Analgesic effects of intravenous flunixin and intrafunicular lidocaine or their combination for castration of lambs

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ABSTRACT

Objective To analyse the effectiveness of intrafunicular lidocaine and intravenous flunixin for reducing pain and signs of stress in lambs undergoing surgical castration.

Design Randomised controlled trial.

Setting One university teaching hospital in Italy.

Participants 30 healthy male lambs, 9–12 weeks old.

Intervention Allocation to five groups: a control group (C), undergoing general anaesthesia but not castration; a surgery group (S), undergoing orchiectomy without analgesic treatment; a surgery-lidocaine group (SL), undergoing orchiectomy and receiving intrafunicular 2 per cent lidocaine solution; a surgery-flunixin group (SF), undergoing orchiectomy and receiving intravenous flunixin; a surgery-flunixin-lidocaine group (SFL), undergoing orchiectomy and receiving both intrafunicular lidocaine and intravenous flunixin.

Main outcome measures Nociception and stress were assessed through intraoperative indicators, serum cortisol concentration, glycaemia, behaviour, immune response and clinical evaluation of the heart rate (HR), respiratory rate and rectal temperature after surgery.

Results Groups S and SL showed increased values of intraoperative HR, mean arterial pressure and postoperative cortisol concentration. In group SFL, cortisol values were similar to those of group C. No other difference could be detected.

Conclusions The combination of intravenous flunixin and intrafunicular lidocaine reduced the pain and discomfort of lambs castrated under general anaesthesia. Intrafunicular lidocaine alone did not prevent pain or discomfort associated with castration.

Trial registration number 30/2012/CEISA/COM.

INTRODUCTION

Considerable research has focused on the efficacy of analgesic strategies for the short-term and long-term management of pain associated with castration and tail docking in various livestock species.[1–4] Legislation has been enacted in the EU and UK to promote the development and use of effective analgesia for castration and tail docking in livestock.[5] Such legislation reflects the growing global concern for the humane management of livestock, and challenges researchers to develop effective methods for pain management that are practical for farming operations.[6]

Routine castration in lambs is achieved through the use of bloodless techniques (Burdizzo and rubber rings) or surgical castration.[7 8] Bloodless techniques cause regional ischaemia with or without testicular atrophy.[9 10] Surgical castration is time consuming and expensive and thus is not suitable for farm practice. Nevertheless, it produces less damage to the tissues and reduces postoperative complications related to dehiscence of infection since the first intention healing is faster.

Pain management during surgical procedures in livestock can be achieved through the use of opioids, ketamine, alpha-2-adrenergic drugs and local anaesthetics, as well as systemic injection of NSAIDs and multimodal treatments. Both bupivacaine and lidocaine have been shown to reduce animal distress responses to Burdizzo forceps and ring castration when injected into the spermatic cord and scrotal neck, with bupivacaine providing a longer period of pain relief than lidocaine.[11–13] In isoflurane-anaesthetised equids, the intratesticular or intrafunicular injection of lidocaine before castration showed positive effects in reducing the cremaster muscle tension and the required concentration of volatile anaesthetic agent.[3 4]

Another common method to achieve analgesia is the parenteral administration of NSAIDs, among which flunixin meglumine was the most frequently used to treat visceral pain in cattle[14] but has recently been replaced by meloxicam and ketoprofen alone or in combination with local anaesthetics.[15–17] Although studies on the use of NSAIDs for pain management in castrated sheep are scant, flunixin, carprofen and meloxicam are recommended for analgesia in sheep.[18 19] Systemic analgesia with carprofen or ketoprofen appears to be effective in

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reducing acute pain derived from Burdizzo castration in bulls,[20 21] whereas intramuscular administration of diclofenac 20 minutes before Burdizzo forceps application reduces the peak plasma cortisol response and time spent trembling or in abnormal postures in lambs.[22] In addition to the previous drugs, meloxicam has been widely investigated, with promising results.[23 24]

Local anaesthesia and parenteral NSAIDs can also be used concurrently to improve analgesia during surgical procedures. There are two main reasons for this association: NSAIDs provide a longer period of analgesia, and their systemic effects provide analgesia to damaged tissues that are not accessible to nerve blockade using local anaesthetics.[12 14 18 20–22] Because pain cannot be directly measured, indirect physiological (serum cortisol concentration, heart rate (HR) and respiratory rate (RR), blood glucose, haematological variables, rectal temperature) or behavioural indices are used to assess pain level and evaluate analgesia induced during invasive procedures. Although plasma cortisol increases under various physiological and pathophysiological conditions, previous studies used this parameter alone or in conjunction with observations of behaviour and posture to assess pain and distress during and after castration.[8 25–28] The use of serum cortisol levels to indicate the magnitude of distress experienced by young lambs after castration and tail docking is supported by positive, although not linear, correlations between the magnitude of cortisol response and the presumed severity of these noxious stimuli.[25 29]

