partial agonist activity, as demonstrated by the higher post-operative pain scores compared to pre-operatives scores. In comparison, fentanyl did not have a ceiling effect, which may explain why postoperative pain scores in fentanyl-treated ewes did not differ from their preoperative values.

To better confirm that the observed abnormal behavior was related to pain, the current study attempted to characterize the nociceptive properties of the analgesic agents at a given plasma drug concentration, which has not previously been reported. The observed relationship between maximum drug concentration and the lowest recorded postoperative pain score for each treatment group substantiates the use of this pain score as a reliable means of capturing and measuring pain in this species. Furthermore, as none of the sheep received rescue analgesia, even when the plasma drug concentrations were at their lowest, we can speculate those concentrations are associated with analgesia in sheep. Plasma concentrations associated with pain relief in sheep have not been established, and although further research is warranted, these findings could be used in comparison with estimates extrapolated from human studies to better understand the therapeutic range of these agents in sheep.

5.2 Pharmacokinetics

Plasma buprenorphine concentration in the ewe was within the suggested therapeutic range one hour after administration and remained above throughout the post-operative period. The observed lagged absorption and variability in Cmax can be expected with intramuscular

administration. The profile of intravenously administered buprenorphine in non-pregnant sheep has previously been described; however, the lack of parallelism and limited parameters reported makes it difficult to directly compare the studies (Nolan, Livingston, & Waterman, 1987). Overall, both studies showed a rapid decline in plasma concentration after reaching Cmax, followed by a slower, steadier phase. These profiles indicate that buprenorphine is rapidly distributed throughout the body before reaching steady-state.

The reported plasma fentanyl concentrations surpassed that which has been extrapolated from humans for analgesia in sheep, 0.5 ng/mL, within 24 hours of application and was maintained for 72 hours after patch application (Ahern et al., 2009; Musk et al., 2017). The observed Tmax supports the suggested optimal time for patch placement to be between 12 and 36 hours prior to surgery (Christou, Oliver, Rawlinson, & Walsh, 2015; Musk et al., 2017). The higher intra-operative fentanyl concentrations observed in this study are consistent with previous findings and could be the result of an increase in absorption and/or a decrease in elimination from anesthetics (Heikkinen et al., 2015), though this is unlikely since a similar study using a TFP on the dorsal thorax of ewes without anesthesia obtained similar concentrations to those achieved in our study (Jen et al., 2017). Although there was not a significant difference in the plasma fentanyl concentrations obtained at induction and its peak two hours later, fentanyl absorption during anesthesia could have been increased due to additional pressure on the patch from the ewe lying in dorsal recumbency while anesthetized.

The highly lipophilic nature of fentanyl facilitates transdermal administration, but as such, drug distribution and the resulting plasma fentanyl concentrations is highly variable in sheep studies (Ahern, Soma, Rudy, Uboh, & Schaer, 2010; Heikkinen et al., 2015; Musk et al., 2017). Transdermal delivery systems, like the one used in this and previous studies, store the

drug in a reservoir to develop a concentration gradient, facilitating movement of the drug through the skin and into circulation. The anatomical site of TFP application can impact the absorption of fentanyl because the permeability of the skin is affected by the composition of the skin, temperature, skin blood flow, and preexisting damage to the skin (Lane, 2013). Sheep skin is composed of the epidermis, dermis, and hypodermis. Within the epidermis is the highly lipophilic stratum cornuem that acts as a second depot for drug absorption. In sheep, the stratum corneum ranges from 2-30μm thick depending on the hydration status of the animal, allowing for variability in absorption (Jen et al., 2017). In addition, fat composition within the hypodermis is patient specific, and it affects the volume of distribution, and thus the elimination of drugs, further contributing to the inter-individual pharmacokinetic variability of transdermal drugs.

We placed the TFP on the dorsal thorax region, at the withers, because the skin temperature and pressure on the patch at that site would remain relatively constant regardless of the sheep's position postoperatively, and it could not be easily manipulated by the sheep. This site was previously utilized in a study using TFP on non-pregnant sheep, and the pharmacokinetic parameters reported parallel closely with those found in the current study (Jen et al., 2017). Other published pharmacokinetic data for TFP in sheep vary considerably. Many studies applied the patch to the ewe antebrachium and reported faster transdermal absorption rates yet lower plasma fentanyl concentrations (Ahern et al., 2010; Heikkinen et al., 2015; Musk et al., 2017). The apparent differences in transdermal absorption rate and elimination rate between antebrachium applied and dorsal thorax applied patches may be explained by the skin composition at those sites. Sheep body condition is scored based on fat and muscle composition along the vertebrae, indicating excess body fat is deposited along the back. Adipose tissue sequesters lipophilic drugs and could explain the longer time it took for fentanyl to reach

systemic circulation and be eliminated from the body when the patch was applied in the dorsal thorax region.

The method of sample analysis is an additional variable that should be taken into consideration when comparing plasma fentanyl concentrations between studies. In the current study, LC-MS analysis was used to detect fentanyl and buprenorphine within the fluid samples, but not all studies utilize this method because of the cost associated and the limited ability to process samples within the lab. Instead, labs choose to utilize commercially available human fentanyl ELISA kits, which are known to cross-react with drug metabolites. A fentanyl pharmacokinetic study on sheep utilized both methods of sample analysis for comparison and found that although the data generated from the ELISA correlated with the LC-MS data, the absolute values were lower than those from LC-MS (Jen et al., 2017). The resulting pharmacokinetic parameters from the LC-MS analysis in that study are similar to the values reported here.

