Full Length Article

Effect of the number of incisions and use of local anesthesia on the physiological indicators of surgically-castrated piglets

Efraín Pérez-Pedraza[a], Daniel Mota-Rojas[b,*], Ramiro Ramírez-Necoechea[b], Isabel Guerrero-Legarreta[c], Julio Martínez-Burnes[d], Karina Lezama-García[b], Patricia Mora-Medina[e], Marcelino Rosas[f], Victor Martínez[g], Miguel González-Lozano[g]

[a] Ph.D. Program in Biological and Health Sciences [Programa de Doctorado en Ciencias Biológicas y de la Salud], Universidad Autónoma Metropolitana (UAM), Mexico City, Mexico
[b] Neurophysiology, Behavior and Assessment of Welfare in Domestic Animals. Department of Animal Production and Agriculture. Universidad Autónoma Metropolitana (UAM), Mexico City, Mexico
[c] Department of Biotechnology, Universidad Autónoma Metropolitana, Unidad Iztapalapa, Mexico City, Mexico
[d] Graduate and Research Department, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tamaulipas, Victoria City, Tamaulipas, Mexico
[e] Department of Livestock Sciences, FESC, Universidad Nacional Autónoma de México, Cuautitlan Izcalli, State of Mexico, Mexico
[f] Biological Sciences Department, Universidad Nacional Autónoma de México, Facultad de Estudios Superiores Cuautitlán, FESC, State of Mexico, Mexico
[g] Centro de Enseñanza, Investigación y Extensión en Producción Porcina (CEIEPP), FMVZ, Universidad Nacional Autónoma de México (UNAM), Mexico City, Mexico

ARTICLE INFO

ABSTRACT

The objective of this study was to determine the effect of local anesthesia and the number of incisions performed on the physiological blood profile of piglets after surgical castration. A total of 60 male piglets were divided into five groups of 12 each, based on the surgical method employed and the use, or not, of local anesthesia, as follows: surgical castration using one horizontal incision in both testicles with (C1 + L) and without (C1) local anesthesia; surgical castration using two vertical scrotal incisions with and without local anesthesia (C2 + L and C2); and control piglets which were removed from their pens and held head-down by their hind limbs for approximately 90 s to simulate castration (SIM). Reference blood samples were drawn 24 h before castration (RV), immediately after surgery or simulated castration (PC), and at 24 and 48 h post-castration, to determine physiological profiles including; pH, hematocrit, glucose, electrolytes, lactate, pCO_2 (mmHg), SO_2 (mmHg), and bicarbonate. Results showed increases in lactate and hematocrit immediately after surgical or simulated castration with decreases in pH, HCO_3- and base excess (BE). Surgical castration produced marked alterations of the physiological profile, detected by reduced pH and HCO_3, higher lactate levels and BE alterations. These changes indicated metabolic acidosis that was greater in the piglets castrated surgically with one horizontal incision than in those castrated with two vertical incisions. More research is needed on the use of lidocaine during surgical castration, as it showed no effect on physiological profile in this study, but did alter hematocrit values.

1. Introduction

In the first days after birth, piglets undergo several production practices that induce pain and stress, so a number of studies have been performed to identify techniques that will cause less harm to their well-being; e.g.: tooth-clipping vs. tooth-grinding [1–3], tearing vs. cutting the spermatic cords during surgery [4], and hot- vs. cold-iron docking of the neonates' tails [5]. Surgical castration is a particularly controversial

production practice, but one performed to obtain several recognized benefits: (a) preventing unwanted breeding; (b) reducing fighting in mature pigs; (c) safer handling of boars; and, (d) reducing the risk of boar taint in meat [6–8]. However, no research has yet been conducted to determine whether the number of scrotal incisions during surgical castration affects the piglets' well-being. The most common castration method involves making a surgical incision in the scrotum to reveal the testes, which are then removed by tearing, cutting or twisting [9]. This

is traditionally done at a young age and without anesthesia [10], but strong evidence suggests that it involves various stressful events that are likely to cause pain, including incisions, extracting the testes, and severing the spermatic cords [11,12]. While there is no consensus on how many scrotal incisions should be made, the technique most often used consists of two, not one [13]. When two incisions are made, they are normally longitudinal in each testicle [11], while the single-incision method requires a transverse cut across both testicles and into the scrotum and tunica vaginalis [7]. However, surgical castration triggers physiological responses that reflect pain during the operation and in the ensuing hours. The stress and pain present during surgery can activate both the hypothalamic-pituitaryadrenal axis and the sympathetic nervous system (SNS) [14]. Physiological changes of this kind are important for evaluating the level of distress that farm animals suffer when exposed to acute noxious circumstances, like castration, tail-docking or dehorning [12,15]. Certainly, these effects and physiological responses can be mitigated by administering general or local anesthesia, while post-operative pain is often relieved with analgesics [16,17].

Most current research focuses on the impact of surgical procedures on normal behavior [18], cortisol levels [15], and immune system responses [11], so no studies have been published on the effect of surgical castration with or without local anesthesia on the physiological profiles of piglets. Hence, the objective of this work was to determine to what extent the number of incisions performed during the surgical castration of piglets affects their physiological profile. The decrease of this response due to the use of local anesthesia during surgery was also assessed.