Since male lambs in livestock practice are preferably castrated in the first days of life, only a few studies have investigated the effect of different castration methods and pharmacological treatment in older lambs.[30] In addition, there is scientific evidence that younger lambs have a less active behavioural response and appear to behave more normally than older ones after castration and tail docking,[31] and that the younger lambs have a reduced cerebrocortical response to castration than their older counterparts and are assumed to perceive less pain.[32]

The objective of this study was to investigate the effect of intrafunicular lidocaine and parenteral flunixin in lambs undergoing surgical castration. To this end, we evaluated some of the intraoperative (HR, RR, mean arterial pressure (MAP)) and postoperative indicators of nociception and pain (HR, RR, rectal temperature, serum cortisol, blood glucose, haematological variables, behaviour) in three-month-old lambs surgically castrated under general anaesthesia and treated with intrafunicular lidocaine and/or intravenous flunixin.

Our hypothesis was that the administration of flunixin and/or lidocaine would reduce nociception and pain during and after castration.

MATERIALS AND METHODS
Animals
All experimental procedures were approved by the Committee for Animal Research and Ethics at both the University of Chieti-Pescara (CEISA) and the University of Teramo (Protocol No 30/2012/CEISA/COM; approval date, July 3, 2012). Thirty male Merinizzata Italiana lambs aged 9–12 weeks and weighing 26.32±3.62 kg were obtained for use in our study. Based on clinical examination, all animals were classified as ASA I, according to the physical criteria established by the American Society of Anesthesiologists.[33 34]

Preoperative management
The lambs were moved to the Department of Veterinary Clinical Science of the University of Teramo two weeks before surgery. They were housed together in a roofed yard for one week after arrival, and in five pens containing six lambs each for another week before surgery. The 3 m² pens were made from wire mesh gates to allow animals in adjacent pens to see, smell and hear each other. Each pen was randomly assigned to a treatment group. To obtain similar body weights between groups, stratification of lambs across treatments was made based on their weight. Food and water were withheld for 24 and 12 hours, respectively, before general anaesthesia was induced.

Immediately before the induction of general anaesthesia, a 20 Gx32 mm venous catheter was aseptically introduced into the cephalic vein of each lamb. General anaesthesia was induced with an intravenous injection of a solution containing ketamine (4.5 mg/kg) (Ketavet; Intervet Productions) and diazepam (0.1 mg/kg) (Valium; Roche). Once endotracheal intubation was achieved with an 8 and 10 mm ID (inner diameter) tracheal tube, anaesthesia was maintained with isoflurane (Isoflo; Esteve) in oxygen with a rebreathing system. To achieve the correct anaesthetic depth, the isoflurane precision vaporiser setting was adjusted to 2 per cent during the first 5–10 minutes and then to 1.5 per cent during the surgical procedure. The adequacy of anaesthesia was determined by standard recognised criteria of anaesthetic depth. Clinical signs, including body movement, position and reflexes of the eye (palpebral and corneal reflexes), rate and depth of breathing, mucosal membrane colour, capillary refilling time, auricular pulse and the animal's response to surgical stimulation, were evaluated. The time given before surgery to each lamb to reach equilibration of the anaesthetic plan was 10 minutes. Once the surgical plane of anaesthesia was achieved, all responses to stimuli were considered reactions to surgical stimulation.

Surgical technique
After trichotomy and aseptic preparation of the surgical field, an open scrotal orchiectomy was performed with the animal in dorsal recumbency. The scrotal raphe was grasped between the thumb and the forefinger, and while applying traction to the ventral aspect of the scrotum, an elliptical portion of the distal skin was excised with a scalpel. The parietal tunic was isolated and incised. The ligament of the epididymal tail was severed, and the *funiculus* was dissected. After funicular

ligation, the testicles were removed, and the vaginal tunic was excised. The subcutis was sutured with 2-0 USP absorbable suture material in a simple continuous pattern, and surgical stainless steel staples were used to close the skin.[35]

At the end of surgery, after trichotomy of the left side of the neck, an 18 G jugular catheter was aseptically placed into the left jugular vein to allow serial blood sampling and avoid repeated venipuncture. A catheter was then sutured to the skin and wrapped with an elastic bandage (Vetrap; 3 M).

