Original Research Paper

Infrared Thermography and Metabolic Changes in Castrated Piglets due to the Effects of Age and the Number of Incisions in the Testicles

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Abstract: The objective of the study was to evaluate infrared temperature and metabolic responses in different-aged piglets castrated surgically with one or two incisions. The piglets were divided into groups according to age (5- and 13-day old) and the number of incisions: simulated castration; castration with one incision (C1I5 and C1I13); and castration via two incisions (C2I5 and C2I13). Temperature evaluations were performed with an infrared thermal thermography camera in the eyes, the thorax and the scrotal area, at 24 h before castration (RV), immediately after castration (PC), 24 and 48 h post-surgery. The study also evaluated plasma glucose and lactate concentrations. The thorax presented lower infrared temperature in the groups C1I13 and C2I13 than those in C1I5 and C2I5 (p<0.05). Regardless of age and type of castration, plasma lactate values increased PC (P=0.0001). The piglets castrated at 5 days of age were less susceptible to presenting temperature changes regardless of the number of incisions, while the animals castrated at 13 days with two incisions showed lower temperatures and imbalances in plasma glucose and lactate concentrations. Castration in piglets of 13 days causes a decrease in temperature in the lacrimal caruncle immediately after performing surgery. The physiological responses caused by castration are less marked in 5-day-old piglets.

Keywords: Castration, Piglets, Infrared Thermography, Metabolic Changes

Introduction

In most countries, pigs reared in pork-producing operations are castrated to obtain several well-recognized benefits: (a) preventing unwanted breeding; (b) behavioral modifications that reduce fighting in mature pigs and make handling easier; and, (c) reducing the risk of boar taint in meat (Rault *et al*., 2011; Kluivers-Poodt *et al*., 2013; O'Connor *et al*., 2014).

While surgical castration is still the most common method used in the pork industry (Godyn *et al*., 2013), there is strong evidence that this surgery propitiates various stressful events that are likely to cause pain. These events include scrotal incisions, extraction of the testes and severing the spermatic cords (Bonastre *et al*., 2016). The stimulation of nociceptors in the scrotal skin activates neurons of various orders which transmit information from the periphery to the brain that can be interpreted as

pain and distress, regardless of the age at which the animals are castrated (McGlone and Hellman, 1988; Sutherland *et al.*, 2010; Mota-Rojas *et al.*, 2016). The most common procedure used by producers involves castrating piglets at 3-5 or 10-14 days of age (Nord *et al.*, 2016). It is well-known that after a painful or stressful short-term handling event, catecholamine and glucocorticoid concentrations increase, stimulating the mobilization of muscular glycogen and the resulting hepatic metabolism that triggers increases in plasma lactate and glucose concentrations (Prunier *et al.*, 2005; Lonardi *et al.*, 2015); increases in metabolic activity that can generate higher core body temperatures (Carroll *et al.*, 2006; Marchant-Forde *et al.*, 2009; Weschenfelder *et al.*, 2013). Infrared Thermography (IRT) is currently being used to detect the effects of painful, stressful and emotionally-upsetting stimuli on temperature changes in several species (Stubsjøen *et al.*, 2009) that are believed to reflect internal body temperature (Kastberger and Stachl, 2003).

There are many reports in the literature of veterinary medicine that describe the benefits of local intra-testicular, scrotal sac and spermatic cord anesthesia for controlling the pain and stress associated with the effects of castration in many species (Haga and Ranheim, 2005; Suriano *et al.*, 2014; Nickell *et al.*, 2015). They do not make recommendations as to how many scrotal incisions should be made, but the most common technique uses two incisions rather than one (Fredriksen *et al.*, 2009). When two incisions are made, they are normally performed longitudinally on each testicle (Marsalek *et al.*, 2015), while the single-incision method requires a transverse cut across both testicles and into the scrotum and tunica vaginalis (Kluivers-Poodt *et al.*, 2013). Considering this background, the objective of the present study was to evaluate infrared temperature changes and metabolic responses in different-aged piglets castrated surgically with one or two incisions.

Material and Methods

Geographical Location of the Experimental Site

This study was conducted at a commercial pig farm in Amecameca located in the foothills of the Sierra Nevada, within the province of the volcanic axis and in the basin of the Moctezuma-Pánuco river. Geographic coordinates are longitude (98° 37' 34" y 98° 49' 10"; latitude 19° 3' 12" y 19° 11' 2").

Ethical Note

All animals were handled humanely throughout the study. All procedures related to the use and care of the animals strictly followed the Mexican regulation norm, NOM-062-ZOO-1999 of Mexico's Department of Agriculture, Ranching, Rural Development, Fishing and Alimentation for animal-based experimentation (NOM, 1999).