2. Materials and methods

2.1. Ethical note

All handling of animals in this study was followed the procedures outlined for the use and care of animals in Mexico's norm NOM-062-ZOO-1999 (Department of Agriculture, Ranching, Rural Development, Fishing and Alimentation for animal-based experimentation [19]. The study took place in a porcine farm located in Amecameca, Mexico (Latitude: 19° 07′ 25.82″ N. Longitude: −98° 45′ 59.36″ W.), following The Code of Ethics of the World Medical Association (Helsinki Declaration) and in strict accordance with the guidelines for the ethical use of animals in applied ethological studies, recorded elsewhere [20].

2.2. Animals

Data were collected from 60, 5-day old male piglets (Yorkshire x Landrace) obtained from twelve different litters delivered by third-parity sows. Neonates with abnormalities in the position of the testicles (unilateral or bilateral cryptorchidism, scrotal hernias, etc.) or signs of systemic disease were excluded. Following the regular handling practices at the host farm, on day 3 post-farrowing, all piglets received a subcutaneous injection of 1 mL (100 mg) of Fe^{3+} in the form of iron dextran. They were not subjected to tooth or tail resection before the experiment. The sows and their litters were held in 1.9–2.5 m^2 farrowing pens. Dimensions were 2.07 m long × 0.60 m wide × 1.01 m high, with a total space of 2 m wide by 2.5 m long. Each pen was equipped with a 50-cm stainless steel feeder and a nipple drinker, and its creep area was warmed by a radiant electric lamp. Each room had 12 pens set up in two rows of six pens each with a dividing aisle. The pens had slatted, wire mesh floors with a plastic carpet over them. Fans and air-heating were installed to control ventilation and temperature (27 °C and 60% relative humidity in the farrowing room). Sows and piglets had *ad libitum* access to water in separate nipple drinkers. On day one of life, all piglets were weighed and identified individually by marking a number on their back in different colors to distinguish the experimental groups. All procedures were carried out by the same person and near the farrowing pens.

Each one of the five study groups consisted of 12 piglets divided according to the number of incisions (one or two) received during surgical castration and with or without the use of the local anesthetic, lidocaine. There was also a control group that underwent simulated castration (SIM). The groups were identified as follows: castration with one horizontal incision in both testicles with and without lidocaine as a local analgesic (C1 + L and C1); castration with two vertical scrotal incisions with and without local anesthesia (C2 + L and C2); and a control group with piglets that were removed from their pens and held head-down by their hind limbs for approximately 90 s (SIM) near the farrowing pen similar to the groups subjected to surgery.

2.3. Surgical procedure

Before surgical castration, the skin of the scrotal area was cleaned with chlorhexidine and isopropyl alcohol. Lidocaine at 10 mg/mL (Pisacaina 2%, Pisa® Laboratories S.A. de C.V., Mexico) was the local anesthetic, with 0.5 mg injected into each testicle, anticipating that it would spread into the spermatic cord [21]. The piglets were returned to the farrowing pen with the others to await castration. Lidocaine was administered at least 10 min before castration following the methodology in Kluivers-Poodt et al. [22].

Surgery for piglets in groups C2 + L and C2 was performed with a scalpel. Two vertical incisions were made in the scrotum, the testicles were extracted, and the spermatic cords were severed [11]. For groups C1 + L and C1, castration involved making just one horizontal incision in the scrotum to expose the testicles. In both cases spermatic cords were scarified with a scalpel, and the testicles removed [23].

All operations were performed by an experienced surgeon on the same day with the piglets restrained in supine position by an assistant. The skin over the scrotum was tautened with one hand to help expose the testicles and incision site. According to treatment group, castration was performed by making one or two incisions (10 mm) on the skin of the scrotum with a scalpel to expose the testicles. Next, the vaginal tunic and spermatic cords were cut, and the testicles removed. Immediately after each castration, a healing agent was applied to the wound (Negasunt Powder 20 Gm®: Coumaphos: 3%, Propoxur: 2%, Prontalbin: 5%). It is important to note that no other post-operative treatment was applied and that castration time was 90 s per piglet.

2.4. Blood samples

Blood samples were collected from the *cava cranialis* vein using 1-mL syringes –pre-prepared with lithium heparin– at five moments: 24 h before castration (RV); immediately after surgery (PC: post-castration); and then at 6, 24 and 48 h after castration. All samples were obtained in less than 15 s while the piglets were physically restrained, by placing them in the same position as for castration. The study personnel who drew the samples had experience and were fully-trained. From each sample, 150 μL were placed in an analyzer to determine the parameters of plasma, gas and electrolytes (GEM Premier, Instrumentation Laboratory Co., Lexington, USA; Instrumentation Laboratory SpA, Milano, Italy). Experimenters processed samples immediately to ascertain the physiometabolic profiles as follows: pH, hematocrit (%), glucose (mg/dL), electrolytes (Na$^+$, K$^+$, Ca^{++} [mmol/ L]), lactate levels (mg/dL), partial pressures of carbon dioxide [PvCO$_2$ (mmHg) and PvO$_2$(mmHg)], and bicarbonate (HCO$_3$ −).

2.5. Statistical analyses

Statistical analyses were performed with the SAS program version 9.3 (SAS Inst. Inc., Cary, NC, USA). A general MIXED linear model was applied to analyze the values of the physiological profiles. Results are presented as means and standard errors.