5.3 Fetal

Transplacental passage of drugs depends on placental structure and drug pharmacology. A human in vitro study found that less than 10% of the maternal buprenorphine dose was transferred to the fetus and that initial transplacental transfer was minimal due to placental tissue sequestering (T. Nanovskaya, Deshmukh, Brooks, & Ahmed, 2002). Following initial buprenorphine administration, maternal concentrations increased to a peak before declining to a plateau 12 hours after. Buprenorphine concentrations in the fetus did not follow this trend; instead, their levels remained steady throughout treatment. Therefore, an initial rate, 2%, and plateau rate, 8%, was calculated to describe the placental transfer of buprenorphine. Since

fentanyl is of similar solubility and also metabolized by CYP34A, it can be assumed that it is metabolized and sequestered in the placenta to some extent as well (T. Nanovskaya et al., 2002). Transplacental fentanyl studies using a sheep model have shown fetal concentrations to range from 50% to 80% that of the maternal concentration (Heikkinen et al., 2015; Musk et al., 2017). Throughout the current study, the fetal-to-maternal fentanyl concentration ratios at given time points ranged from 13% to 25%. The fetal fentanyl values fell into similar ranges reported in previous sheep studies, and fentanyl accumulation was also not observed (Heikkinen et al., 2017; Musk et al., 2017). Therefore, the lower ratios observed in this study are likely due to the higher maternal fentanyl concentrations. This suggests a transplacental rate limiting step is involved in the cross of fentanyl (Sastry, 1995).

Distribution in the fetal compartment relies on the drug composition. Both drugs are highly lipid soluble and readily cross the placenta where they are equally distributed throughout fetal tissue. However, the two agents differ in their protein affinity; buprenorphine primarily binds to glycoproteins while fentanyl primarily binds to albumin (T. N. Nanovskaya, Bowen, Patrikeeva, Hankins, & Ahmed, 2009; Wiesner, Taeger, & Peter, 1996). Fetal urine is excreted into the allantoic cavity, which has been found to be highly concentrated with plasma proteins, specifically albumin (Wales & Murdoch, 1973). This would allow fentanyl to bind and become trapped in the allantoic fluid, preventing diffusion into the amniotic cavity, possibly explaining why fentanyl was not detected in all amniotic samples. Buprenorphine binds to alpha and beta globulins; however, the presence of those plasma proteins in allantoic fluid is minute. Therefore, buprenorphine could have readily passed into the amniotic cavity, where the fluid is highly saturated with globulin proteins (Tong et al., 2009). The molecular weight of buprenorphine may have also prevented any intramembranous reabsorption from amniotic fluid back into fetal

circulation, further adding to accumulation within amniotic fluid (Thorburn, 1994). This in combination with protein binding could explain why the detected buprenorphine concentration in amniotic fluid was so high; however, further investigation is needed.

The novel amniotic catheter proved to be an effective technique for collecting amnion and remained functional throughout the study. Buprenorphine was detected in all amniotic samples from the respective ewes, yet fentanyl was detectable in only three of the six ewes' amniotic fluid. The concentrations for both drugs were sporadic, showing no clear correlation or pattern. Although no literature is available on buprenorphine, to the authors' knowledge, there have been two human studies attempting to characterize fentanyl in amniotic fluid (Cooper, Jauniaux, Gulbis, Quick, & Bromley, 1999; Shannon et al., 1998). Those studies showed similar concentration variability in the detected samples and also had patients with undetected fentanyl concentrations in the amnion.

Recorded fetal heart rate and blood pressure suggests the fetuses were stable, and these parameters did not differ based on the analgesic administered to the ewe. The correlation between drug concentration and fetal heart rate is consistent with findings in human studies, showing fentanyl or buprenorphine exposure to increase the risk of fetal heart rate deceleration (Gaiser, McHugh, Cheek, & Gutsche, 2005; Ngamprasertwong et al., 2016).

5.4 Anesthetic Monitoring

Analgesic agent appeared to influence the anesthetized ewe's cardiovascular system, yet the recorded values remained in adequate ranges and were similar to those reported in pregnant ewes under similar conditions (Mohamadnia, Hughes, & Clarke, 2008). A previous study comparing intramuscular buprenorphine to TFP applied 12 hours before surgery, did not find any

intraoperative differences based on treatment group (Ahern et al., 2009); while this report did measure plasma fentanyl concentrations, it is presumed that the fentanyl concentrations in the current study were higher in the anesthetized ewe's circulation, and thus a had greater impact on anesthesia. Ewes in both groups achieved cardiovascular stability, suggesting that buprenorphine and fentanyl both provided analgesia during the surgical procedure. Similar hemodynamic values have been reported in sheep following fentanyl administration intravenously and through the patch system (Funes et al., 2015; Lepiz et al., 2017).

Fentanyl-induced isoflurane MAC sparing effects were previously reported in sheep and are consistent with the current findings (Funes et al., 2015). The previous report did not record recovery times; however, in the current study, ewes receiving fentanyl were also extubated and standing sooner than those treated with buprenorphine. Because they required less anesthetic, rapid recovery may be explained by a relatively faster wash out of isoflurane from the ewe's body. It was also noted that several fentanyl-treated ewes appeared to experience excitation during recovery; however, the recovery qualities were clinically acceptable and this observation should just be a precaution when using the transdermal fentanyl patch for invasive surgical procedures.

