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Assessment of sedation and cardiorespiratory variables in sheep using different anesthetic protocols during artificial insemination

Avaliação da sedação e variáveis cardiorrespiratórias em ovinos submetidos a diferentes protocolos de anestesia durante a realização da inseminação artificial

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Highlights

Acepromazine with meperidine promoted greater cardiovascular instability.
Acepromazine alone promoted mild sedation.
Sedation protocols reduced agitation at times of higher pain stimulus.

Abstract

Artificial insemination (AI) is an important technique in sheep breeding. Since it is an invasive procedure, sedation and analgesia are essential. In the present study, 75 Texel sheep, standardized for weight (kg) and age (months) were used. The animals were randomly allocated to five groups: Acepromazine Group (AG); Acepromazine and Butorphanol Group (ABG); Acepromazine and Morphine Group (AMG); Acepromazine and Meperidine Group (AMRG) and Saline group (SG). The following parameters were assessed: sedation score, agitation level, heart rate (HR); respiratory rate (*f*), rectal temperature (T°R), latency time and time to perform artificial insemination (AI). Assessments times were M-20, M0, M1 and M2. Significant differences were considered when $p<0.05$. There were no significant differences for weight and age. HR increased by 30.6, 34.2 and 42.5% from M-20 to M2 for the AG, ABG and AMRG, respectively. At M0, the AMRG obtained higher values, reaching 41.4% above the other groups. For *f* there was a decline of 21.8 and 26.9% in M1 in relation to M-20 for the AMRG and AMG, respectively, and a decrease of 20 and 25% between M-20 and M2. A comparison between SG *f* values showed an increase of 106.3 and

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68.8% between M-20 and M1 and M2, respectively. The f values obtained at M1 and M2 for the SG were higher than those of the other groups. Although there were no intergroup differences in sedation level, the values obtained are clinically relevant. There were intergroup differences in agitation level, whereby at M1, AMRG and AMG obtained a score of 2 and SG 0, while at M2, AMG scored 2 and SG 0. There were no statistical differences for latency and AI times. Thus, under the conditions of the present study, the AMRG protocol was the least safe among the options assessed. The pre-anesthetic medication protocols promoted mild sedation and did not reduce the time required for AI.
Key words: Artificial insemination. Sheep. Sedation protocols.

Resumo

A inseminação artificial (IA) é um importante técnica para melhoramento genético em ovinos. Em função de ser uma técnica invasiva, a tranquilização e analgesia são fundamentais. No presente estudo foram utilizados 75 ovinos, da raça Texel, padronizados quanto a peso (kg) e idade (meses). Os animais foram distribuídos aleatoriamente em cinco grupos: Grupo Acepromazina (GA); Grupo Acepromazina e Butorfanol (GAB); Grupo Acepromazina e Morfina (GAMF); Grupo Acepromazina e Meperidina (GAME) e Grupo Salina (GS). Os parâmetros avaliados foram: escore de sedação, grau de agitação, frequência cardíaca (FC); frequência respiratória (*f*), temperatura retal (T°R), tempo de latência e tempo para realização da IA. Os momentos de avaliação foram: M-20, M0, M1 e M2. Diferenças significativas foram consideradas quando $p<0,05$. Não houveram diferenças significativas para peso e idade. Com relação a FC observou-se um aumento de 30,6%, 34,2% e 42,5% de M-20 para M2 para os grupos GA, GAB e GAME, respectivamente. Entre grupos, observou-se que em M0 o grupo GAME apresentou valores superiores, chegando a um valor de 41,4% acima dos demais grupos. Para a *f* destaca-se uma diminuição de 21,8% e 26,9% em M1 com relação a M-20 para os grupos GAME e GAMF, respectivamente. Já entre M-20 e M2 para GAME e GAMF destaca-se uma diminuição de 20% e 25%, na devida ordem. Quando comparados os valores de f do grupo GS, observa-se que entre o M-20 e os momentos M1 e M2 houve um aumento de 106,3% e 68,8% respectivamente. Os valores de *f* obtidos nos momentos M1 e M2 para GS foram maiores que os demais grupos. Quanto ao grau de sedação não houveram diferenças entre os grupos, contudo os valores obtidos são de relevância clínica. Com relação ao grau de agitação, houveram diferenças entre grupos, em M1 os grupos GAME e GAMF pontuaram 2, enquanto GS pontuou 0, em M2 o grupo GAMF pontuou 2 e o grupo GS 0. Para o tempo de latência e tempo para realização da IA não houveram diferenças estatísticas. Assim, nas condições do presente estudo o protocolo em GAME apresentou-se como o menos seguro dentre as opções avaliadas. Os protocolos instituídos de medicação pré-anestésicas promoveram discreta sedação dos pacientes e não diminuíram o tempo para a realização da técnica de IA.
Palavras-chave: Inseminação artificial. Ovelha. Protocolos de sedação. Texel.