To evaluate the effect of the number of incisions and the use of

lidocaine on the physiological profiles, and to directly model and direct the structure of covariance in order to obtain valid standard errors and efficient statistical tests, we utilized the mixed linear model methodology to analyze the repeated measures data of the metabolites that were examined in the blood from the same experimental units (piglets) 24 h pre-castration, immediately post-surgery, and then at intervals of 6, 24 and 48 h. The mixed linear model applied to analyze the blood values included the fixed effects of: 1) the number of incisions made during castration; 2) treatment group; 3) measurement interval; and 4) the first and second order interactions among these factors, taking the piglet as the random effect. To model the structure of covariance, we utilized the PROC MIXED of the Statistical Analysis System (SAS, 2012), where the between-animal variation was specified using the RANDOM instruction, and the covariation within animals was specified using the REPEATED instruction. The analyses focused on comparisons between treatment groups and measuring intervals pre- and post-surgery.

The personnel responsible for all evaluation procedures, and those who gathered the results of the study were not informed as to the treatments involved and had no role in dividing the groups of piglets or in the final analysis of the data. Also, the researcher who was in charge of the statistical analyses was unaware of the treatment regimens applied. In all statistical tests, the level of significance was set at a two-tailed $P < .05$.

3. Results

Piglets in the two-incision group showed fewer alterations and better recovery in terms of acid-base balance at all sampling intervals. Regarding hematocrit values, this group also showed greater stability between sampling times when lidocaine was applied as a local anesthetic prior to castration. Hematocrit values showed a similar behavior in the group castrated with one incision when lidocaine was used; however, no between-group differences were found in terms of blood gases exchange. Finally, the pH buffer system showed within-group differences between the different sampling times (reference $vs.$ post-castration values).

3.1. Acid-Base balance (pH and blood lactate)

Blood pH showed a strong decrease after castration in all groups, even SIM (samples: RV $vs.$ PC). For the piglets in groups C1 + L ($P < .0001$) and C1 ($P < .0001$), the difference was 0.18 lower, while for those in groups C2 + L ($P = .0092$) and C2 ($P = .0019$), the decrease was 0.14. The decrease for group SIM, meanwhile, was 0.13 ($P < .0001$). pH was re-established at six hours after castration in all piglets regardless of group (Fig. 1a).

All groups, including SIM, showed statistically-significant increases in blood lactate levels immediately after castration (samples: RV $vs.$ PC). The increase in group C1 + L was 139.3% ($P < .0001$), while for group C1 it was 134.8% ($P < .0001$) when post-castration values were compared to reference values (PC $vs.$ RV). Of the piglets castrated with two incisions, group C2 + L showed the smallest increase in lactate, at 28.3% ($P = .0039$), upon comparing RV to PC. The increase in group C2 was 172.7% ($P < .0001$), the highest value observed (RV $vs.$ PC). The piglets that underwent simulated castration (SIM) showed a 95.8% increase in lactate ($P < .0001$). Though no significant differences in lactate levels were found among groups at the same sampling times, CL + 2 (surgical castration with two incisions and lidocaine) showed a lower increase than SIM at the post-castration and post-simulated castration times (Fig. 1b).

3.2. Blood gas measurements

The blood gas exchange only showed changes in the piglets castrated using one incision and lidocaine as anesthetic (group C1 + L). The increase in $PvCO_2$ was 28.4% immediately after castration (RV $vs.$
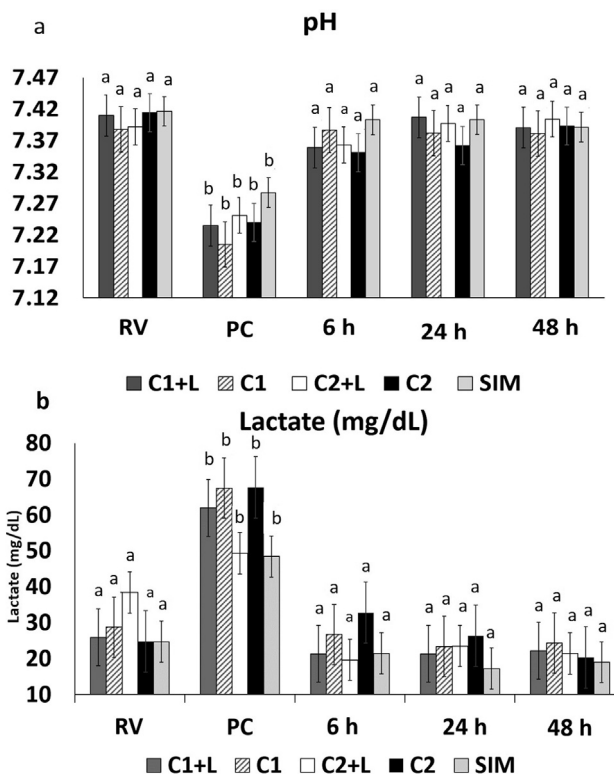

Fig. 1. Acid-Base balance in surgically-castrated piglets after one or two incisions, and with or without local anesthesia. C1: surgically-castrated piglets with one scrotal incision; C2: surgically-castrated piglets with two scrotal incisions; + L: Lidocaine use; SIM: Simulated castration. RV: Reference Values; PC: Blood sample obtained immediately after castration; 6 h, 24 h and 48 h: Blood samples taken at 6, 24 and 48 h after castration. Different letters indicate significant differences between sampling times within groups (same color bars).