5.5 Clinical Application

In veterinary medicine, drug availability, financial costs, and administration protocol associated with treatment plays a role in designing perioperative regimens. Because both fentanyl and buprenorphine are controlled substances, schedule II and III classes, respectively, the availability of these drugs may be a concern. In addition, fentanyl's high potential for abuse is a liability the clinician should take into consideration. In the current study, postoperative

treatment with buprenorphine averaged $241.47 per ewe and transdermal fentanyl averaged $57.89. Using transdermal fentanyl would decrease the price of analgesic treatment by almost 75%, while providing adequate analgesia. Another important consideration when choosing an analgesic regimen is the amount of animal handling required, as handling adds additional stress to the animal and increases time and labor required for patient care. The fentanyl patch required extra skin preparation and bandaging materials for application, yet no additional handling was needed through the postoperative period; this could be particularly beneficial in a clinical setting when an animal is ready to be discharged but still needs pain medication. In contrast, buprenorphine administration necessitated restrain of the ewe and administration of an intramuscular injection every eight hours, for 48 hours.

5.6 Summary

In conclusion, transdermal fentanyl patch application to the dorsal thorax region 24 hours before surgery required less time, labor, patient handling, and costs than buprenorphine. It provides a noninvasive method of continuous analgesic administration while maintaining adequate plasma fentanyl concentrations, with intra and post-operative analgesic effects equivalent, or superior, to those produced by buprenorphine. Fetal opioid's exposition is higher with fentanyl; however, no cardiovascular side effects were detected with either analgesic. Further research is necessary to follow up later potential pre and postnatal effects. Furthermore, additional considerations concerning anesthetic technique, patient pregnancy, costs, and animal handling should be taken into account when designing perioperative analgesic regimens. The use of composite pain scoring system coincides with opioid maternal plasma levels.

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APPENDIX A

PAIN SCORE CATEGORIES	
Comfort	
0	Awake and alert, interested in surroundings
1	Dull, not very interested in surroundings, rises readily when approached
2	Depressed, not at all interested in surroundings, lethargic, ears drooped, not chewing cud, teeth grinding
3	Recumbent, no response when approached, fixed look and staring or eyes half closed, little response when gently prodded
Movement	
0	Standing or gets up from sitting as soon as approached, full weight bearing
1	Does not get up promptly when approached, able to stand
2	Standing and walking with assistance
3	Recumbent, unable to stand
Palpation of Wound	
0	Normal in posture, no response to palpation of abdominal wound
1	Slight tucking of abdomen, slight flinching of the skin and abdominal muscle when gently palpating the wound, turns head
2	Moderate tucking up of abdomen, moderate flinching of the skin and abdominal muscle when gently palpating the abdominal wound, attempts to walk away
3	Severe tucking up of abdomen, guarding wound, kicks, abdominal muscles very tense, attempts to walk away
Feeding/appetite	
0	Eating and drinking normally, ruminating and normal rumen sounds
1	Some decrease in food and water intake, ruminating
2	Minimal food and water intake, quiet/infrequent rumen sounds
3	Not eating or drinking at all, no rumination
Posture:	
Standing	
0	Normal standing/walking
1	Slightly abnormal standing/walking
2	Extremely abnormal standing/walking
3	'Statue standing' immobile standing with an obvious withdraw from interaction
Sternal	
O	Sternal lying, head up
1	Sternal lying, head down
2	Abnormal lying, partial leg extension
3	Abnormal lying, full leg extension
Lateral	
2	Lateral position, head up
3	Lateral position, head down

Table A-1. Pain score parameters for pregnant ewes.
Categorical scores were added together for a total pain score, maximum of 15.

	SEDATION SCORE VARIABLES
0	Standing, alert, normal behavior
1	Standing, alert, reduced head and ear movements
2	Standing, slight head drop
3	Standing, moderate head drop
4	Standing, severe head drop, ataxia
5	Standing, severe head drop, severe ataxia (stumbling)
6	Sternal recumbency, head up
7	Sternal recumbency, unable to support head
8	Lateral recumbency, occasional attempts to obtain sternal recumbency
9	Lateral recumbency, uncoordinated head and leg movements
10	Lateral recumbency, no movements

Table A-2. Sedation score system for pregnant ewes.

Variable	*Buprenorphine*	*Fentanyl*
*MAP (mmHg)**	85.0 ± 4.79	72.7 ± 8.28
*HR (bpm)**	113 ± 5.37	91.3 ± 4.75
*etCO2 (%)**	43.5 ± 1.68	40.3 ± 2.98
SpO2 (%)	93.8 ± 1.61	94.5 ± 1.39
*Iso (%)**	1.42 ± 0.28	1.16 ± 0.23

Table A-3. Intraoperative data obtained from anesthetized sheep.
Ewes were treated for pain with buprenorphine, IM, (n=6) or transdermally administered fentanyl (n=6). Data is presented as mean ± SD and * denotes $p<0.05$ between treatment.