Introduction

Sheep farming has enormous potential for growth and expansion in meat and wool production (Lizarraga & Chamber, 2012). For this growth to continue, reproduction techniques such as artificial insemination (AI) have been increasing to ensure faster breeding.

Advantages of AI include faster animal production, improved weight gain, decline in venereal disease transmission, shorter interval between generations, added zootechnical value to the herd, increased profitability and maximum use of high-quality breeding animals, obtained by diluting the ejaculate collected for semen processing. Despite these advantages, it is important to note that a limitation is the low fertility index of sheep submitted to AI (Feranti et al., 2013).

In ewes, especially the Crioulo Lanada breed, cervical anatomy is a barrier that precludes their large-scale use in transcervical intrauterine AI, due to the difficulty in passing the insemination pipette through the four to eight funnel-shaped rings (Ramiro et al., 2017). The closer the fertilization site for semen deposition, the higher the pregnancy rate. Thus, good AI results are achieved when semen is deposited directly into the uterus, using laparoscopy (Ramiro et al., 2017). Laparoscopic artificial insemination is currently applied using two or three skin incisions (previously anesthetized with lidocaine and no vasoconstrictor at the access points), with subsequent use of a coil catheter for complete access to install the optics and one or two working points (Feranti et al., 2013).

The low laparoscopic AI success rate may be related to the invasiveness of the technique, the longer time required for its execution and the need for a highly qualified professional (Padilha et al., 2011). In addition to the need for physical containment, maintaining the animal in the Trendelenburg position increases the stress response and creates risks such as carbon dioxide insufflation, which may lead to important hemodynamic alterations in the animal (Alvares et al., 2015). Elevating the animal´s pelvic region may favor serious hypoventilation and regurgitation, with aspiration of food content that may result in death (Alvares et al., 2015). Without administering sedatives or tranquilizers, it is impossible to control the animal`s agitation, thereby lengthening the AI procedure (Padilha et al., 2011).

Thus, in order to avoid stress, pre-anesthetic drugs can be used to reduce anxiety, promoting mild to moderate sedation and muscle relaxation, as well as pre, trans and post AI analgesia. It is believed that controlling acute stress through pharmacological intervention will make the animal less agitated and keep them immobile, allowing AI to be successfully applied (Musk & Wilkes, 2018).

The use of popular veterinary drugs, such as acepromazine and phenothiazine, may reduce acute stress, mediated by the sympathetic autonomic nervous system. The recommended dose for small ruminants is 0.05mg kg^{-1}, promoting calmness and relaxing muscles, albeit not exhibiting analgesic properties (Lemke, 2013). Despite the tranquilizing effects, the competitive antagonism in alpha-1 adrenergic receptors

typically causes hypertension, which, through compensatory mechanisms, may lead to reflex tachycardia, reduced systemic vascular resistance and an increase in circulating catecholamines (Grasso et al., 2015). Additionally, body temperature often drops, a negative inotropic effect with myocardial depression and splenic vasodilation (Lemke, 2013). In sheep, respiratory depression does not occur with the use of acepromazine only, but PaO_2 increases when associated with opioids (Nishimura et al., 2017).