PC: $P = .0082$). In no other group did the blood gas measurements show significant differences, regardless of the number of incisions or the use of local anesthesia, and between groups as well as between sampling times within each group. However, the decrease in PvO_2 at post-castration, and the later increase, were noted in every group compared to the remaining sampling times within groups, including SIM (Table 1).

3.3. Acid-Base disorders

Table 2 shows significant blood bicarbonate concentration decreases in all groups immediately after castration (PC $vs.$ RV samples). Despite this immediate post-castration decrease, these values returned to normal levels after six hours. By group, the C1 piglets showed the sharpest decrease –6.73 mmol/L ($P < .0001$)– followed by group C2 at 5.9 mmol/L ($P = .0017$), and groups C2 + L and C1 + L with decreases of 4.9 mmol/L ($P = .0015$) and 3.6 mmol/L ($P < .0001$), respectively. The lowest decrease was in SIM at 3.21 mmol/L ($P < .0008$, Table 2).

Regarding base excess (BE), while no significant differences were found between groups when sampling times were compared, differences were observed in RV $vs.$ PC in most groups. The decreases in base excess levels compared to reference values were as follows: 9.88, 7.28, 6.81, and 4.06 mmol/L ($P < .0001$ in all cases) for groups C1, C1 + L, C2 + L and SIM, respectively. No significant differences were found in C2 ($P = .1145$) (Table 2).

3.4. Glucose and hematocrit

No significant differences were found in hematocrit values between groups, though C1 showed a significant increase of 5.91% ($P = .0298$)

Table 1
Blood gas exchange of surgically-castrated piglets after 1 or 2 incisions.

PvO_2(mm/Hg) Group	Castrated				SIM Mean $\pm$ SE	P-value
	One incision		Two incisions			
	C1 + L Mean $\pm$ SE	C1 Mean $\pm$ SE	C2 + L Mean $\pm$ SE	C2 Mean $\pm$ SE		
RV	37.10 $\pm$ 6.36	35.36 $\pm$ 6.07	38.50 $\pm$ 6.36	39.00 $\pm$ 5.38	55.00 $\pm$ 7.61	0.3274
PC	28.58 $\pm$ 4.29	31.07 $\pm$ 4.72	27.00 $\pm$ 6.25	39.70 $\pm$ 5.59	33.36 $\pm$ 5.33	0.5226
6 h	35.30 $\pm$ 4.94	32.55 $\pm$ 5.21	26.44 $\pm$ 5.21	37.81 $\pm$ 4.71	38.00 $\pm$ 4.71	0.4722
24 h	31.50 $\pm$ 4.33	31.18 $\pm$ 4.13	28.36 $\pm$ 4.13	36.66 $\pm$ 4.57	32.11 $\pm$ 4.57	0.7621
48 h	41.00 $\pm$ 3.83	33.33 $\pm$ 4.43	42.57 $\pm$ 5.02	31.66 $\pm$ 5.42	35.71 $\pm$ 5.02	0.4195
P-value	0.3348	0.9592	0.1054	0.8898	0.2503	
$PvCO_2$(mm/Hg)						
RV	38.66 $\pm$ 2.70[a]	42.40 $\pm$ 3.23	38.80 $\pm$ 3.24	40.23 $\pm$ 2.80	39.85 $\pm$ 2.29	0.8219
PC	49.64 $\pm$ 1.96[b]	48.35 $\pm$ 2.73	44.75 $\pm$ 3.62	44.10 $\pm$ 3.20	44.36 $\pm$ 1.83	0.6857
6 h	42.55 $\pm$ 2.56[ab]	42.55 $\pm$ 3.41	45.66 $\pm$ 3.41	43.72 $\pm$ 3.05	38.70 $\pm$ 1.92	0.5402
24 h	42.20 $\pm$ 2.56[ab]	42.27 $\pm$ 3.08	40.81 $\pm$ 3.08	42.60 $\pm$ 3.20	40.44 $\pm$ 2.02	0.9576
48 h	39.90 $\pm$ 2.44[a]	44.25 $\pm$ 3.62	38.28 $\pm$ 3.87	40.60 $\pm$ 4.52	42.42 $\pm$ 2.29	0.3870
P-value	0.0082	0.5261	0.4569	0.8718	0.2660	

C1: surgically-castrated piglets with one scrotal incision; C2: surgically-castrated piglets with two scrotal incisions; +L: Lidocaine use; SIM: Simulated castration.
RV: Reference Values; PC: Blood sample obtained immediately after castration; 6 h, 24 h and 48 h: Blood samples taken at 6, 24 and 48 h after castration.
Different letters indicate significant differences between sampling times within groups (same column).

after castration, while group C2 had an increase of 8.17% (P = .0462) compared to reference values (Table 3). Glucose, Na^+, K^+, and Ca^{++} concentrations showed no differences among groups or sampling times within groups.

4. Discussion

The results of this study show physiological alterations (pH, lactate, PvO_2, $PvCO_2$, HCO_3-, HCT) in all groups after surgical castration or simulated castration, an effect that was especially strong on the acid-base balance. Two particularly important results were the decrease in pH values and the increase in lactate levels, which more than doubled the RV for each group, except C2 + L, which showed greater profile stability. As a consequence, a marked reduction in HCO_3- and BE levels was observed, especially in the piglets in the groups castrated surgically with one scrotal incision and those castrated surgically without pre-surgery anesthesia. Likewise, the hematocrit values were significantly higher values in the groups that were castrated surgically without lidocaine.