Parameters	Maternal	Fetal
T_{max} *(hr)*	4.5 (26-32)	5 (28-32)
C_{max} *(ng)*	3.62 (0.5-17.9)	0.07 (0.06-0.12)
Λz (/hr)	0.01 (0.01-0.03)	0.002 (0.002-0.01)
$t_{1/2\lambda z}$ *hr*	60.0 (26.3-121)	197 (78-281)
$AUC_{0\text{-}last}$ *(hr*ng/mL)*	132 (32.4-381)	8.1 (2.8-18.3)
$AUC_{0\text{-}inf}$ *(hr*ng/mL)*	134 (70.7-385)	28.1 (19.8-35.1)
AUC % Extrap (%)	24.1 (1.1-66.4)	47.8 (31.9-69.1)
MRT_{last} *(hr)*	299 (33.4-905)	X
$MRT_{0\text{-}inf}$ *(hr)*	300 (33.6-901)	646 (322-1388)

Table A-4. Pharmacokinetic parameter estimates of buprenorphine in pregnant ewes. Values are represented as median (range). X denotes values that were not calculated.

Parameters	Maternal	Fetal	Atypical Ewe	Atypical Fetus
T_{max} *(hr)*	26 (24-72)	32 (28-60)	48	12
C_{max} *(ng)*	4.15 (2.1-7.9)	0.72 (0.5-0.8)	0.3	0.1
Λz (/hr)	0.03 (0.017-0.049)	0.04 (0.01-0.09)	X	X
$t_{1/2\lambda z}$ *hr*	21.5 (14.3-40.2)	18.8 (8.1-38.8)	X	X
$AUC_{0\text{-}last}$ *(hr*ng/mL)*	180 (81.4-326)	23.0 (15.8-35.7)	3.4	0.2
$AUC_{0\text{-}inf}$ *(hr*ng/mL)*	211.6 (186-331)	34.4 (21.6-54.2)	X	X
AUC % Extrap (%)	5.19 (1.7-18.2)	9.5 (0.8-53.9)	X	X
MRT_{last} *(hr)*	44.9 (41.1-61.0)	49.1 (31.1-60.6)	56	0.2
$MRT_{0\text{-}inf}$ *(hr)*	55.7 (43.9-64.9)	61.7 (33.8-101.2)	X	X
$AUMC_{0\text{-}last}$ *(hr*ng*ng/ml)*	7626 (9124-19857)	1168 (608-2162)	188	8
$AUMC_{0\text{-}inf}$ *(hr*ng*ng/ml)*	11083 (9248-21007)	2215 (730-4186)	X	X

Table A-5. Pharmacokinetic parameter estimates of fentanyl in pregnant ewes.
Values are represented as median (range). X denotes values that were not calculated.
Ewe and fetus with atypical time-concentration curve were excluded from group analysis.

APPENDIX B

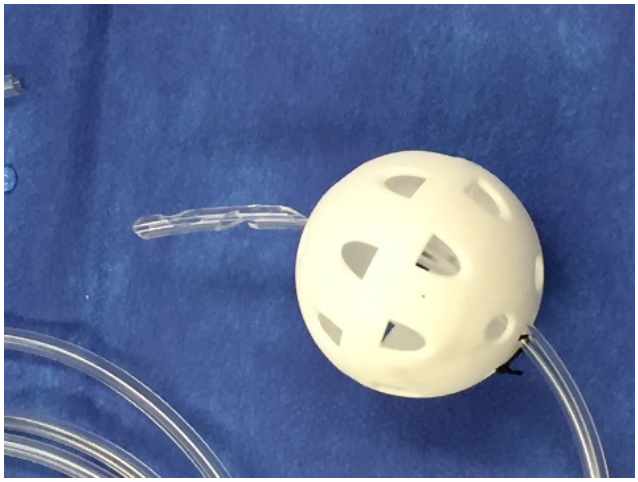

Figure B-1. Catheter used for collecting amniotic fluid.