Treatment groups

Twenty-four out of 30 lambs were surgically castrated and equally assigned to one of five groups (n=6/each group): the surgery (S) group; the surgery and lidocaine (SL) group; the surgery and flunixin (SF) group; and the surgery, flunixin and lidocaine (SFL) group. The remaining six lambs were assigned to the control group (C), which underwent general anaesthesia but not castration. Groups S and C received no analgesia. To obtain complete data recording, the length of anaesthesia was planned to be 35 minutes or all groups. If the animal showed intraoperative movement in response to surgical manipulation or a more than 30 per cent increase in HR, MAP or RR above control values, a bolus of ketamine (1 mg/kg) was given as a rescue drug.[36] If lack of appetite, overt suffering or depression was noticed during the postoperative period, rescue flunixin (1.1 mg/kg) (Flunifen; Ceva Vetem) was injected intravenously. In the SL and SFL groups, a solution of 2 per cent lidocaine (Lidocaine 2%; Esteve) in 0.9 per cent sodium chloride was percutaneously injected into each spermatic cord before castration. The final lidocaine dose was 2 mg/kg in a total volume of 5 ml for each spermatic cord. For this purpose, 10 minutes before skin incision, regional intrafunicular anaesthesia was performed. To guide injection, the spermatic cord was palpated and identified at the level of the neck of the scrotum, and a 21 G 25 mm needle was inserted percutaneously into the spermatic cord. The solution was injected into the *funiculus spermaticus* in a fan-shaped manner, taking care to not perforate the funicular vessels. Intravenous administration was avoided by aspiration before injection of local anaesthetics. During surgery, once the tunica vaginalis was divided and the funiculus spermaticus exteriorised with the testicle, oedema and translucency of the funiculus were considered signs of correct injection. In the SF and SFL groups, 1 hour before surgery and once a day for two days after surgery, flunixin (1 mg/kg every 24 hours) (Flunifen; Ceva Vetem) was administered intravenously through a venous catheter.

Evaluation of the indicators of nociception and surgery-related stress

A timetable of the intraoperative and postoperative collection times is reported in Table 1.

TABLE 1: Time schedule of intraoperative evaluation and blood sampling throughout the experiment

Cortisol (c)	Tc0		Tc1	Tc2	Tc3		Tc4	Tc5						
Glycaemia (g)	Tg0					Tg1	Tg2	Tg3	Tg4					
Haematology (h)	Th0									Th1	Th2	Th3		
Anaesthesia (a)		Ta1	Ta2	Ta3	T4	Ta5	Ta6	Ta7						
Time	−20 minutes	−15 minutes	−10 minutes	−5 minutes	0	5 minutes	10 minutes	15 minutes	20 minutes	30 minutes	90 minutes	2 hours	3 hours	4 hours	6 hours	9 hours	12 hours	24 hours	48 hours	72 hours
		Anaesthetic induction	Intrafunicolar block		Skin incision			Skin staples	End of surgery						

c: cortisol (Tc0: preoperative; Tc1: 30 minutes after surgery; Tc2: 90 minutes after surgery; Tc3: 3 hours after surgery; Tc4: 6 hours after surgery; Tc5: 9 hours after surgery; Tc6: 9 hours after surgery).
g: glycaemia (Tg0: preoperative; Tg1: 2 hours after surgery; Tg2: 4 hours after surgery; Tg3: 6 hours after surgery; Tg4: 12 hours after surgery).
h: haematology (Th0: preoperative; Th1: 24 hours after surgery; Th2: 48 hours after surgery; Th3: 72 hours after surgery).
a: anaesthesia (Ta1: 10 minutes before skin incision; Ta2: 5 minutes before skin incision; Ta3: skin incision; Ta4: 5 minutes after skin incision; Ta5: 10 minutes after skin incision; Ta6: skin staple application/15 minutes after skin incision).

Intraoperative evaluation

Lambs were connected to a multiparametric monitor for monitoring pulse oximetry, end-tidal carbon dioxide concentration (Et_{CO_2}), fraction of inspired oxygen (FiO_2), HR, RR, fraction of expired isoflurane (F_EISO) and electrocardiography. Systolic arterial pressure, diastolic arterial pressure and MAP were monitored using an indirect oscillometric technique by placing a cuff over the median artery. The HR, RR, arterial blood pressure, oxygen saturation, FiO_2, Et_{CO_2}, F_EISO, eye reflex and surgically induced movements were recorded at five-minute intervals for 35 minutes (Ta1, Ta2, Ta3, Ta4, Ta5, Ta6 and Ta7) (Table 1).

Postoperative evaluation

After surgery, nociception and surgery-related stress were assessed through the following:

► Postoperative clinical evaluation.
► Haematobiochemical assays:
  – Serum cortisol.
  – Blood glucose.
  – Haematology.
► Objective behavioural assessment of pain.

Clinical and behavioural assessments were performed by qualified medical staff who were experienced in sheep handling and unaware of treatment. Concerning the postoperative clinical evaluation, following recovery from general anaesthesia, the lambs were housed in separate pens for five days in order to avoid social transmission of behavioural and physiological responses between groups.[37] Every day, a general examination of each animal was performed, while the HR, RR and rectal temperature were measured three times per day for five days and recorded. To evaluate the serum cortisol concentration, blood samples (3 ml each) were collected through a venous catheter immediately before surgery (Tc0) and at 30, 90, 180, 360 and 540 minutes (Tc1, Tc2, Tc3, Tc4, Tc5) after the initial incision (Table 1). Samples from Tc1 to Tc5 were collected postoperatively. Blood was collected into plain tubes and chilled before centrifugation at 3000x*g* for 10 minutes. The serum was collected and stored at −20°C. Serum samples were analysed for cortisol concentrations four weeks after collection. The concentration of cortisol was determined using an ELISA with a detection limit of 10 ng/ml.