Blood gas exchange observed in this study can be explained by the considerable $PvCO_2$ increase found in all groups, including SIM. This increase has been observed previously in pigs in association with a physiological response to stressful stimuli like handling and weaning at 8 and 15 days [24]. Descriptions of this phenomenon emphasize the incidence of metabolic acidosis that the animals attempted to modulate through hyperventilation [25].

Blood pH values decreased immediately after surgical castration in all groups including SIM; however, the imbalance was greater in the piglets castrated with one incision than in those castrated using two. The increase in lactate was apparent at PC sampling in both the surgically-castrated groups and the simulated group. Thus, the decrease in pH may be due to the fact that the tissues affected by castration are innervated, so the damage caused by surgical castration probably produces painful stimuli in response to the activation of the sympathetic nervous system [26]. When pigs find themselves in stressful conditions, they may shift from aerobic to anaerobic metabolism, which means producing ATP from muscle glycogen [27]. As a consequence of catecholamine stimulation, glycogen is mobilized, leading to a transient increase in lactate from muscles [28]. Blood pH then decreases due to the increase in lactate [29], which was observed in both surgical

Table 2
Blood acid-base balance of surgically-castrated piglets after 1 or 2 incisions, with and without administration of a local anesthetic (lidocaine).

HCO_3- (mmol/L) Group	Castration				SIM Mean $\pm$ SE	P-value
	One incisión		Two incisions			
	C1 + L Mean $\pm$ SE	C1 Mean $\pm$ SE	C2 + L Mean $\pm$ SE	C2 Mean $\pm$ SE		
RV	24.42 $\pm$ 0.96[a]	25.31 $\pm$ 0.91[a]	23.19 $\pm$ 0.91[a]	24.44 $\pm$ 0.96[a]	24.20 $\pm$ 0.0.96[a]	0.6057
PC	20.82 $\pm$ 0.79[b]	18.58 $\pm$ 0.87[b]	19.10 $\pm$ 1.15[b]	18.53 $\pm$ 1.03[b]	20.99 $\pm$ 0.98[b]	0.1597
6 h	24.65 $\pm$ 0.82[a]	25.21 $\pm$ 0.98[a]	25.16 $\pm$ 0.94[a]	23.81 $\pm$ 1.01[a]	24.41 $\pm$ 0.7[a]	0.6575
24 h	26.58 $\pm$ 0.86[a]	24.82 $\pm$ 0.88[a]	24.83 $\pm$ 0.80[a]	23.38 $\pm$ 1.06[a]	25.27 $\pm$ 0.79[a]	0.5710
48 h	23.75 $\pm$ 0.58[a]	25.55 $\pm$ 0.67[a]	23.71 $\pm$ 0.76[a]	24.82 $\pm$ 0.90[a]	25.48 $\pm$ 0.76[a]	0.2103
P-value	< 0.0001	< 0.0001	0.0015	0.0017	0.0008	
Base excess (BE) (mmol/L)						
RV	0.61 $\pm$ 1.24[a]	0.79 $\pm$ 1.17[a]	−0.84 $\pm$ 1.17[a]	0.84 $\pm$ 1.24	−0.22 $\pm$ 1.24[a]	0.8209
PC	−6.67 $\pm$ 1.13[b]	−9.09 $\pm$ 1.25[b]	−7.65 $\pm$ 1.65[b]	−4.61 $\pm$ 1.56	−4.28 $\pm$ 1.41[b]	0.0853
6 h	−0.36 $\pm$ 0.88[a]	0.72 $\pm$ 0.93[a]	0.42 $\pm$ 0.93[a]	−1.26 $\pm$ 0.84	0.37 $\pm$ 0.84[a]	0.5018
24 h	2.09 $\pm$ 0.96[a]	0.34 $\pm$ 0.92[a]	0.69 $\pm$ 0.92[a]	−1.58 $\pm$ 0.96	1.20 $\pm$ 1.01[a]	0.1156
48 h	−0.44 $\pm$ 0.76[a]	0.92 $\pm$ 0.87[a]	−0.17 $\pm$ 0.99[a]	0.03 $\pm$ 1.07	0.97 $\pm$ .99[a]	0.5364
P-value	< 0.0001	< 0.0001	< 0.0001	0.1145	0.0011	

C1: surgically-castrated piglets with one scrotal incision; C2: surgically-castrated piglets with two scrotal incisions; +L: Lidocaine use; SIM: Simulated castration.
RV: Reference Values; PC: Blood sample obtained immediately after castration; 6 h, 24 h and 48 h: Blood samples taken at 6, 24 and 48 h after castration.
Different letters indicate significant differences between sampling times within groups (same column).

Table 3

Hematocrit (HTC) percentage in surgically-castrated piglets after 1 or 2 scrotal incisions with and without administration of a local anesthetic (lidocaine).