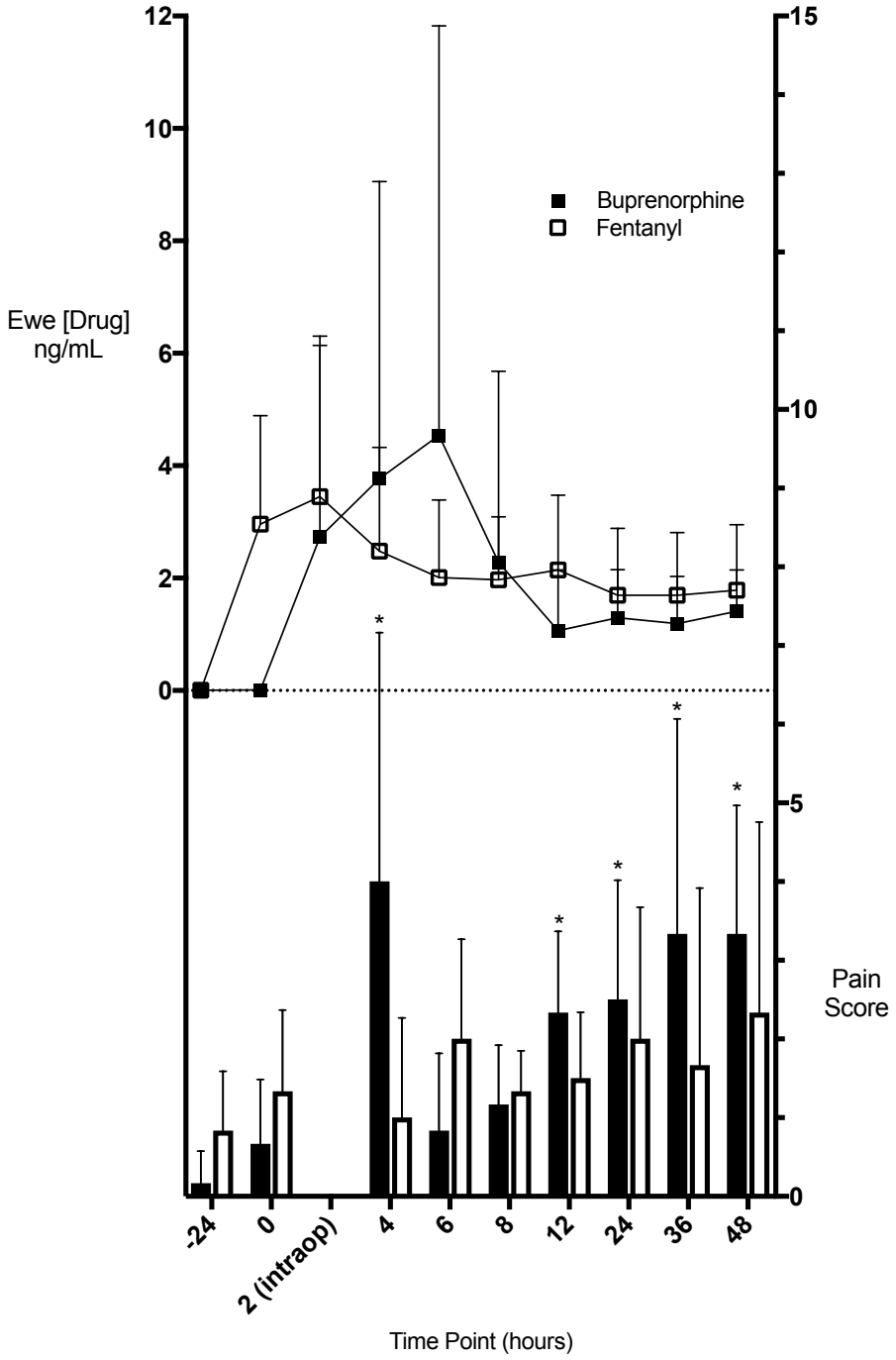

Figure B-2. Ewe plasma drug concentration with parallel pain score at each time point.
Top: Ewe mean ± SD plasma concentration of buprenorphine and fentanyl.
Bottom: Mean total pain score for each treatment group. Ewes were anesthetized during time 2, so pain score was not assigned. * denotes significant difference, $p<.05$, from preoperative value (t=-24)

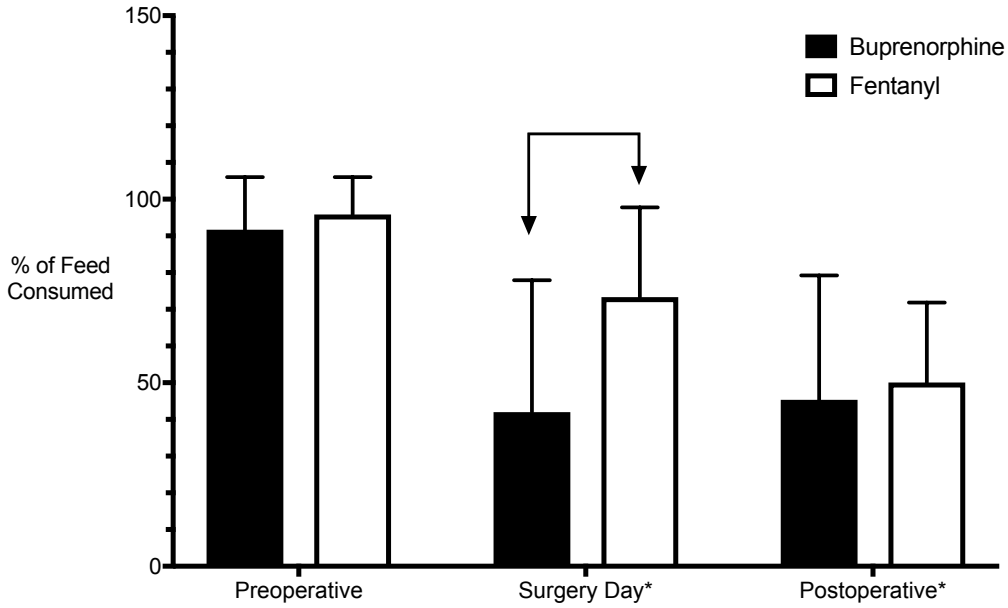

Figure B-3. Average feed consumption of pregnant ewes.
Data is shown as a percent of feed consumed from what was available to the ewe. Preoperative and postoperative values are averaged over two days. *denotes significant difference, $p<0.05$, from preoperative value. Bracket denotes significant difference, $p<0.05$, between treatment groups.