To quantify the blood glucose concentration, five whole blood samples were collected from each lamb through the jugular venous catheter immediately before (Tg0) and 120, 240, 360 and 720 minutes after surgery (Tg1, Tg2, Tg3, Tg4) (Table 1). The samples were immediately analysed after collection using a commercial glucometer with reactive glucose test strips (coefficient of variation 2.7–4 per cent). Whole blood samples were collected through the jugular venous catheter in EDTA-treated tubes immediately before (Th0) and 24, 48 and 72 hours after surgery (Th1, Th2, Th3) (Table 1). The blood samples were analysed using a cell counter to determine the red blood and white blood cell counts, differential

TABLE 2: Description of lamb active behaviour, postures and indices recorded during the experiment

Parameter	Description
Behaviour	
Foot stamping/ kicking	The leg is lifted and forcefully placed on the ground while walking.
Easing quarters	The leg is moved in a cautious manner.
Standing up/lying down	Standing up and lying down in succession, each unit scored includes both the act of rising and lying down.
Head-turning	Movement of the head beyond the shoulder
Vocalisation	Vocal sounds
Postures	
Normal lying	Ventral recumbency: lying on the sternum and abdomen with all four legs tucked in
Abnormal ventral lying	Lying on the sternum with the hindlegs extended, or ventral recumbency during which the scrotal region does not contact the ground (dog-sitting)
Abnormal lateral lying	Lying on one side with one or both forelegs and both hindlegs stretched out laterally
Normal standing/ walking	Standing and walking with no apparent abnormalities
Abnormal normal standing/walking	Standing or walking unsteadily, swaying, arched back, hindlimbs positioned apart and caudal to the pelvis
Statue standing	Standing without walking for more than 10 seconds
Tail stretching	Holding the tail erect (not recorded during defecation and urination)
Other	
Food intake	Eating or rumination

white blood cell count, lymphocyte/neutrophil ratio, haematocrit and haemoglobin concentration.

The behaviour of each individual lamb was recorded for 12 hours after castration using a digital camera. The collected videos were retrospectively analysed based on the scan sampling method[37] by a single-blinded observer. Active pain-avoidance behaviours were observed for a one-minute period at five-minute intervals for a total of one hour after castration. Postural behaviours were observed at 10-minute intervals for 12 hours, consisting of three periods of four-hour duration. Observation time points for each lamb were aligned with its individual castration time. Final observational data (expressed as per cent) referred to the time spent performing a specific behaviour with respect to all the expressed behaviours. The behavioural analysis was carried out to identify the occurrence of active pain avoidance and posture behaviour that were previously associated with lamb and sheep pain (Table 2).[28] Moreover, clinical monitoring for suffering and lack of appetite was undertaken after surgery.

Statistical analysis

Data were stored in electronic spreadsheets (Microsoft Excel, Redmond, WA, USA) and analysed using a

commercial statistical program (SPSS, V.13.0). Behavioural data, expressed as the percentage of time spent performing a specific behaviour, were not normally distributed; therefore, they were analysed with the Kolmogorov-Smirnov test. All other data were tested for normal distribution using the Shapiro-Wilk test. Since those data did not satisfy the criteria, they were normalised and underwent multivariate repeated measure analysis of variance followed by a post hoc Tukey test to detect differences between treatment and time-related differences within groups. Comparative results with P≤0.05 were considered to represent statistically significant differences.

RESULTS

All animals underwent the anaesthetic and surgical procedures without complications. No indications for the administration of rescue drugs were noticed during the intraoperative or postoperative period, since none of the animals manifested intraoperative movement in response to surgical manipulation, more than a 30 per cent increase in HR, MAP or RR above control values, or lack of appetite, overt suffering or depression after surgery.

Intraoperative evaluation

After reaching an F_EISO ranging from 1.3 to 1.4 per cent, 10 minutes elapsed before incision to allow equilibration. No intergroup or intragroup significative differences were detected throughout the surgical procedure for F_EISO. Mean values of F_EISO during surgery were as follows:

▶ Group C: 1.21±0.1.
▶ Group S: 1.32±0.2.
▶ Group SL: 1.33±0.1.
▶ Group SF: 1.25±0.2.
▶ Group SFL: 1.31±0.1.

The results of the intraoperative evaluation of the treatment groups are shown in Table 3. At Ta6, which corresponds to the application of skin staples, group SL had a higher value of HR and MAP compared with all other groups except for the group S (P=0.021; P=0.04), and higher values were maintained at Ta7 as well, even though

TABLE 3: Intraoperative physiologic value indicators of nociception. Intraoperative values of FEISO, HR, RR and MAP for each group of lambs are shown