This study was conducted at a commercial pig farm located in central Mexico, after obtaining approval from the Doctoral Commission of Biological Sciences of the Universidad Autonoma Metropolitana Iztapalapa-Xochimilco in Mexico City in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). All procedures were conducted in accordance with the guidelines for the ethical use of animals in applied ethological studies, described elsewhere (Sherwin *et al.*, 2003).

Animals

Data were collected from 72, 5- and 13-day-old male piglets (Yorkshire x Landrace) that weighed 1.68 ±0.40 and 5.46±1.01 kg, respectively. Piglets were obtained from twelve different litters delivered by third-parity sows. Piglets 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, then they were returned to the farrowing pen. The piglets 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 cages and housed in individual farrowing crates made of tubing. Dimensions were 2.07 m long × 0.60 m wide × 101 m high, with a total space of 2 m wide by 2.5 m long. Each cage was equipped with a 50-cm stainless steel feeder and a nipple drinker. An electric radiant lamp was used to heat the creep area in each crate. Each room held 12 far rowing crates, arranged in two rows of six crates separated by an aisle. The crates had fully-slatted floors made of wire mesh, covered with a plastic carpet. Ventilation and temperature were controlled automatically by fans and air heating (27°C and 60% relative humidity in the maternity room). Sows and piglets had *ad libitum* access to water in separate nipple drinkers. From day one of post-partum life, all piglets were weighed and identified individually by marking a number on their back. Different colors were used to distinguish the experimental groups. All these procedures were conducted by the same person.

Each one of the six study groups consisted of 12 piglets divided according to age and the number of incisions made during surgical castration, as follows: simulated castration (SC5 and SC13): a control group in which the 5- and 13-day old piglets, respectively, were removed from their pens and held in a head-down position by the legs for 120 s; castration with one horizontal incision in both testicles in 5- and 13-day old piglets (C1I5 and C1I13, respectively); and castration with two vertical scrotal incisions in 5- and 13-day old piglets (C2I5 and C2I13).

Surgical Procedure

Before performing surgical castration, the scrotal area was superficially cleaned with chlorhexidine surgical scrub and isopropyl alcohol. Lidocaine at 10 mg/ml (Pisacaina 2%, Pisa® Laboratorios, S.A. de C.V., Mexico) was used as the local anesthetic, with 0.5 ml being injected into each testicle Hansson *et al.* (2011), with the expectation that it would spread from there into the spermatic cord. Those piglets were then returned to the crate with the other ones awaiting castration. Lidocaine was administered at least 15 min before castration in accordance with the methodology employed by Kluivers-Poodt *et al.* (2012).

Surgery for the piglets in groups C2I5 and C2I13 was performed with a scalpel to make two incisions in the scrotum, extract the testicles and sever the spermatic cords (Bonastre *et al.*, 2016). For groups C1I5 and C1I13, castration was carried out by making one incision in the scrotum to expose the testicles. The spermatic cord was scarified with a scalpel and the testicles removed (Sturlini Barticciotto *et al.*, 2016).

All surgical procedures were performed by experienced surgeons on the same day with the piglets restrained in the supine position by an assistant. The skin over the scrotum was tautened with one hand to help expose the testicles and incision site. Depending on the treatment group, castration was performed by making one or two incisions (2-3 cm) 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 mention that no other post-operative treatment was applied.

Infrared Thermal Camera

Evaluation of the piglets' body temperature was performed with a portable infrared thermal camera (ThermaCam E45; FLIR Systems, Boston, MA, USA). Before beginning the study, the camera was calibrated for the temperature and relative humidity of the room where measurements were to be taken. The emissivity value used was 0.98, as recommended by Soerensen and Pedersen (2015).

To avoid the confounding effect of ambient conditions on ocular temperature variation, a data logger; registering ambient temperature and relative humidity was attached to the camera while images were captured.

To optimize the accuracy of the thermographic image and to reduce sources of artifacts, before every testing session the same image of a Lambert Surface (An ideal, perfectly diffusing surface for which the intensity of reflected radiation is independent of direction) was taken to define the radiance emission and to nullify the effect of surface reflections on tested animals. All the electric radiant lamp and the ventilation system were turned off during the castration of the pigs and each time the infrared thermal camera was used. The sows were kept at an average room temperature of 26°C±2 with a relative humidity of 60%. Lighting was set at 39.8 candelas.