Acepromazine associated with opioids is frequently used in dogs to strengthen sedation and provide adequate pain relief, a synergism called neuroleptanalgesia (Reis et al., 2017). When morphine, which is classified as a pure opioid agonist because it acts on mu receptors (μ), is injected intramuscularly, it provides pain relief for around 4 hours. Its main adverse effects are decreased heart rate, stimulation of the chemoreceptor trigger zone and respiratory depression. In dogs, intramuscular latency is approximately 30 minutes, with hepatic metabolism and renal excretion (Fantoni & Garofalo, 2012).

Meperidine is also classified as a synthetic opioid and like morphine, acts on μ receptors and is a pure agonist. Although it acts in a similar manner, this drug lasts between 1 and 2 hours and its analgesic potency is 10-fold less than that of morphine (Fantoni & Garofalo, 2012). Its analgesic effect starts around 10 minutes after intramuscular morphine administration. The adverse effects caused by the use of meperidine are similar to those of morphine, but may cause more mast cell degranulation with histamine release. Histamine release is also directly related to the dose (high doses) and administration pathway (mainly intravenous) (Reis et al., 2017).

Butorphanol is frequently used in veterinary medicine and acts as an agonist-antagonist opioid, with analgesic potential 3 to 5 times greater than that of morphine (Epstein, 2015). Given its low activity in μ receptors and higher in their kappa (κ) counterparts, butorphanol promotes less evident cardiovascular depression than agonist opioids (Tranquilli et al., 2013). Clinical studies in dogs demonstrate that butorphanol produces visceral analgesia with less somatic action (Epstein, 2015).

In dogs, the synergetic effect of the acepromazine-opioid combination promotes greater feelings of calmness than when used separately (Gomes et al., 2018). Opioid sedation depends on drug selectivity to its respective receptors, the pharmacokinetics of each drug, the individual response of the animal and the method used to classify sedation level. There are literature reports that the effectiveness of opioids is not well described in sheep, promoting mild analgesia, thereby requiring more studies to determine the analgesia level of this drug class on the species in question, mainly due to the difficulty in assessing pain in these animals (Lizarraga & Chambers, 2012; Murdoch et al., 2013). Thus, the aim of the present study was to assess the sedative effect, agitation level and influence on cardiorespiratory parameters after intramuscular administration of different sedation protocols in sheep submitted to AI.

Materials and Methods

A total of 75 female Tecel sheep were used, with average weight and age of 55.7±7.7 kg and 35.5±16.3 months, respectively, submitted to AI. They were classified according to the American Society

of Anesthesiology (ASA) as ASA I patients, confirmed by clinical and complementary examinations including complete hemogram, kidney function (urea and creatinine) and liver function tests (albumin, aminotransferase aspartate and alkaline phosphatase), conducted one week before the experimental procedure.

The animals were kept in collective bays on a private property, located in Lages-SC, fed balanced ration and maize silage twice a day, with access to pasture paddocks throughout the day and ad libitum water. Food and water fasting were 24 and 12 hours, respectively.

In order to synchronize the estrous cycle, fixed-time artificial insemination (FTAI) was used. All the ewes were submitted to hormonal treatment using intravaginal sponges impregnated with 60 mg of acetate and medroxyprogesterone. The devices remained inside the animals for 14 days and when removed, 300 UI of equine chorionic gonadotrophin (eCG) was administered to stimulate ovarian activity (Padilha et al., 2011).

Estrous was detected with the help of a vasectomized ram, initiated 12 hours after the devices were removed, and typical estrous signs were monitored every four hours until all the animals demonstrated the respective signs. The following clinical signs were observed: swollen vulva, tail wagging and standing still to be mounted, after which the sheep underwent AI (Padilha et al., 2011).