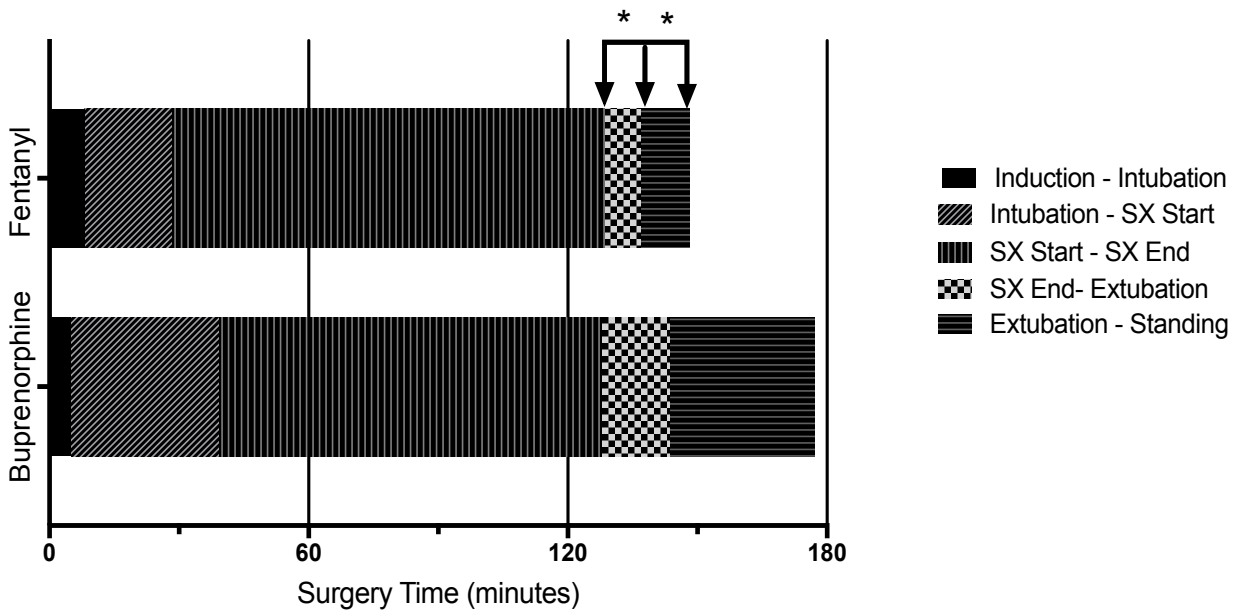

Figure B-4. Average times between designated surgical markers.
*denotes significant difference, $p<0.05$, between treatment groups.

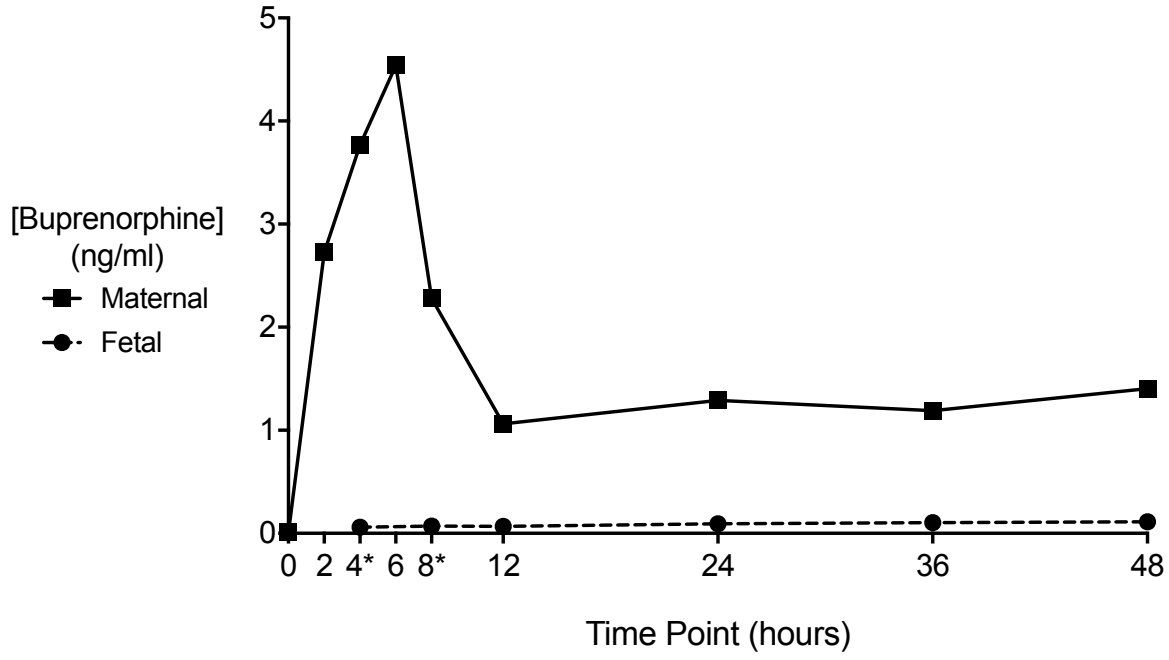

Figure B-5. Ewe and fetus mean serum concentration of buprenorphine.
* denotes significant difference, $p<0.05$, between maternal/fetal plasma drug concentration.