While the piglets were restrained by the assistant and after drawing the blood samples, a second assistant –who was blind to the treatments– took three photographs of each region, as follows: the piglets' faces, to allow evaluation of both eyes (the area of the medial posterior palpebral border of the lower eyelid and the *caruncula lacrimalis*), the right flank and the scrotal area (Fig. 1). Three images of each body region were taken each time. The infrared thermographic images were captured at a distance of 50 cm from the piglets at the following intervals: 24 h prior to castration (Reference values, RV), immediately after castration (PC) and at 24 and 48 h post-surgery. A total of 900 images were recorded as JPEG files using ThermaCam Researcher Basic image analysis software (FLIR Systems) to obtain minimum, average and maximum temperatures of the skin areas sampled. One clear infrared image (precise location and perfect focus) was chosen for each animal.
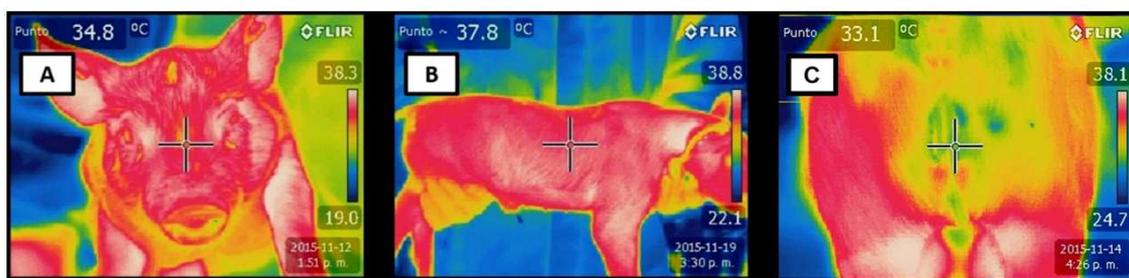
Fig. 1: Infrared thermographic images of surgically-castrated piglets. A = frontal infrared thermographic image showing measurement of the surface temperature of the eyes (small areas around the posterior border of the eyelid and the *caruncula lacrimalis*), B = infrared thermographic image showing the right side of the piglet to obtain the surface temperature of the thorax, C = infrared thermographic image of the scrotal wound made to perform surgical castration

Blood Samples

Blood samples were collected immediately after the simulated or surgical castration from the *cava cranialis* vein using 3-ml syringes at four sampling time points: 24 h before castration (RV), immediately after surgery (0h) and at 24 and 48 h post-castration. All samples were obtained in less than 20 s under physical restraint by placing the piglets in the same position as for castration. All personnel involved in sampling were experienced and had received training previously. Once obtained, 150 µL of the samples from both age groups and type of castration were placed in a plasma gas and electrolyte parameter analyzer (GEM Premier, Instrumentation Laboratory Co., Lexington, USA and Instrumentation Laboratory SpA, Milano, Italy). Trained personnel processed all samples immediately to determine plasma glucose (mg/dL) and lactate levels (mg/dL).

Statistical Analysis

Normality assays were performed (PROC UNIVARIATE, SAS 9.0) for all variables examined, the two methods of castration considered and the two age groups to demonstrate the following: that errors had normal distributions and the existence of a null mean with a typical deviation (α). To test for the effect of age and castration method on temperature, a repeated measures ANOVA was performed (SAS 9.0). The researchers who carried out the evaluation and collected the study outcomes were not aware of the treatments and did not participate in selecting the animals or data analysis. Likewise, the researcher responsible for analyzing the data was unaware of the treatments. A two-tailed $p<0•05$ was considered significant in every test.

Results

Infrared Thermography of the Caruncula Lacrimalis in the Castrated Piglets

Table 1 shows the infrared temperature values obtained for the *caruncula lacrimalis* of the piglets due to the effects of age and type of castration at the different sampling time points. Significant differences were observed among the age groups and types of castration, but only at 24 h post-castration in all groups.

Table 1: Temperature assessment (°C) of the eyes (small areas around the posterior border of the eyelid and the *caruncula lacrimalis*) in castrated piglets by scrotal surgery with one or two incisions at 5 and 13 days of age

	Piglets 5d			Piglets 13d			
	SC5	C1I5	C2I5	SC13	C1I13	C2I13	
TIME	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	P-Value
RV	35.35±0.42[a1]	35.72±0.26[a1]	35.97±0.28[a1]	36.30±0.37[a1]	36.40±0.25[a1]	35.95±0.24[a1]	0.25
PC	35.35±0.58[a1]	35.33±0.38[a1]	35.50±0.52[a1]	34.72±0.44[a2]	34.54±.33[a2]	34.31±.33[a2]	0.22
24 h	36.00±.56[ab1]	35.50±0.35[a1]	35.07±0.39[a1]	35.46±0.50[ab12]	34.33±0.34[b2]	33.98±0.32[b2]	0.0069
48 h	35.20±0.86[a1]	35.84±0.54[a1]	33.20±1.22[a1]	35.80±0.61[a12]	35.08±0.35[a,12]	35.34±0.35[a12]	0.48
P-Value	0.51	0.62	0.09	0.003	0.0002	0.0029	