Immediately before the procedure, the animals were assessed for their behavior, agitation level and cardiorespiratory variables, then randomly allocated to five groups of 15 animals each. The Acepromazine Group (AG) received an intramuscular injection of 0.05mg kg^{-1}, and the Acepromazine and Butorphanol Group (ABG) 0.05 and 0.1mg kg^{-1} of acepromazine and butorphanol, respectively. The Acepromazine and Meperidine Group (AMRG) received an intramuscular injection of acepromazine and meperidine at doses of 0.05 and 5mg kg^{-1}, respectively, and the Acepromazine and Morphine Group (AMG) were intramuscularly injected with 0.05 and 0.5mg kg^{-1} of acepromazine and morphine, respectively. All the drugs were prepared and administered using a single syringe. The Saline Group (SG) received a 5mL intramuscular injection of a saline solution. The veterinarian anesthetist in charge of assessing the parameters was blinded to the treatments.

The ewes were sheared from the ventral region to the abdomen and local infiltration anesthesia of lidocaine (6 mL) with no vasodilator was administered (half the volume on the right and half on the left side of the abdomen) and AI was started 10 minutes after the anesthesia was applied by the same experienced veterinarian. The animals remained in dorsal decubitus, immobilized and placed in the Trendelenburg position (head lower than feet) to allow the bowel loops and rumen to be projected cranially. Next, antiseptics (alcohol/iodine/alcohol) were applied, with posterior skin incision and muscle fascia with a number 11 scalpel, followed by trocar insertion on the left side of the animal. After AI, the skin was sutured with a 2.0 nylon thread. Samples of frozen semen from the same batch were used for AI, stored in a specific cylinder with daily control of nitrogen levels, in order to preserve sperm viability.

The following parameters were assessed: sedation score based on the sedation assessment scale (Kästner et al., 2003); the Musk and Wilkes (2018) agitation assessment scale, stethoscope heart rate (bpm); respiratory rate (breaths per minute) by observing rib cage movement; rectal temperature (ºC) using a digital thermometer; latency time and AI duration.

Assessments were carried out immediately before pre-anesthesia drug administration (M-20); 20 minutes after (M0); 5 minutes before local infiltration anesthesia (M1) and immediately after AI (M2).

The data were analyzed with Prisma software and submitted to the Shapiro-Wilk normality test, in order to verify normal distribution. One-way RM ANOVA was applied to the parametric data between assessment times in the group, followed by Dunnett´s test. In regard to nonparametric data, the Friedman and Dunn`s tests were conducted between assessment times in the group, and the Kruskal-Wall and Dunn`s tests for intergroup times ($p<0.05$).

Results and Discussions

Due to the animal homogeneity between the study groups, there were no statistical differences for weight and age, with average values of 55.7±7.7 kg and 35.5±16.3 months, respectively (Table 1).

Table 1
Means and standard deviations for weight in kilograms and age in months, of animals that were pre-medicated with intramuscular injections of 0.05 mg kg^{-1} of acepromazine (AG) or 0.05 mg kg^{-1} of acepromazine and 0.1 mg kg^{-1} of butorphanol (ABG) or 0.05 mg kg^{-1} of acepromazine and 5 mg kg^{-1} of meperidine (AMRG) or 0.05 mg kg^{-1} of acepromazine and 0.5 mg kg^{-1} of morphine (AMG) or saline solution (SG), submitted to laparoscopic AI

Variable	Treatments				
	AG	ABG	AMRG	AMG	SG
WEIGHT (kg)	61.1±7.9	53.3±7.5	54.4±10.9	56.2±7.7	53.3±4.4
AGE (months)	43.6±27.2	29.3±17.3	38.8±16	32.4±10.3	33.2±10.6

Heart rate (HR) (Table 2) increased by 30.6, 34.2 and 42.5% for the AG, ABG and AMRG groups, respectively, between M-20 and M2. In M2, the HR values of the groups that received acepromazine alone or associated with the opioid were 21.5, 14.6, 39.2 and 13.1% higher than those of the SG for AG, ABG, AMRG and AMG, respectively. The first hypothesis that explains this increased HR at M2 when compared to M-20 for all the groups, may be related to the end of the AI procedure at this time, resulting in greater patient movement due to the time taken and removal from the stretcher and Trendelenburg