HCT (%) Group	Castrated				SIM Mean ± SE	P-value
	One incision		Two incisions			
	C1 + L Mean ± SE	C1 Mean ± SE	C2 + L Mean ± SE	C2 Mean ± SE		
RV	33.42 ± 2.38	32.66 ± 2.07[ab]	31.16 ± 3.35	29.63 ± 2.03[a]	31.00 ± 2.30	0.6460
PC	38.00 ± 1.80	38.57 ± 1.66[b]	31.42 ± 3.10	37.80 ± 2.13[b]	36.63 ± 1.96	0.2093
6 h	34.90 ± 1.99	33.77 ± 2.07[ab]	31.33 ± 2.73	30.45 ± 2.03[a]	31.63 ± 1.96	0.6575
24 h	34.60 ± 1.99	33.81 ± 1.88[ab]	33.27 ± 2.47	31.77 ± 2.24[a]	30.66 ± 2.17	0.5710
48 h	33.00 ± 1.90	29.28 ± 2.35[a]	38.16 ± 3.35	36.50 ± 3.37[a]	33.40 ± 2.91	0.2044
P-value	0.2876	0.0298	0.5167	0.0462	0.2382	

C1: surgically-castrated piglets with one scrotal incision; C2: surgically-castrated piglets with two scrotal incisions; +L: Lidocaine use; SIM: Simulated castration. RV: Reference Values; PC: Blood sample obtained immediately after castration; 6 h, 24 h and 48 h: Blood samples taken at 6, 24 and 48 h after castration. Different letters indicate significant differences between sampling times within groups (same column).

castration and SIM groups.

Blood pH levels normally remain within narrow limits because of the action of various corporal buffer systems [30], including HCO_3-. When an increase in the production or concentration of hydrogen ions occurs, HCO_3- takes additional hydrogen ions from the environment, thus forming carbonic acid, which dissolves in water and carbonic anhydride and is then liberated through respiration [31]. Hence, the pH decrease and lactate increase in SIM and piglets castrated surgically with one or two incisions could alter the bicarbonate concentrations and base-excess values as found in this study.

On this issue, Ritter et al. [32] observed that as pigs are exposed to additional stressors, their rectal temperature and blood lactate levels increased linearly, while blood pH, bicarbonate and base-excess values declined, also in a linear fashion. This suggests that restraint and castration could cause more pronounced physiological alterations than those result from restraint exclusively. This argument is consistent with the findings of this study in terms of the higher lactate levels detected in the surgically-castrated piglets compared to the neonates in the simulated castration group, though this difference was not significant.

It is important to consider that the testicular protection provided by the scrotum depends on three connective tissue structures (skin, the dartos fascia and the scrotal fascia). Also, the testicles are separated by a significant amount of connective and fat tissues that form the scrotal wall [16]. For these reasons, the restraining method used during surgical castration, and the type of surgical incision made, could have additional consequences for blood gases and metabolites, as we observed in this study. Thus, a surgical incision made directly over the scrotal raphe (C1 groups) turns out to be a more invasive method due to the aforementioned anatomical characteristics and the polymodal nociceptors, when compared to the castration technique that makes two incisions, one over each testicle (i.e., C2 + L), since those incisions sever only three connective tissue structures.

Lidocaine, a local anesthetic, has typically been evaluated by analyzing vocal parameters and plasma cortisol. Applying lidocaine reduces the pain and stress involved in the castration procedure [22]. Regarding the effect of administering local anesthesia prior to surgical castration, our results show no statistical differences between the surgically-castrated groups with and without the use of lidocaine; however, Haga and Ranheim's [10] findings suggest that when castration is performed with no local anesthesia the nociceptive response generated is more pronounced than when lidocaine is applied. This suggests that injecting this anesthetic does, indeed, decrease pain and modulate cardiovascular responses to castration. All local anesthetics act by interrupting the onset and propagation of action potentials in nerve cells by blocking the increase in $Na+$ conductance which, of course, is voltage-dependent [33]. Thus, when lidocaine is injected into the testis and/or spermatic cord it proved to be effective in reducing the acute pain that castration induces [10].

The unexpected result of this study was the absence of any effect of lidocaine use on physiological responses. One possible explanation of this finding is that the lidocaine did not flow evenly into all the tissues affected by castration, with the result that the sensory block achieved was uneven [10]. It should be noted that we administered the lidocaine 10 min prior to surgery, but Ranheim and Haga [34] found only low lidocaine concentrations in the cremaster muscle ten minutes after injection into the testes. Therefore, the 10-minute interval may be insufficient to achieve a complete sensory block, a condition that might also affect other tissues, including the scrotal ligament and the intra-abdominal area of the spermatic cord.

Although no differences were found in the state of acidosis and blood gas diffusion in response to the use of local anesthesia, it is important to note that the hematocrit was significantly higher immediately after surgery in the piglets castrated without lidocaine. This can be attributed to two reactions: first, splenic contraction and, at least in part, the lower volume of blood plasma. Splenic contractions are triggered by the catecholamines liberated whenever the sympathetic branch of the autonomic nervous system is stimulated [35]. Additionally, the piglets in groups C1 and C2 showed the largest decrease in serum bicarbonate values compared to groups C1 + L and C2 + L. The drop in serum bicarbonate produces an excess of free protons, and when a lower than normal blood pH is found, metabolic acidemia is evidenced.