Parameter	Time	Treatment				
		C	S	SL	SF	SFL
HR	Ta1 (−10 minutes)	110±20	126±27.7	124±12.3	115±35.2	125±19.2
	Ta2 (−5 minutes)	115±11.8	133±17.1	127±17.5	107±21.3	119±19.2
	Ta3 (skin incision)	120±25.4	131±12.5	125±21.4	138±10.9	116±21.1
	Ta4 (5 minutes)	116±21.6	136±13.9	121±20.5	125±11.6	113±16.2
	Ta5 (10 minutes)	117±24.1	135±13.9	130±20.8	117.50±10.6	115±15.5
	Ta6 (15 minutes)	108[b]±22.6	131[a]±12.1	142[a]±13.2	116[b]±18.6	113[b]±11.7
	Ta7 (20 minutes)	106	130±12.9	155±8.9	115±27.5	118±14.2
RR	Ta1 (−10 minutes)	43±15.1	37±17	62±31.7	38±9.3	33±6
	Ta2 (−5 minutes)	32[b]±5.7	34[b]±16.6	53[a]±18.4	40[ab]±5	28[b]±2.9
	Ta3 (skin incision)	33±1	34±10	45±8.1	40±13.8	31±6.9
	Ta4 (5 minutes)	35±3	36±10.4	50±13.4	49±20.8	34±8.1
	Ta5 (10 minutes)	37	41±26.3	45±8.7	57±25.6	33±10.1
	Ta6 (15 minutes)	40	43±23.2	44±6.7	46±10	33±6
	Ta7 (20 minutes)	30	37±16.1	42±7	40±10.6	36±8.4
MAP	Ta1 (−10 minutes)	81±14.5	92±13.9	97±16.7	97±23.7	105±11.1
	Ta2 (−5 minutes)	74±19.5	94±12	92±20.1	106±23.7	84±20.3
	Ta3 (skin incision)	84±19.3	90±20.4	89±16.4	103±16.1	77±14.5
	Ta4 (5 minutes)	81±10	103±6.6	90±18.2	87±17.6	79±10.4
	Ta5 (10 minutes)	82±17.6	103±6.9	107±10.2	93±16.1	88±13.3
	Ta6 (15 minutes)	81[c]±17	106[a]±7.5	105[a]±10.7	90[bc]±10.2	90[bc]±20.3
	Ta7 (20 minutes)	69	110±10.3	100±30	87±11.2	99±19.1

Data are presented as the mean values±sd.
Different letters on the same row indicate significantly different results (a, b, c: P<0.05).
Six animals were allocated for each group (n=6/each group).
C, control group (n=6); HR, heart rate; MAP, mean arterial pressure; RR, respiratory rate; S, surgery group (n=6); SF, surgery-flunixin group (n=6); SFL, surgery-flunixin-lidocaine group (n=6); SL, surgery-lidocaine group (n=6).

TABLE 4: Cortisol values expressed in ng/ml and treatment-related differences at different time points between treatment groups of lambs

Time	Treatment groups				
	C	S	SL	SF	SFL
T0	23.8±9.1	25.6±10.3	18.2±11.6	38.2±12.6	18.6±10.6
T1 (30 minutes)	40.8^C±24.3	106.2^A±36.3	87.6^B±26.25	98.1^B±28.5	64.9^{BC}±25.6
T2 (90 minutes)	27.1^C±26	85.9^A±22.4	69.6^B±49.33	58.3^B±46.4	23.3^C±15.9
T3 (3 hours)	28.8^C±18.6	81.1^A±25.5	72.7^B±45.4	52.3^{BC}±31.8	17.2^C±6.4
T4 (6 hours)	27.1^b±15	49.4^a±22.3	56.6^a±70	31.1^b±19	19.9^b±9.2
T5 (9 hours)	26.4^b±18.6	43.0^a±22.8	22.1^b±48.25	28.9^b±20	14.0^b±9.5

Data are presented as the mean values±sd.
Different letters on the same row indicate significant differences (a, b: P<0.05; A, B, C: P<0.01).
C, control group (n=6); S, surgery group (n=6); SF, surgery-flunixin group (n=6); SFL, surgery-flunixin-lidocaine group (n=6); SL, surgery-lidocaine group (n=6).

they did not reach statistical significance. Group SFL had lower values of HR and MAP that were not different from the SF and C groups. Concerning other intraoperative variables, no time-related or treatment-related difference could be observed.

Postoperative evaluation
Serum cortisol, glycaemia and haematology
The mean serum cortisol concentration was not significantly different between the treatment groups before surgery (Tc0; Table 4). Thirty minutes after castration (Tc1), cortisol concentrations increased significantly in all the castrated groups (Table 4), except for group C. Group S showed an increased cortisol concentration from 30 minutes up to nine hours after induction, while in groups SL and SF, similar values were detected at Tc1–Tc2; these values were significantly higher than those in group C and lower than those in group S. While cortisol decreased significantly six hours after surgery (Tc4) in group SF, its value in group SL returned to baseline levels only at Tc5 (nine hours after surgery). Cortisol curves were similar between groups C and SFL at all time points (P>0.05). No significant intergroup or intragroup differences in the haematological or glycaemic parameters were observed throughout the observational period.