position, culminating with lower sedation scores at this assessment time (previously described) and an increase in agitation level, thereby raising HR because of adrenergic stimulation in all the groups at this time. The second hypothesis is related to the four animal groups that received an intramuscular injection of acepromazine, suggesting that the higher HR values result from reflex tachycardia, a compensatory mechanism that aims to maintain adequate tissue perfusion in situations where systolic volume (SV) and cardiac output (CO) may decline, since HR values were higher in these groups when compared to their SG counterparts. It is known that phenothiazines can decrease CO due to their antagonist action in adrenergic alpha-1 receptors, which results in vascular smooth muscle relaxation and a decrease in systemic vascular resistance (Sinclair & Dyson, 2012). Additionally, the negative inotropic action of acepromazine and the effect of splenic vasodilation may contribute to lowering systolic volume (SV) and CO (Auger et al., 2019). SV, in turn, depends on the pressures and ventricular filling (pre-load), myocardial contractility and ventricular emptying resistance (post-load) (Stanzani & Otto, 2018). Thus, given the equation CO = SV x HR, it is expected that declines in CO and SV result in a higher HR in patients with no morbidities. The data obtained show that the highest HR values at M2 were obtained in the AMRG group, suggesting that reflex tachycardia was exacerbated by the vasodilator synergism that may occur with meperidine. The use of meperidine, even by intramuscular administration, is commonly related to mastocyte degranulation and the rise in histamine levels in the blood stream (Dippenaar & Naidoo, 2015). In the blood stream, histamine acts on H1 and H2 receptors, leading to peripheral vasodilation, contributing to lower CO and SV (Jones, 2016). This occurrence may be reinforced when intergroup assessment times are compared, where at M0, the AMRG values were 23.9; 30.8, 33.3 and 41.4% higher than those of the AG, ABG, AMRG and SG, respectively. The SG exhibited the smallest change in HR at all the assessments, with the largest increase occurring between M0 and M2 (19.3%), the lowest percentage of the groups. The increase in HR coincided with the times of highest handling and greatest agitation in the animals of this group (Table 3), possibly because of adrenergic stimulation that released endogenous catecholamines as a function of agitation, decubitus discomfort and pain.

Table 2
Means and standard deviations for heart rate (HR) in beats per minutes, respiratory rate (f) in breaths per minute and body temperature (BT) in degrees Celsius in sheep pre-medicated with intramuscular injections of 0.05 mg kg^{-1} of acepromazine (AG) or 0.05 mg kg^{-1} of acepromazine and 0.1 mg kg^{-1} of butorphanol (ABG) or 0.05 mg kg^{-1} of acepromazine and 5 mg kg^{-1} of meperidine (AMRG) or 0.05 mg kg^{-1} of acepromazine and 0.5 mg kg^{-1} of morphine (AMG) or saline solution (SG), submitted to laparoscopic AI

Variable	Treatments	M-20	M0	M1	M2
HR (beats/min)	AG	121±22	113±33abcd	140±35ab	158±37Aab
	ABG	111±27	107±17acd	130±33ab	149±33Aab
	AMG	127±39	140±40b	155±40a	181±41Aa
	AMRG	106 ±30	105±31acd	107±26b	147±55ab
	SG	109±25	99 ±21acd	129±42ab	130±29b
f (breaths/min)	AG	41 ±8a	53±22abc	42±11a	42±11a
	ABG	45 ±11ab	42±16ac	38±11a	40±7a
	AMRG	55 ±15b	46±11abc	43±14Aa	44±11Aa
	AMG	52 ±11ab	39±22ac	38±9Aa	39±14Aa
	SG	48 ±11ab	67±29b	99±38Ab	81±34Ab
BT (ºC)	AG	38.8±0.5	38.7±0.4	39.1±0.5A	39.1±0.5A
	ABG	39±0.3	38.8±0.4A	39.2±0.3A	39.4±0.4A
	AMRG	39.1±0.4	38.9±0.4	39.3±0.5A	39.4±0.6A
	AMG	39.1±0.3	38.8±0.3A	39.1±0.4	39.1±0.3
	SG	39±0.3	38.9±0.3	39.3±0.5A	39.4±0.4A

Upper case letters mean statistical differences between moments within the same group, using RM ANOVA followed by Dunnett's test.
Lower case letters indicate intergroups differences at a same moment, using ANOVA followed by Tukey`s test ($p< 0.05$ for both).