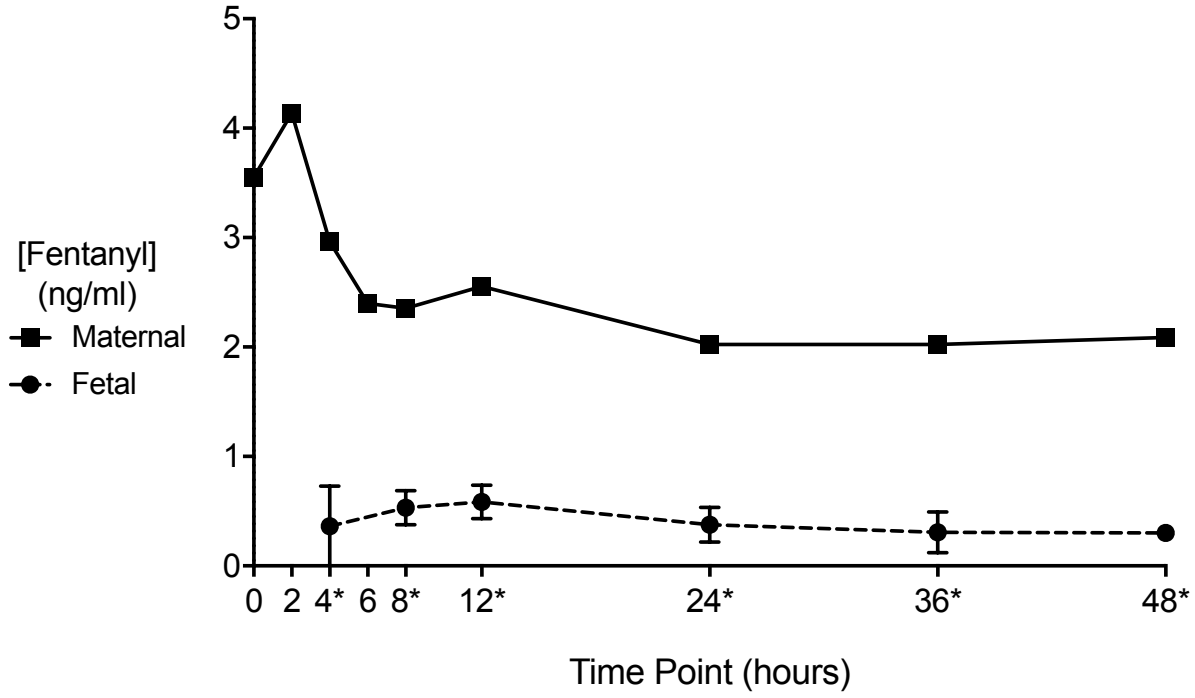

Figure B-6. Ewe and fetus mean serum concentration of fentanyl.
* denotes significant difference, $p<0.05$, between maternal/fetal plasma drug concentration.

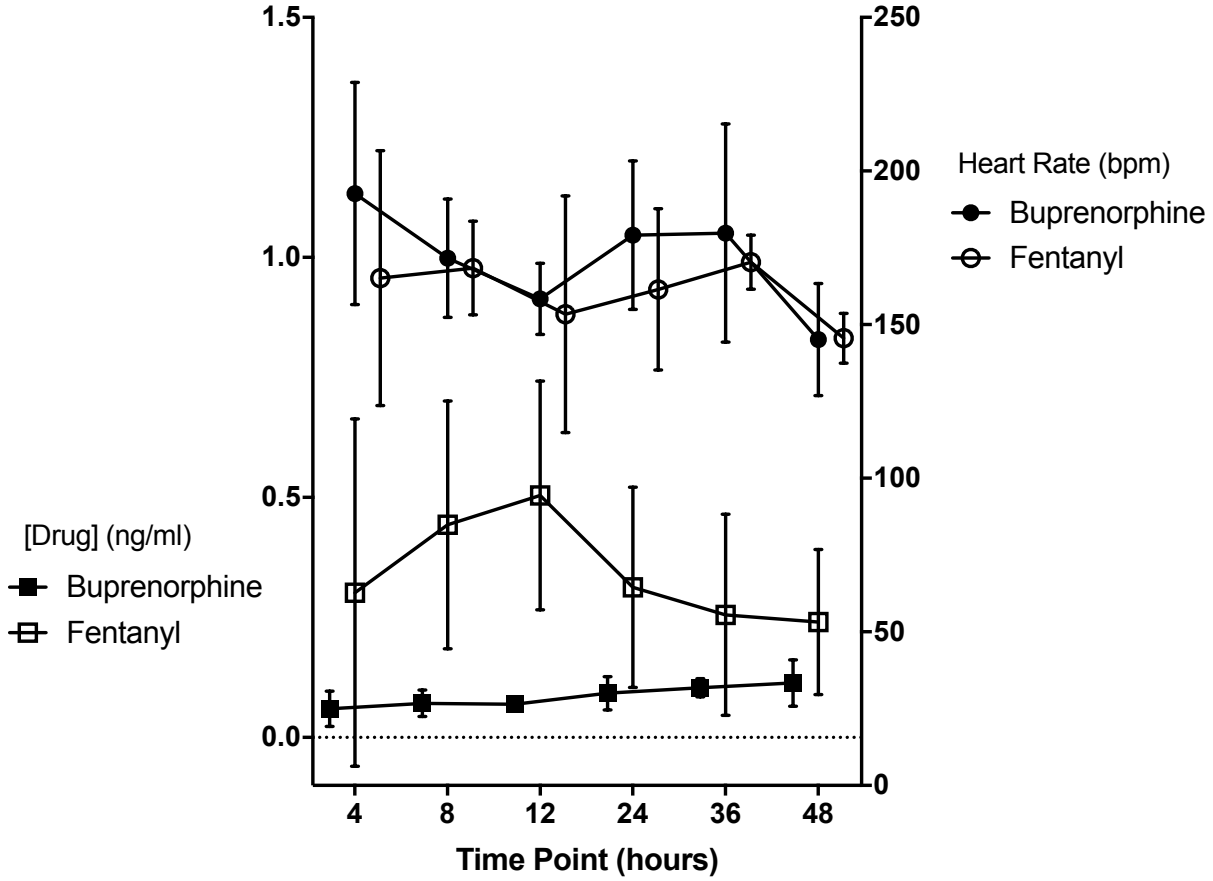

Figure B-7. Fetal heart rate in correlation with fetal plasma drug concentration.
Top: Fetal heart rate, mean ± SD.
Bottom: Fetal serum concentration, mean ± SD, following buprenorphine or fentanyl administration in pregnant ewes.