Postoperative clinical evaluation and objective behavioural assessment of pain
In all the castrated lambs, the HR was significantly higher on days 1 and 2 compared with days 3–5 (P<0.00) (Table 5), whereas the RR showed higher values on D1 in all groups except for group C (P<0.05) (Table 6). No difference in the treatment effect on the mean HR, RR and rectal temperature between groups could be detected at any time point. Abnormal ventral lying (previously defined as lying on the sternum with hindlegs extended or ventral recumbency, during which the scrotal region does not contact the ground) was the most common distress-related behaviour observed following castration. Although no significant difference in the frequency of abnormal ventral lying was observed between the SL, SFL and SF groups (6, 7, 8 per cent), the highest frequency of this posture was observed in the S group (12 per cent). Abnormal ventral lying was not observed in the control group at any time (Fig 1). Quantitative results of the behavioural assessment are provided in Table 7.

DISCUSSION
In this study, the effects of intravenous flunixin and intrafunicular lidocaine on pain and discomfort in lambs

TABLE 5: Postoperative heart rate (HR) expressed as bpm

	D1	D2	D3	D4	D5
C	78.56^A±12.2	69.94^A±13.7	70.50^B±8.2	77.44^B±10.2	64.22^C±7.6
S	88.44^A±9.3	78.67^A±7.6	75.00^B±12.6	78.22^B±11.9	68.89^C±6.9
SL	89.22^A±12.7	83.11^A±11	70.72^B±8.3	70.67^B±4	66.50^C±8.6
SF	86.11^A±12.2	85.44^A±12.7	74.50^B±12	80.44^B±11.3	73.78^C±11.2
SFL	80.78^A±15	88.11^A±25.4	83.56^B±20.5	74.11^B±14.3	71.89^C±14.4

Data are presented as the mean values±sd.
Different letters on the same row indicate significant differences (A, B, C: P<0.001).
C, control group (n=6); D1, day 1 postsurgery; D2, day 2 postsurgery; D3, day 3 postsurgery; D4, day 4 postsurgery; D5, day 5 postsurgery; S, surgery group (n=6); SF, surgery-flunixin group (n=6); SFL, surgery-flunixin-lidocaine group (n=6); SL, surgery-lidocaine group (n=6).

TABLE 6: Postoperative respiratory rate (RR) expressed as breaths/minute

	D1	D2	D3	D4	D5
C	30.56±11.5	30.28±7.5	27.56±4.8	27.89±3.4	36.67±6.7
S	42.17^a±20.13	26.93^b±13.3	31.67^b±12.8	28.96^b±17.9	27.78^b±4.6
SL	38.18^a±19.7	37.89^b±11.9	37.67^b±15.9	35.00^b±9.5	36.20^b±9.5
SF	33.83^a±3.3	32.21^b±13.6	32.94^b±5.9	31.89^b±7.1	30.89^b±5.6
SFL	35.27^a±6.2	35.67^b±6.4	32.06^b±5.5	34.44^b±7.7	34.33^b±7.9

Different letters on the same row indicate significant differences (a, b: P<0.05).
Data are presented as the mean values±sd.
C, control group (n=6); D1, day 1 postsurgery; D2, day 2 postsurgery; D3, day 3 postsurgery; D4, day 4 postsurgery; D5, day 5 postsurgery;
S, surgery group (n=6); SF, surgery-flunixin group (n=6); SFL, surgery-flunixin-lidocaine group (n=6); SL, surgery-lidocaine group (n=6).

undergoing surgical castration were compared. The combination of systemic flunixin and intrafunicular lidocaine proved to be the best method, reducing postoperative pain and discomfort to levels similar to those in lambs not castrated (control group). The administration of lidocaine or flunixin alone caused a moderate reduction in pain but neither drug alone was as effective as the association.

The main limitations of the study are based on the low sample size, single observer measurements and absence of a strongly reliable pain assessment method. Due to ethical reasons and the invasiveness of the experimental procedure, we opted for a restricted sample size. Even though behavioural evaluations were based on a single observer, the blinding of this observer to the treatment and the specific knowledge of the topic reduced the potential individual bias. Pain evaluation lacks an objective and reliable method, so we combined different non-specific methods to increase the likelihood of reasonable results.

All the drugs used during the experiment were allowed on an extralabel use basis but not registered for use in food production animals in the EU.[38] In our experimental model of surgical castration, general anaesthesia was adopted to minimise restraint and responses to external and environmental stimuli that could affect the stress response to surgical manipulation and nociception. Lambs were maintained at F_EISO concentrations reported to be suitable to maintain general anaesthesia in ruminants that were not sedated before induction.[39] The use of general anaesthesia also allowed having a positive control (group S) receiving only the nociceptive stimuli without treatment. In case the surgical stimulation evoked too much pain, the rescue protocol would have ensured a proper deepening of the anaesthetic plan, avoiding suffering. Nevertheless, no group experienced such a level of pain during the procedure. Moreover, even if only six animals were allocated to this treatment, the use of a surgery group allowed the collection of cortisol data in three-month-old lambs castrated under general anaesthesia, which are lacking in the literature.