Table 3
Median, minimum and maximum values for sedation level and agitation based on the sedation scale (Kästner et al., 2003) and agitation assessment scale (Musk & Wilkes, 2018) in sheep pre-medicated with intramuscular injections of 0.05 mg kg^{-1} of acepromazine (AG) or 0.05 mg kg^{-1} of acepromazine and 0.1 mg kg^{-1} of butorphanol (ABG) or 0.05 mg kg^{-1} of acepromazine and 5 mg kg^{-1} of meperidine (AMRG) or 0.05 mg kg^{-1} of acepromazine and 0.5 mg kg^{-1} of morphine (AMG) or saline solution (SG), submitted to laparoscopic AI

Variable	Treatments	M0	M1	M2
Sedation	AG	2 [0-6]	-	-
	ABG	2 [0-4]	-	-
	AMRG	3 [0-4]	-	-
	AMG	2 [0-6]	-	-
	SG	0 [0-3]	-	-
Agitation	AG	2 [0-4]	2 [0-4]ab	2 [0-4]ab
	ABG	2 [0-3]	1 [0-4]ab	2 [0-4]ab
	AMRG	2 [0-3]	2 [0-4]a	2 [0-4]ab
	AMG	1 [0-4]	2 [0-4]a	2 [0-4]a
	SG	0 [0-2]	0 [0-3]b	0 [0-3]b

Lower case letters (a,b) indicate intergroup differences at a same moment, using the Kruskal-Wallis test followed by Dunn`s test ($p<0.05$ for both).

Analysis of respiratory rate *(f)* (Table 2) shows that all the means obtained are above normal physiological values for sheep, between 20 and 34 breaths per minute (bpm) (Reece et al., 2015). This increase in *f* at M-20 likely occurred because of the inevitable handling of these animals during basal data collection. Since sheep are considered prey in nature, they exhibit a flight reaction when faced with a threat. This response corresponds to activation of the sympatho-adrenomedullary (SAM) system, which secretes catecholamines such as adrenaline and noradrenaline, which, via dilation and contraction of different blood vessels, redirect blood flow to the brain, heart and muscles in a flight situation, resulting in increased HR and f (Aleixo et al., 2017). Although f values are high, there was a 21.8, 20.0. 26.9 and 25.0% decline in M-20 in relation to M1 and M2 for the AMRG and AMG, respectively. The decreases found are similar to the previously mentioned mean reference values. Associating these AMRG and AMG data to the agitation level (Table 3) reveals that at M1 and M2, both protocols obtained good agitation scores, with sheep less agitated and only slight resistance to positioning. The use of total agonist opioids such as morphine and meperidine may promote bulbar depression (Epstein, 2015). However, it is suggested that in the present study, f values declined due to less agitation, corroborating Nishimura et al. (2017), who also demonstrated a drop

in respiratory rate in sheep that received acepromazine and morphine in the same dose and administration route as the present study, showing minimal changes in $PaCO_2$, remaining within the physiological range of 35 to 45 mmHg, indicating that this decrease does not cause significant complications. A comparison between respiratory rate at M-20 with those at M1 and M2 for the SG shows an increase of 106.3 and 68.8% respectively. Additionally, the respiratory rates of SG at M1 and M2 were higher than the means of the other groups, which did not differ (AG, ABG, AMRG and AMG), with a 146 and 96% increase at M1 and M2, respectively. Given that the SG animals received only a saline solution of pre-anesthetic medication and exhibited the highest agitation between M and M2, caused by the stress of the Trendelenburg position, the values obtained can be explained by the lack of sedation and analgesia. The median of 0 obtained in agitation assessment of the SG at M1 and M2 (Table 3) reinforces the idea that this group was more agitated than those that received acepromazine alone or associated with opioids. In the decubitus used for artificial insemination, the animals remained with their pelvic region raised. The resulting cranial displacement of abdominal organs could have caused the increased respiratory rate at M1 and M2 via diaphragmatic compression, generating a decline in respiratory complacency and minute volume, leading to compensatory tachypnea. However, it was expected that increases in f would occur in all the groups, since all the animals were submitted to the same procedure, decubitus and for the same amount of time. Thus, given that the increase was obvious only in SG, it was attributed to the previously cited stress factors.