SC5, simulated castration 5-day old piglets; C1I5, castration with one horizontal incision in both testicles in 5-day old piglets; C2I5, castration with two vertical scrotal incisions 5-day old piglets; SC13, simulated castration 13-day old piglets; C1I13, castration with one horizontal incision in both testicles in 13-day old piglets; C2I13, castration with two vertical scrotal incisions 13-day old piglets. SEM, standard error of the mean, RV, reference values (24 hours prior to castration); PC, post castration (immediately); 24 h, 24 hours before castration; 48 h, 48 hours post-surgery.
[a, b, c] Different letters in the same row indicate statistically significant differences among groups at the same time. [1,2]Different numbers in the same column indicate statistically significant differences among times in the same group, Tukey (p<0.05).

Table 2: Temperature assessment (°C) of the thorax surface in piglets castrated by scrotal surgery with one or two incisions at 5 and 13 days of age

	Piglets 5d			Piglets 13d			
	SC5	C1I5	C2I5	SC13	C1I13	C2I13	
TIME	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	P-Value
RV	37.52±0.45[a1]	37.51±0.28[a1]	37.41±0.30[a1]	36.58±040[a1]	36.78±0.27[a1]	37.13±0.26[a1]	0.26
PC	37.00±0.67[a1]	36.65±0.47[a1]	37.46±0.54[a1]	35.90±0.42[a1]	35.69±0.30[a1]	35.74±0.27[a2]	0.05
24 h	37.40±0.59[ab1]	37.63±0.37[a1]	37.68±0.42[a1]	35.90±0.53[ab1]	35.74±0.34[b1]	35.74±0.34[b2]	0.0003
48 h	37.30±0.61[a1]	36.98±0.38[a1]	36.20±0.86[a1]	35.45±0.43[a1]	36.51±0.27[a1]	36.48±0.24[a1]	0.13
P-Value	0.93	0.08	0.32	0.33	0.11	0.0048	

SC5, simulated castration 5-day old piglets; C1I5, castration with one horizontal incision in both testicles in 5-day old piglets; C2I5, castration with two vertical scrotal incisions 5-day old piglets; SC13, simulated castration 13-day old piglets; C1I13, castration with one horizontal incision in both testicles in 13-day old piglets; C2I13, castration with two vertical scrotal incisions 13-day old piglets. SEM, standard error of the mean, RV, reference values (24 hours prior to castration); PC, post castration (immediately); 24 h, 24 hours before castration; 48 h, 48 hours post-surgery
[a, b, c]Different letters in the same row indicate statistically significant differences among groups at the same time. [1,2]Different numbers in the same column indicate statistically significant differences among times in the same group, Tukey (p<0.05).

At that time, the piglets in group C1I13 presented a temperature that was 1.17°C below that of the piglets in group C1I5. Also, the piglets in group C2I13 presented an infrared temperature that was 1.09°C lower than that of the piglets in group C2I5 (p<0.05). The C2I13 piglets had lower infrared temperatures immediately after castration and at 24 h post-surgery, compared to the RV; however, infrared temperatures returned to RV at 48 h post-castration.

Infrared Thermography of the Thorax Surface in Castrated Piglets

The infrared temperature values from the surface of the thorax are shown in Table 2 at 24 h post-castration. The C1I13 and C2I13 piglets presented an average infrared temperature that was 1.64°C lower than that of the piglets in group SC13 and 1.12°C lower than the piglets in groups C1I5 and C2I5. Also, the SC13 and C2I13 piglets had reduced infrared temperatures immediately after castration and at 24 h post-surgery, compared to the RV. However, temperatures in all groups returned to RV at 48 h post-castration.

Infrared Thermography of the Scrotal Surface in Castrated Piglets

Table 3 shows the infrared temperature values for the scrotal region of the piglets due to the effects of age and type of castration at the different sampling time points. Our results may suggest that immediately after castration, the piglets in C1I13 and C2I13 presented infrared temperatures that were 1.95°C and 2.44°C lower, respectively, than the temperature values determined for SC13.