Concerning the intraoperative observations, the only relevant results were derived from the clinical signs (HR and RR), whereas F_EISO did not differ between groups; therefore, we cannot conclude that any of the treatments produced an ISO-sparing effect, differently from what was observed in a similar study on donkeys.[4] Even in this case, a larger sample size is necessary to examine in depth this result. Despite the lack of significance of the F_EISO
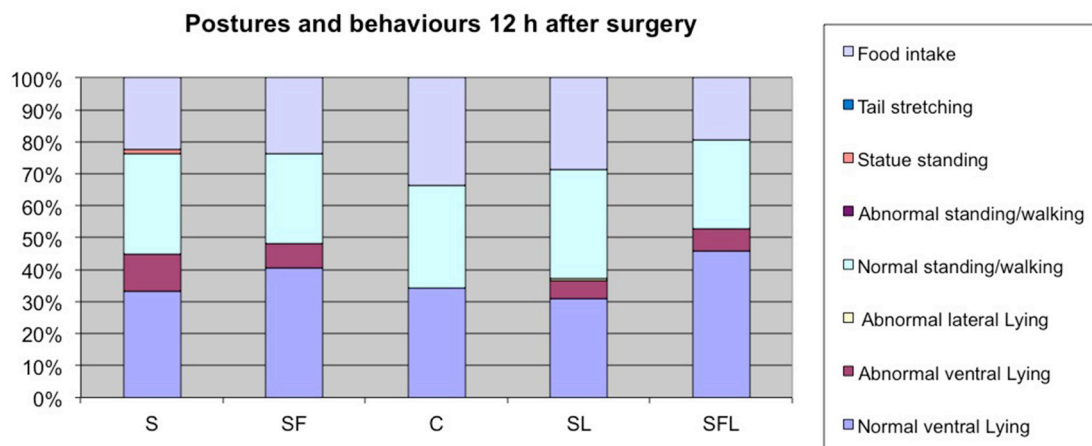
FIG 1: Postures and behaviour 12 hours after surgery in lambs belonging to the different treatment groups. Abnormal ventral lying was the most representative abnormal posture in the castrated animals (S 12 per cent, SF 8 per cent, SL 6 per cent, SFL 7 per cent). It was never observed in the C group. C, control group (n=6); S, surgery group (n=6); SF, surgery-flunixin group (n=6); SFL, surgery-flunixin-lidocaine group (n=6); SL, surgery-lidocaine group (n=6).

TABLE 7: Quantitative results of behavioural assessment of lambs during the 12-hour observation period after castration

	C	S	SL	SF	SFL
Normal ventral lying	34.11±0.88	33.14±0.89	30.86±1.34	41.02±1.24	45.88±2.13
Abnormal ventral lying	0.00[c]±0.00	11.83[a]±1.15	5.71[b]±1.09	7.51[b]±0.56	7.06[b]±0.89
Abnormal lateral lying	0.00±0.00	0.00±0	0.57±0.00	0.00±0.00	0.00±0.00
Normal standing/walking	32.56±0.91	31.36±1.6	34.29±1.94	28.03±0.89	27.65±1.78
Abnormal standing/walking	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Statue standing	0.00±0.00	1.18±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Tail stretching	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Food intake	33.34±0.94	22.49±1.89	28.57±2.00	23.43±0.94	19.41±1.56

Values are expressed as the percentage (%)±sd of total behaviours performed by lambs of each group during 12 hours after surgery. Different letters on the same row indicate significant differences (a, b, c: P<0.05).
C, control group (n=6); S, surgery group (n=6); SF, surgery-flunixin group (n=6); SFL, surgery-flunixin-lidocaine group (n=6); SL, surgery-lidocaine group (n=6).

data, groups S and C showed a tendency to have higher HRs and RRs compared with the other groups, although these differences were statistically significant only at Ta6 (application of skin staples) and Ta2, respectively; no differences were found between groups SF, SFL and C. This tendency suggests a treatment effect, although a larger sample size is needed to make stronger statements.

In contrast to our findings, the intrafunicular injection of lidocaine in donkeys undergoing castration had a sparing effect on isoflurane and prevented an increase in HR and MAP.[4] This difference could be ascribed to a different surgical approach or intraoperative evaluation system. The surgical castration of donkeys in the study of Suriano *et al*[4] is considered an inguinal approach, whereas the castration of the lambs in this study was achieved through a scrotal approach. It can, therefore, be hypothesised that cutaneous pain perception in the inguinal region is different from that of the scrotal region, although research on the density of sensitive innervation in these regions is needed to support this statement. Moreover, in Suriano's paper, a direct blood pressure monitoring was performed, while we relied on an indirect oscillometric measurement that did not allow a continuous evaluation of MAP. In addition, since we recorded intraoperative data every five minutes, it is possible that we were not able to detect an early variation in parameters that had to be ascribed to the acute autonomic response that occurred within two minutes after the stimuli, as described in cattle.[24]