The body temperatures (BT) described in Table 2 showed an increase at M1 and M2 in relation to M-20 for the AG, ABG, AMRG and SG treatments. Although statistically different, the clinical values obtained were not relevant, given that all the average values were within the reference range for adult sheep of 38.5 to 39.9 ºC (Reece et al., 2015). Since the rise in temperature coincided with the times of greatest animal handling (M1 and M2), it is suggested that increased temperatures occurred due to activation of the sympathetic autonomous nervous system, thereby increasing the metabolic activity of the animals. Muscle activity, in turn, is an important endogenous factor related to body temperature (Damatto et al., 2019), and most likely accounts for the rise in temperature. Temperature increases show that the protocols used promoted sedation and slight muscle relaxation.

In relation to sedation level, using the sedation assessment scale (Kästner et al., 2003) in sheep showed no statistical intergroup difference (Table 3). However, the AG, ABG and AMRG treatments obtained a median of 2 points, indicating that a large number of these animals were in season at M0, exhibiting slight lowering of the head. The AMRG obtained a median of 3, suggesting that a significant number were in season, with moderate lowering of the head, while the SG exhibited a median of 0, demonstrating that these animals were in season, alert and behaving normally. The median sedation level of 2 for the AG, ABG, AMG and 3 for the AMRG indicates that the protocols at the respective doses applied promoted mild sedation, since the scale used has a maximum score of 10 points, where the animals would be in lateral

decubitus and motionless. It is known that the use of acepromazine alone in ruminants promotes calmness and mild muscle relaxation (Lemke, 2013), also observed by Nishimura et al. (2017), when they used an intramuscular acepromazine dose of 0.05mg kg^{-1}, and both corroborate the data obtained here. The association between acepromazine and opioids is well documented in dogs and the combination increases sedation and provides pain relief, given that phenothiazines have no antinociceptive properties (Lemke, 2013). It was expected that phenothiazines associated with morphine or butorphanol administered in sheep at the doses proposed could increase the sedative effect. However, as observed in the data obtained in the present study, there was no significant rise in sedation level when compared to the use of acepromazine alone, agreeing with Nishimura et al. (2017), who assessed the sedative effect of acepromazine alone or associated with morphine or methadone or tramadol in sheep. In the aforementioned study, the animals that received acepromazine (0.05mg kg^{-1}) associated with morphine (0.5mg kg^{-1}) obtained slightly higher median values than when acepromazine (0.05mg kg^{-1}) was used alone, albeit not statistically different.

In order to assess agitation level (Table 3), the agitation assessment scale (Musk & Wilkes, 2018) was used. It is important to note that SG animals obtained a median of 0 at all three assessment times (M0, M1 and M2), indicating that these animals were agitated, and difficult to maintain in the Trendelenburg position. The difficulty is potentially greater when the animals undergo AI, since decubitus in a head down position is in itself quite uncomfortable for the animal, thereby requiring that thoracic and pelvic members be tied with a rope to immobilize the animal during the procedure. In addition to the uncomfortable position, the absence of analgesia in pre-anesthetic medication for SG animals results in greater agitation. At all agitation assessment times, AG, ABG, AMRG and AMG animals obtained a median of 2, indicating that the animals were less agitated, but with some resistance to position, except for the AMG at M0 and ABG at M1, where the median was 1 in both. In this case, the animals resisted occasionally to positioning and were more agitated. In addition, the AMRG and AMG were different from the SG at M1, and the AMRG differed at M2, demonstrating that among the protocols tested, they were the most effective in controlling sheep agitation at times of greatest handling and nociception. Although opioids did not positively influence sedation level, when associated with phenothiazines they were important in promoting higher scores on the agitation scale. It is suggested that the positive effect of morphine and meperidine on agitation occurred due to their analgesic activity, especially since they are pure agonists. It is well documented that in terms of analgesia, opioids have more affinity for the μ receptors and promote more pain relief when compared to butorphanol, an agonist-antagonist with higher activity in κ receptors, lower analgesic potential and greater sedative potential (Warne et al., 2013; Tranquilli et al., 2013).