At 24 h post-castration, the C1I13 group presented an infrared temperature that was 2.32°C lower than that of group C1I5. Also, group C2I13 had an infrared temperature 2.14°C lower than group C2I5 (p<0.05). In contrast, the measurements taken at 48 h post-surgery showed an infrared temperature that was 1.48oC lower in group C1I13 than in C1I5 (p=0.029).

In relation to the infrared temperatures evaluated within each age group at the different sampling time points, observations showed that values decreased immediately after castration by one or two incisions in the C1I5 and C2I5 piglets, but no alterations at 24 and 48 h post-castration, compared to the RV (p<0.05). The C2I13 piglets, in contrast, showed a marked decrease in their temperature values immediately after castration that were maintained up to 24 h (p<0.05).

Glucose

Table 4 presents the plasma glucose values of the piglets in both age groups castrated by one or two incisions. The study found significant differences between the baseline values of the 5- and 13-day-old piglets, with the latter registering higher plasma glucose values (p<0.05). Other observations showed that immediately after surgery, the C2I13 piglets presented higher plasma glucose values than the 5-day-old piglets castrated by one or two incisions (p<0.05).

Lactate

Figure 2 shows the plasma lactate values for the piglets in both age groups castrated by one or two incisions. These results indicate that immediately after surgery, C1I13 animals had higher plasma lactate levels than the C1I5 piglets castrated by the same technique and the piglets in the simulation group. In other results, at 24 h, the study found that the piglets C2I5 had higher plasma lactate values than the 13-day-old animals in the simulated castration group and the piglets C1I13 (p<0.05). Regarding evaluation time, results show that, regardless of age and type of castration, plasma lactate values increased immediately after surgery and in the piglets with simulated castration (p=0.0001).

Table 3: Temperature assessment (°C) of the scrotal surface in castrated piglets by scrotal surgery with one or two incisions at 5 and 13 days of age

	Piglets 5d			Piglets 13d			
	SC5	C1I5	C2I5	SC13	C1I13	C2I13	
TIME	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	P-Value
RV	37.75±0.62[a1]	37.33±0.44[a1]	36.64±0.41[a1]	36.94±0.56[a1]	36.07±0.37[a1]	36.58±0.36[a1]	0.18
PC	36.55±0.65[a1]	35.38±0.34[ab2]	35.33±0.53[abc2]	36.08±0.41[a1]	34.13±0.32[bc1]	33.64±0.27[c3]	0.0001
24 h	36.47±0.67[ab1]	36.97±0.42[a1]	36.80±0.47[a1]	35.25±0.67[ab1]	34.65±0.42[b1]	34.66±0.40[b23]	0.0005
48 h	37.65±0.59[a1]	36.960.37[a1]	35.90±0.84[ab12]	35.86±0.48[ab1]	35.48±0.28[b1]	35.30±.24[b12]	0.029
P-Value	0.2	0.0005	0.02	0.07	0.05	0.0001	

SC5, simulated castration 5-day old piglets; C1I5, castration with one horizontal incision in both testicles in 5-day old piglets; C2I5, castration with two vertical scrotal incisions 5-day old piglets; SC13, simulated castration 13-day old piglets; C1I13, castration with one horizontal incision in both testicles in 13-day old piglets; C2I13, castration with two vertical scrotal incisions 13-day old piglets. SEM, standard error of the mean, RV, reference values (24 hours prior to castration); PC, post castration (immediately); 24 h, 24 hours before castration; 48 h, 48 hours post-surgery.
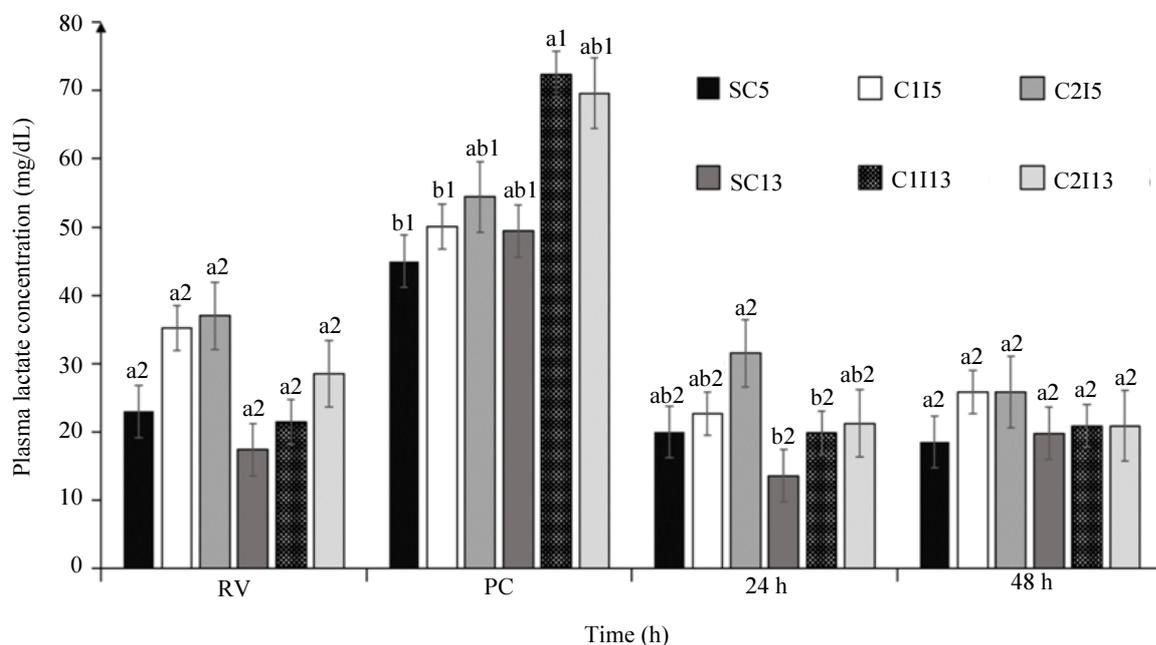[a, b, c] Different letters in the same row indicate statistically significant differences among groups at the same time. [1,2]Different numbers in the same column indicate statistically significant differences among times in the same group, Tukey (p<0.05)