In previous studies on the castration of lambs, pain relief and stress reduction were described after lidocaine injection into both the spermatic cord and scrotal neck[11 12] or into the scrotal neck alone.[9] As the scrotal somatic sensory innervation is due to nerves travelling in the subcutis of the scrotal neck,[40] failure of intrafunicular lidocaine alone to block sensory input to the scrotum can explain the different response to the anaesthetic injection in our study. Intrafunicular lidocaine, while establishing anaesthesia of the testicle and the epididymis through sensory blockade of the superior and inferior spermatic

nerves from the intermesenteric plexus, does not establish anaesthesia of the overlying skin that is provided by the iliohypogastric, ilioinguinal and genitofemoral nerves along with a branch of the pudendal nerves on the caudal surface of the scrotal sac.[41] For this reason, the absence of differences in intraoperative variables (HR, MAP and electroencephalographic curve) between piglets undergoing surgical castration under general anaesthesia with an intrafunicular or intratesticular lidocaine block can be ascribed to the subcutaneous injection of a local anaesthetic into the scrotum, achieving skin desensitisation.[42]

In other studies,[25 29] serum cortisol levels were considered to assess the relative magnitude of acute distress in animals receiving various analgesic treatments. In our study, pretreatment cortisol values were within the ranges previously reported in the literature,[43 44] indicating no stress related to the management and housing before surgery. In our castrated lambs, the combination of flunixin and intrafunicular lidocaine was the most effective method for reducing postoperative stress, with serum cortisol levels statistically similar to the baseline levels and to those of the control group. This association significantly reduced the 30-minute plasma cortisol peak and allowed for a rapid return to the presurgical level of cortisol. Consistent with previous findings,[11] we found that the administration of flunixin one hour before surgery did not eliminate the increased concentration of serum cortisol 30 minutes after surgery but allowed the return to baseline levels six hours after surgery. Cortisol values in group SL were similar to those in group SF but significantly higher than the baseline for a longer time compared with group SF.

Although severe acute pain induces autonomic and neuroendocrine responses that alter carbohydrate homeostasis and lead to glycogenolysis augmentation, gluconeogenesis and insulin resistance,[34] blood glucose was not affected at any time by surgery nor treatment. Findings regarding the relationship between haematological parameters and the level of pain associated with different types of castration are inconsistent.[45] In our study, we observed that

haematology was not affected at any time by open scrotal orchiectomy or by the pain management regimens used and therefore did not reflect the differences found in the plasma cortisol concentration.

The five-day postoperative clinical evaluation of lambs did not reveal any significant difference related to the treatment. However, on days 1 and 2, the HR and RR were significantly higher in all groups receiving surgery, indicating that the lambs experienced significant discomfort on these days compared with days 3–5. Although to the authors' knowledge, no references about postcastration variation of HR and RR in lambs are reported, these increases (HR and RR) can be ascribed to the intermediate phase of the surgical inflammatory response, when injured tissues are infiltrated by inflammatory cells with concurrent activation of haemostasis, complement cascades and lymphocytes, which are all oxygen-demanding processes.[46–48]

Although no 'gold standard' for pain exists, scales that have been used to assess sheep include the simple descriptive scale,[49] the numerical rating scale[50] and the visual analogue scale.[49 51] Recently, coding and quantification of facial expression in lambs were investigated using the Lamb Grimace Scale, with promising results in a small number of animals.[52] Since the results of pain evaluation with these systems in the literature seem to be controversial and subjective, postcastration pain and discomfort assessments in our study were achieved through physiological and behavioural responses associated with pain perception in sheep.[8 11 27 37 53]

Behaviour is a valuable indicator of distress, and pain-related behaviours have been proven to be useful indices of the duration and phases of a distressing experience. Previous studies[8] indicated that abnormal ventral lying is the posture most frequently observed in animals experiencing pain from castration. In our study, validation of pain behaviour was accomplished on the basis of previous literature[28] and appropriate training of the observer. In line with the results of other studies, our observations showed that after castration, lambs receiving no analgesic treatment exhibited abnormal ventral lying at a significantly higher frequency than did lambs of the other treatment groups. Even if the behavioural pattern used to identify the need of a rescue protocol (lack of appetite, overt suffering or depression after surgery) was not listed in Table 2, the animals were also observed for their general condition, to avoid the risk of underestimating pain and discomfort. The rescue protocol was not necessary in any case in both the preoperative and postoperative periods because the pain was controlled and moderate. Nevertheless, this fact may be a limitation of the study because moderate pain is not a good discriminant for testing an analgesia protocol.

In conclusion, we can say that in the absence of analgesic treatment, surgical castration produced an important and persistent negative effect on lambs up to nine hours after surgery. In agreement with other reports,[54 55] our results revealed that the association of flunixin and lidocaine was a reliable method for reducing pain and distress during and after castration. Neither the preoperative intrafunicular injection of lidocaine alone nor the preoperative flunixin systemic administration significantly reduced pain or distress, even though they proved to provide mild analgesia to surgically castrated lambs.

Despite the several limitations, the present study produced interesting results that deserve further future research in order to clarify some aspects and overcome the effects of said limitations.

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