Latency time (Table 4) is the time in minutes between treatment administration and manifestation of the first signs of calmness via behavioral changes related to the sedation assessment scale (Kastner et al., 2003) in sheep. Decreased head and ear

movements correspond to a score of 1 and were the signs used to delimit latency. The animals that exhibited no behavioral changes 20 minutes after administration of pre-anesthetic medication were declassified in terms of latency time. Data analysis found no statistical intergroup differences. However, the SG showed unexpected shorter latency (12.2 minutes), given that these animals only received saline solution as pre-anesthetic medication. The short latency time for the SG may be because the present study was blind, where the assessors did not know which treatments were administered. Additionally, the signs used to delimit latency are subjective and may have been influenced by the pain caused during intramuscular injection, even of saline solution. Although the shortest latency was observed for the SG, only 40% of the animals in this group displayed latency, while 86.7% of ABG and AMG animals and 80% of their AG and AMRG counterparts showed signs of having received some tranquilizer.

Table 4
Means and standard deviations for latency time, AI duration and percentage of animals that exhibited latency, in sheep pre-medicated with 0.05 mg kg^{-1} of acepromazine (AG) or 0.05 mg kg^{-1} of acepromazine and 0.1 mg kg^{-1} of butorphanol (ABG) or 0.05 mg kg^{-1} of acepromazine and 5 mg kg^{-1} of meperidine (AMRG) or 0.05 mg kg^{-1} of acepromazine and 0.5 mg kg^{-1} of morphine (AMG) or saline solution (SG), submitted to laparoscopic AI

Treatments	Latency (minutes)	Latency (%)	Insemination (minutes)
AG	17.8±4.2	80	3.1±1.3
ABG	17.1±5.2	86.7	3±1.7
AMRG	14.6±5.7	80	3.9±1.9
AMG	14.3±5.6	86.7	2.9±1
SG	12.2±5.5	40	3.2±1.3

With respect to insemination time (minutes) (Table 4), there was no statistical difference between treatments. In order to assess this parameter, the time was measured from the first skin incision to removal of the laparoscopic trocars. It is important to note that artificial insemination was conducted by the same experienced veterinarian. Procedure time was expected to be shorter in animals from the groups that obtained higher scores on the agitation and sedation scales, but that did not occur. The disadvantage of laparoscopic AI is the need for a skillful qualified professional (Feranti et al., 2013). As such, this parameter is significantly influenced by the veterinarian. As previously described, the protocols used promoted mild sedation. Many animals were resistant when physically contained and placed in the Trendelenburg position. Nociceptive stimulation by the skin

incision and insertion of the trocar into the abdominal cavity exacerbated agitation, even following local anesthetic with lidocaine and no vasoconstrictor at the incision site. Drug combinations that produce higher sedation and agitation scores may directly influence procedure time and reduce risks such as perforating large vessels or abdominal viscera.

Conclusions

Under the conditions of the present study, the AMRG exhibited the greatest changes in cardiorespiratory variables and was therefore, the least safe option of those assessed. The absence of calmness and analgesia in SG animals demonstrated the real need for sedation to perform AI. Acepromazine alone produced mild sedation and when associated with meperidine or morphine or butorphanol did not significantly increase calmness. The analgesic activity of opioids was important in decreasing animal agitation at the times of greatest pain stimulus, but the pre-anesthetic medication protocols at the doses used in the study did not reduce laparoscopic artificial insemination time.

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