Table 4: Plasma glucose concentration (mg/dL) in piglets castrated by scrotal surgery with one or two incisions at 5 and 13 days of age during a period of 48h post-surgery

| TIME | Piglets 5d | | | Piglets 13d | | | |
	SC5 Mean ± SEM	C1I5 Mean ± SEM	C2I5 Mean ± SEM	SC13 Mean ± SEM	C1I13 Mean ± SEM	C2I13 Mean ± SEM	P-Value
RV	89.00±5.38[d1]	94.81±3.63[cd1]	97.27±3.63b[cd1]	117.80±5.38[a1]	111.27±3.63[ab1]	109.63±3.63[abc1]	0.0002
PC	102.20±8.20[ab1]	99.90±5.53[b1]	94.90±5.80[b1]	100±60±8.20[ab1]	118.63±5.53[ab1]	124.45±5.53[a1]	0.003
24 h	104.20±6.72[a1]	101.12±5.31[a1]	99.55±5.01[a1]	107.00±6.72[a1]	105.72±4.53[a1]	106.18±4.53[a1]	0.89
48 h	109.75±7.21[a1]	98.63±4.35[a1]	107.33±4.80[a1]	120.75±7.21[a1]	109.72±4.35[a1]	102.50±4.56[a1]	0.14
P-Value	0.33	0.65	0.17	0.29	0.27	0.06	

SC5, simulated castration 5-day old piglets; C1I5, castration with one horizontal incision in both testicles in 5-day old piglets; C2I5, castration with two vertical scrotal incisions 5-day old piglets; SC13, simulated castration 13-day old piglets; C1I13, castration with one horizontal incision in both testicles in 13-day old piglets; C2I13, castration with two vertical scrotal incisions 13-day old piglets. SEM, standard error of the mean, RV, reference values (24 h prior to castration); PC, post castration (immediately); 24 h, 24 h before castration; 48 h, 48 hours post-surgery.
[a, b, c] Different letters in the same row indicate statistically significant differences among groups at the same time. [1,2]Different numbers in the same column indicate statistically significant differences among times in the same group, Tukey (p<0.05)

Fig. 2: Plasma lactate concentration (mg/dL) in piglets castrated by scrotal surgery with one or two incisions at 5 and 13 days of age during a period of 48h post-surgery. SC5 = simulated castration 5-day old piglets, C1I5 = castration with one horizontal incision in both testicles in 5-day old piglets, C2I5 = castration with two vertical scrotal incisions 5-day old piglets, SC13 = simulated castration 13-day old piglets, C1I13 = castration with one horizontal incision in both testicles in 13-day old piglets, C2I13 = castration with two vertical scrotal incisions 13-day old piglets
[a, b, c] Different letters indicate statistically significant differences among groups at the same time. [1,2]Different numbers indicate statistically significant differences among times in the same group, Tukey (p<0.05)

Discussion

The results of this study include the observation that the age at which castration was performed added to the number of incisions made in the scrotum generated changes in the infrared thermographic temperatures in different areas of the body, as well as modifications of plasma glucose and lactate levels. These alterations can be interpreted as indirect indicators of stress.