Alterations of the physiological profiles differed statistically only in the blood samples obtained from each group immediately after castration. Other studies have shown that castration causes reactions –both physiological and behavioral– that reflect pain. These reactions may be quite marked during surgery and the early hours post-castration, though they diminish quickly. Some behavioral alterations, however, have been seen to persist for a few days [34]. In their evaluation of the effect of lidocaine on cortisol levels during surgical castration in piglets as an indicator of stress and pain, Marsalek et al. [11] found that concentrations of cortisol increased in a significant manner at 1 h after the surgery was performed, and that administering lidocaine did not reduce those concentrations. In this regard, Davis et al. [36] found fewer physiological alterations (i.e., cortisol levels) 30 min after simulated castration in piglets compared to surgically-castrated animals. Similarly, Prunier et al. [16] showed that the plasma adrenocorticotropic hormone (ACTH) increased and then peaked at 5 min after surgery, but returned to the pre-surgery level within 3 h.

5. Conclusions

Regardless of the number of incisions, scrotal surgical castration produces alterations in the blood physiological profiles of piglets. In fact, a tendency towards more pronounced blood physiological profile mismatches is observed in neonates castrated with one horizontal incision. Likewise, though to a lesser degree, simulated castration also causes alterations in the physiological profile compared to surgically-

castrated piglets. Therefore, alterations in the physiological profile of piglets that underwent surgical castration are evident only immediately after castration, and return to normal values within 6 h of surgery. Clearly, more research is needed on the use of lidocaine during surgical castration because, even though our results did not reach statistical significance for the complete physiological profile, they do show an effect on hematocrit values and the state of acidosis. Thus, we conclude that lidocaine can be helpful in reducing alterations in the physiological profile of the blood in surgically-castrated piglets.

Competing interests

There is no competing interest to declare.

References

[1] Hay M, Rue J, Sansac C, Brunel G, Prunier A. Long-term detrimental effects of tooth clipping or grinding in piglets: a histological approach. Anim welfare 2004;13:27–32.

[2] Holyoake P, Broek D, Callinan A. The effects of reducing the length of canine teeth in sucking pigs by clipping or grinding. Aust Vet J 2004;82:574–6.

[3] Moya SL, Boyle L, Lynch P, Arkins S. Influence of teeth resection on the skin temperature and acute phase response in newborn piglets. Anim walfare 2006;15:291–7.

[4] Taylor AA, Weary DM. Vocal responses of piglets to castration: identifying procedural sources of pain. Appl Anim Behav Sci 2000;70:17–26.

[5] Sutherland M, Bryer P, Krebs N, McGlone J. Tail docking in pigs: acute physiological and behavioural responses. Animal 2008;2:292–7. https://doi.org/10.1017/S1751731107001450.

[6] Rault JL, Lay D, Marchant-Forde J. Castration induced pain in pigs and other livestock. Appl Anim Behav Sci 2011;135:214–25. https://doi.org/10.1016/j.applanim.2011.10.017.

[7] Kluivers-Poodt M, Zonderland JJ, Verbraak J, Lambooij E, Hellebrekers J. Pain behaviour after castration of piglets; effect of pain relief with lidocaine and/or meloxicam. Animal 2013;7:1158–62.

[8] O'Connor A, Anthony R, Bergamasco L, Coetzee J, Gould S, Johnson A, et al. Pain management in the neonatal piglet during routine management procedures. part 2: grading the quality of evidence and the strength of recommendations. Anim Health Res Rev 2014;15:39–62. https://doi.org/10.1017/S1466252314000073.

[9] Godyn D, Herbut E, Walczak J. Infrared thermography as a method for evaluating the welfare of animals subjected to invasive procedures – a review. Ann Anim Sci 2013;13:423–34. https://doi.org/10.2478/aoas-2013-0027.

[10] Haga HA, Ranheim B. Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. Vet Anaesth Analg 2005;32:1–9.

[11] Marsalek P, Svoboda M, Bernady J, Vecerek V. Concentrations of neopterin, biopterin, and cortisol associated with surgical castration of piglets with lidocaine. Czech J Anim Sci 2015;60:473–8. https://doi.org/10.17221/8555-CJAS.

[12] Mota-Rojas D, Ceballos M, Orihuela A, Corredor M, Pérez E, Ramírez R, et al. Painful practices in farm animals. In: Mota-Rojas D, Velarde A, Huertas-Canen S, Cajiao MN, editors. Animal welfare (in spanish). España: Elsevier; 2016. p. 137–54.

[13] Fredriksen B, Furnols MFI, Lundstrom K, Migdal W, Prunier A, Tuyttens FAM, et al. Practice on castration of piglets in Europe. Animal 2009;3:1480–7.

[14] Bonastre C, Mitjana O, Tejedor M, Calavia M, Yuste AG, Úbeda L, et al. Acute physiological responses to castration-related pain in piglets: the effect of two local anesthetics with or without meloxicam. Animal 2016;10:1474–81. https://doi.org/10.1017/S1751731116000586.

[15] Marchant-Forde J, Lay D, McMunn K, Cheng HW, Pajor A, Marchant-Forde RM. Postnatal piglet husbandry practices and well-being: the effects of alternative techniques delivered in combination. J Anim Sci 2009;92:1150–60. https://doi.org/10.2527/j as. 2008-1080.

[16] Prunier A, Bonneau M, Von Borell E, Cinotti S, Gunn M, Fredriksen B, et al. A review of the welfare consequences of surgical castration in piglets and the evaluation of non-surgical methods. Anim welfare 2006;15:277–89.

[17] Von Borell E, Baumgartner J, Giersing M, Jaggin N, Prunier A, Tuyttens F, et al. Animal welfare implications of surgical castration and its alternatives in pigs. Animal 2009;3:1488–96. https://doi.org/10.1017/S1751731109004728.