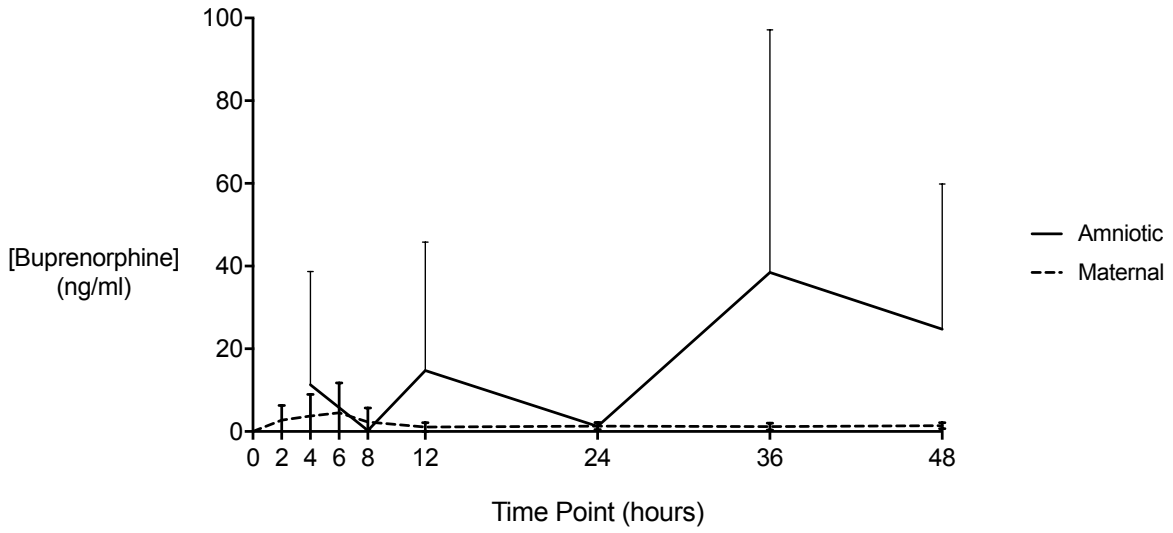

Figure B-8. Amniotic fluid and ewe plasma buprenorphine concentration.

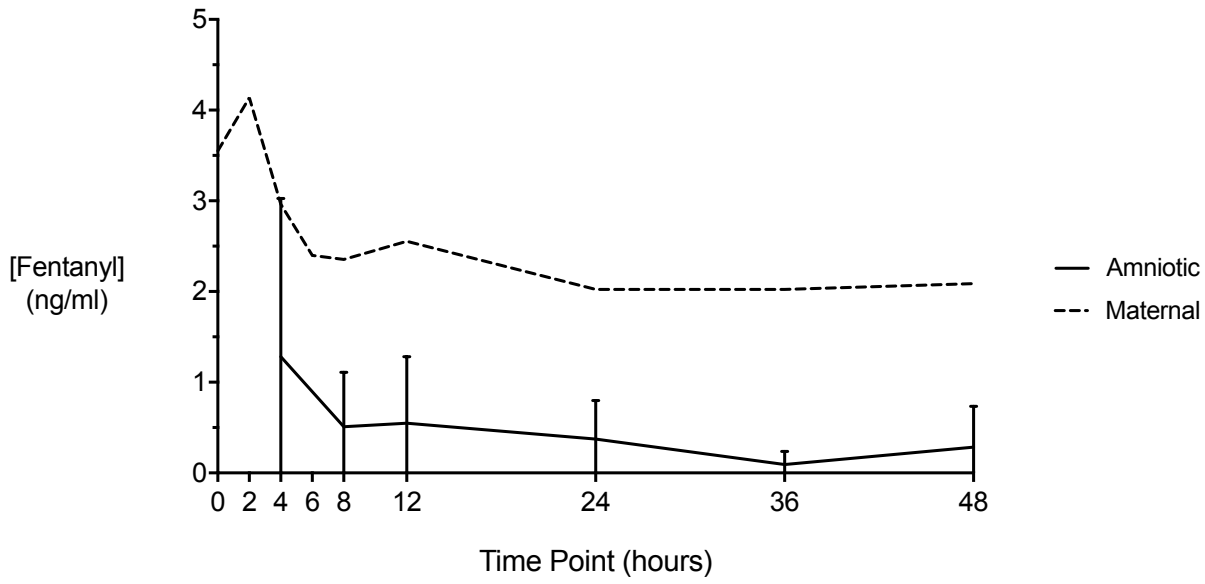

Figure B-9. Amniotic fluid and ewe plasma fentanyl concentration.