Our results indicate that castrating piglets at 13 days of age provoked changes in infrared temperatures when compared to the piglets that had surgery at 5 days of age. In this regard, Guatteo *et al.* (2012) reported that the age of animals plays a role in pain responses to castration, as these are less pronounced in younger subjects than older ones. Likewise, Janczak *et al.* (2012) observed that 14-day-old piglets had a higher mechanical nociceptive pain

threshold, which may be related to their higher age and weight compared to younger, 7-day-old, piglets and could produce a lower capacity for responding to pain in younger piglets. McGlone and Hellman (1988), meanwhile, compared castration in piglets at 2 vs. 7 weeks of age. They found that using local anesthesia eliminated pain-induced behaviors in the younger subjects, but appeared to be ineffective in the older ones (White *et al.*, 1995; Von Borell *et al.*, 2009). Findings of this nature may help explain the results of our study with respect to the alterations in infrared temperatures and energy metabolism that the younger piglets manifested, despite the application of local anesthesia. The results presented here in could be interpreted as suggesting that it is more advisable to perform castration under anesthesia when animals are younger. Also, Taylor *et al.* (2001) indicate that piglets experience no less pain when castrated at less than 1 week of age vs. 2 or 3 weeks.

In other findings, the responses to castration observed in our study include decreased temperatures in the eye, thorax and scrotal wound. In this regard, changes in skin temperature have been shown to be associated with both clinical and emotional responses in humans and other animal species, while rapid changes in blood flow can be associated with stress responses (Yarnell *et al.*, 2013). When an animal becomes stressed, the HPA axis is activated and the generation of heat, due to increases in catecholamine and cortisol levels and blood flow responses, produces changes in temperature and heat loss (Schaefer *et al.*, 2012; Mota-Rojas *et al.*, 2011). Stress-induced hyperthermia occurs in numerous species, characterized by an increase in core body temperature but a decrease in surface temperature of 0.5-to-1.5°C within 10-15 min of the onset of "emotional stress" (Edgar *et al.*, 2013; Mota-Rojas *et al.*, 2011, 2012b; 2016). This coincides with the results of our study, where the surface temperature of the piglets decreased immediately after castration, regardless of the number of incisions made. In this respect, studies of castrated piglets by Lonardi *et al.* (2015) reported an increase in the temperature of the eye 20 minutes after surgery, or a simulation of this, which differs from the findings of our study, where the temperature reduction in the 13-day-old piglets continued up to 24 h post-surgery. It is important to note that decreases in eye temperature have also been reported in other species faced with different stressor stimuli; for example, eye temperatures in birds decreased with handling (Herborn *et al.*, 2015) and it has been suggested that the drop in eye temperature seen in dehorned bovines following disbudding without local anesthetic may be caused by sympathetic vasoconstriction (Stewart *et al.*, 2008). The decrease in eye temperature may also be due to the presence of a network of venous blood from the nasal cavity that is cooler and whose function is to lower the temperature of

the blood that irrigates the brain. Hence, this blood may also decrease the temperature of the eye and the small areas around the posterior border of the eyelid and the *caruncula lacrimalis* (Loughmiller *et al.*, 2001; Stewart *et al.*, 2007). We should point out that in addition to the decrease in eye temperature, the 13-day-old piglets also had lower thorax temperatures. This phenomenon may be explained by the activation of SNS, which produces pupillary dilatation, higher blood pressure and heart rate, peripheral vasoconstriction and, consequently, a decrease in skin surface temperatures, while activation of the sympathetic branch induces an increase in core temperature (brain and upper organs), but decreases in the temperatures of more peripheral body areas, such as the nose, face and ears. This is caused by vasoconstriction, which controls the conduction of heat to the skin by the blood in the arterioles and arteriovenous anastomoses that supply it to the venous plexus of the skin (Guyton and Hall, 1996; McCafferty *et al.*, 2011; Yarnell *et al.*, 2013; Bonastre *et al.*, 2016; Travain *et al.*, 2016). Soerensen and Pedersen (2015) evaluated the surface temperature of piglets after removal of their incisors. They found that this practice causes increased levels of noradrenaline, whose vasoconstrictor effect can reduce blood flow to the skin and, as a result, decrease temperatures. Menzel *et al.* (2014) presented the hypothesis that reduced thorax temperatures could be caused by physiological ventilation of lung tissue with cool air. Thus, with regard to the temperature changes in the wound caused by castration, our study found an evident decrease in all groups that underwent surgery, but that this was more pronounced in the 13-day-old piglets. This result may be due to the fact that vasoconstriction in the skin in response to a stressful stimulus is a specific regional response (Herpin *et al.*, 2004); that is, an initial acute response to stress that, presumably, acts to increase perfusion pressure and redirect blood flow to organs and the skeletal musculature, areas that have more urgent metabolic requirements. Vasoconstriction of the blood vessels that supply the skin also protects against excessive loss of blood in the event of damage to external tissue (Stewart *et al.*, 2007). This mechanism for preventing excessive blood loss could explain the more marked temperature reduction in the C2I13 piglets. It is important to note that the first response to wounds is hemostasis, which occurs within a matter of minutes to stop bleeding and seal off the wound through the action of three mechanisms: the aggregation of platelet clumps, vasoconstriction and the formation of a fibrin clot (Clark *et al.*, 1996; Kimani, 2013). It is probable that when a reduction of blood flow to the wound occurs, white blood cells are trapped at the capillary level. This results in the plugging of capillary loops that produce areas of localized ischemia. Finally, in the inflammatory phase, platelets aggregate and form clots to minimize blood and fluid loss at the wound site (Paz and West, 2013).