[18] Moya SL, Boyle LA, Lynch PB, Arkins S. Effect of surgical castration on the behavioural and acute phase responses of 5-day-old piglets. Appl Anim Behav Sci 2008;111:133–45. https://doi.org/10.1016/j.applanim.2007.05.01.

[19] NOM-062-ZOO-1999. Technical specifications for production, carea and use of animals. México: Diario oficial de la federación. México D. F.

[20] Sherwin CM, Christiansen SB, Duncan IJ, Erhard HW, Lay DC, Mench JA, et al. Guidelines for the ethical use of animals in applied ethology studies. Appl Anim Behav Sci 2003;81:291–305.

[21] Hansson M, Lundeheim N, Nyman G, Johansson G. Effect of local anaesthesia and/or analgesia on pain responses induced by piglet castration. Acta Vet Scan 2011;53:34–42.

[22] Kluivers-Poodt M, Houx B, Robben S, Koop G, Lamboij E, Hellebrekers L. Effects of a local anaesthetic and NSAID in castration of piglets, on the acute pain responses, growth and mortality. Animal 2012;6:1469–75. https://doi.org/10.1017/S1751731113000086.

[23] Sturlini Barticciotto L, Loureiro Luna SP, Rodolfo de Sá Lorena SE, Garcia F, Antonio D. Weight gain, behavioral and cortisol changes after orchiectomy with or without local anaesthesia in piglets. Semina: Ciências Agrárias 2016;37:1307–16. https://doi.org/10.5433/1679-0359.2016v37n3p1307.

[24] Roldan P, Trujillo-Ortega M, Borderas-Tordesillas F, Martínez-Rodriguez R, Mora-Medina P, et al. Physiometabolic responses to road transport in weaned piglets for a short period and the effects of straw bedding. Anim Sci J 2015;86:563–71.

[25] Kiwull-Schöne H, Kiwull P, Frede S, Wiemann Martin. Role of brainstem sodium/proton exchanger 3 for breathing control during chronic acid–base imbalance. Am J Resp Crit Care 2007;176:513–9. https://doi.org/10.1164/rccm.200703-347OC.

[26] White R, DeShazer J, Tressier C, Borcher G, Davey S, Waninge A, et al. Vocalization and physiological response of pigs during castration with or without a local anesthetic. J Anim Sci 1995;73:381–6.

[27] Hedrick HB, Aberle ED, Forrest JC, Judge MD, Merkel RA. Principles of Meat Science. 3rd ed.Dubuque, IA: Kendall/Hunt Publishing Company; 1994. p. 87–92.

[28] Hay M, Vulin A, Génin S, Sales P, Prunier A. Assessment of pain induced by castration in piglets: behavioral and physiological responses over the subsequent 5 days. Appl Anim Behav Sci 2003;82:201–18. https://doi.org/10.1016/S0168-1591(03)00059-5.

[29] Mota-Rojas D, López A, Martínez-Burnes J, Muns R, Villanueva-García D, Mora-Medina P, et al. Invited review: Is vitality assessment important in neonatal animals? CAB Reviews 2018;13(36):1–13. https://doi.org/10.1079/PAVSNNR13036.

[30] Islas-Fabila P, Mota-Rojas D, Martínez-Burnes J, Mora-Medina P, González-Lozano M, Roldan-Santiago P, et al. Physiological and metabolic responses in newborn piglets associated with the birth order. Anim Reprod Sci 2018;197:247–56. https://doi.org/10.1016/j.anireprosci.2018.08.037.

[31] Martínez-Rodríguez R, Roldan-Santiago P, Orozco-Gregorio H, Trujillo-Ortega M, Mora-Medina P, González-Lozano M, et al. Physiological responses and blood gas exchange following long-distance transport of piglets weaned at different ages over unpaved or paved roads. Livest Sci 2015;172:69–78. https://doi.org/10.1016/j.livsci.2014.12.013.

[32] Ritter M, Ellis M, Anderson D, Curtis S, Keffaber K, Killefer J, et al. Effects of multiple concurrent stressors on rectal temperature, blood acid-base status, and longissimus muscle glycolytic potential in market-weight pigs. J Anim Sci 2009;87:351–62. https://doi.org/10.2527/jas.2008-0874.

[33] Stevens B, Posner L, Jones C. Lascelles B, Comparison of the effect of intratesticular lidocaine/bupivacaine vs. saline placebo on pain scores and incision site reactions in dogs undergoing routine castration. Vet J 2013;196:499–503.

[34] Ranheim B, Haga HA. Local anaesthesia for pigs subject to castration. Acta Vet Scand 2006;48:S13. https://doi.org/10.1186/1751-0147-48-S1-S13.

[35] Mota-Rojas D, Becerril-Herrera M, Roldan-Santiago P, Alonso-Spilsbury M, Flores-Peinado S, Ramírez-Necoechea R, et al. Effects of long distance transportation and CO$_2$stunning on critical blood values in pigs. Met Sci 2012;90:893–8. https://doi.org/10.1016/j.meatsci.2011.11.027.

[36] Davis K, Seddon Y, Creutzinger K, Bouvier M, Brown J. An investigation into the use of sucrose to reduce castration pain in piglets. Can J Anim Sci 2017;97:439–47. https://doi.org/10.1139/cjas-2016-0170.