With regards to the increase in plasma glucose levels in the 13-day-old piglets immediately after castration, it is well-known that this index is considered an indirect indicator of stress in pigs, because in response to that condition glu¬cose levels rise due to catecho¬lamine and glucocorticoid secretion (Pollard *et al*., 2002; Mota-Rojas *et al*., 2011; 2012b). In addition, Von Borell *et al*. (2009) mention that castration induces physiological and behavioral reactions that are indicative of pain, while also generating stress and discomfort before and after surgery. These reactions are of great magnitude during surgery and in the initial hours following it. Other studies point out that increases in plasma glucose concentrations may occur due to the breakdown of glycogen from the liver, or the depletion of glycogen reserves from skeletal muscles (Tadich *et al*., 2005; Averós *et al*., 2008; Martínez-Rodríguez *et al*., 2015; Mota-Rojas *et al*., 2011; 2012b). These findings suggest that castration is more stressful for piglets when performed at higher ages. However, it is important to remember that assessing pain requires an integrated, multi-disciplinary approach that includes both behavioral and physiological approaches, because of the different ways in which individual animals respond to stressors (Gottardo *et al*., 2016).

Turning now to plasma lactate levels, all the castrated piglets –regardless of age– presented an increase in this metabolite immediately after castration. In this regard, several studies of adult (Becerril-Herrera *et al*., 2010; Mota-Rojas *et al*., 2012a) and neonate (Martínez-Rodríguez *et al*., 2011) pigs have demonstrated that increases in plasma lactate concentrations can result from exposure to different stressors. Lactate is a metabolite that causes muscular glycogenolysis due to a lack of glucose phosphatase 6, which is necessary for glycogen synthesis (Mota-Rojas *et al*., 2012b). The lactate that forms in the muscle is transported through the bloodstream to the liver, where it is transformed into glucose (Moberg, 2000; Pollard *et al*., 2002). Prunier *et al*. (2005) found higher concentrations of blood lactate in piglets following castration compared to non-castrated animals, since a significant proportion of the stress/discomfort that they experience results from handling procedures (Leidig *et al*., 2009). In light of this evidence, we can conclude that the physiological changes seen in the piglets in the present study could be associated with the pain generated by castration when this surgery is performed at a younger age, regardless of the number of incisions.

Conclusion

In conclusion, the castration of piglets at 13 days of age with one incision generates more metabolic imbalances associated with stress and pain, the lower infrared temperature values in the *caruncula lacrimalis* and thorax. Likewise, piglets castrated by two incisions at 5 and 13 days of age have adverse effects to a lesser degree. Although castrations by one incision in piglets of 5 days have less adverse effects, it is necessary to continue investigating other clinical indicators.

Therefore, our recommendation is that castration should be done in piglets during the first 5 days postpartum to prevent imbalances in their energy metabolism and changes in surface temperature.

Author's Contributions

Daniel Mota-Rojas: Participated in the conception and project design.

Efraín Pérez Pedraza: Collected field samples, generated the data and statistically analysed the data.

Isabel Guerrero-Legarreta, Julio Martínez Burnes, Ramiro Ramírez-Necoechea, Miguel González-Lozano, Patricia Mora-Medina, Daniel Mota-Rojas, Efraín Pérez Pedraza and Rosy Cruz-Monterrosa: Interpreted the data and wrote the draft paper.

DMR, JMB, IGL, RRN and MGL: Provided substantial intellectual input and edited the manuscript.

DMR, IGL, RRN, MGL and PMM: Financed the project. All authors gave final approval for publication.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and there are no ethical issues involved.

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