Development of effective and minimally invasive surgical techniques for the preparation of intact, sterile boars

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Summary

Objective: The objective of this study was to evaluate three surgical procedures to produce intact, sterile boars.

Materials and methods: Boars (n = 39) were allocated to one of four treatment groups: no surgery (control), epididymectomy by removal of the epididymis tail (TE), vasectomy via scrotal access (VS), and vasectomy via inguinal access (VI) at 63 days of age. Selected physiological, hematological, and endocrine responses were monitored after surgeries to evaluate the different techniques’ relative safety and effectiveness.

Results: Libido and testosterone concentrations were not affected by surgical treatment and were similar to those observed in the control group. The TE and VS procedures required the least and most time to complete, respectively, while VI was intermediate (P < .001). Both lactate and cortisol concentrations were elevated at the time of surgery compared with the control group, but had decreased by 2 days post surgery (P = .02).

Implications: Considering the surgical time and ease, the TE procedure is suggested as the choice technique for producing intact, sterile boars. The swine industry is shifting from individual crates to the use of group pen housing of sows. Use of intact, sterile boars could be implemented to improve estrus detection in group pen housing systems.

Keywords: swine, teaser boar, vasectomy, epididymectomy, estrus detection.

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Resumen - Desarrollo de técnicas quirúrgicas eficaces y mínimamente invasivas para la preparación de sementales intactos y estériles

Objetivo: El objetivo de este estudio fue evaluar tres procedimientos quirúrgicos para producir sementales intactos y estériles.

Materiales y métodos: Los sementales (n = 39) se asignaron a uno de cuatro grupos de tratamiento: sin cirugía (control), epididimectomía mediante extracción de la cola del epidídimo (TE), vasectomía por acceso escrotal (VS) y vasectomía por acceso inguinal (VI) a los 63 días de edad. Se seleccionaron y monitorearon respuestas fisiológicas, hematológicas y endocrinas después de las cirugías para evaluar la seguridad y la efectividad relativas de las diferentes técnicas.

Resultados: La libido y las concentraciones de testosterona no se vieron afectadas por el tratamiento quirúrgico y fueron similares a las observadas en el grupo de control. Los procedimientos TE y VS requirieron el menor y mayor tiempo, respectivamente, mientras que el VI fue intermedio (P < .001). Tanto las concentraciones de lactato como de cortisol estaban elevadas en el momento de la cirugía en comparación con el grupo de control, pero habían disminuido 2 días después de la cirugía (P = .02).

Implicaciones: Considerando el tiempo quirúrgico y la facilidad, se sugiere el procedimiento TE como la técnica de elección para producir sementales intactos y estériles. La industria porcina está pasando de las jaulas individuales al uso de corrales grupales de cerdas. Se podría implementar el uso de sementales intactos y estériles para mejorar la detección del estro en los sistemas de alojamiento de corrales grupales.

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Brazil is the fourth largest producer and exporter of pork meat in the world. This became possible due to intensive management systems, the climate, and the country’s animal health status. Despite the significant advance in the Brazilian pig industry’s productivity, reproductive management failure is common. The most frequent failures are associated with estrus detection and insemination timing resulting in increased re-cycle rates and reduced farrowing rates and number of pigs born alive. In addition, advances in pig production and reproduction are constantly forcing producers to adapt to new systems and technologies.

Gestating-sow housing systems using individual crates is a topic of discussion due to animal welfare concerns surrounding the limited freedom to express natural animal behavior. The use of group housing for sows at different gestational stages has been emphasized to minimize stress and maximize animal welfare. In this group system, animals have a larger walking area allowing them to interact with other animals creating a better social environment. Group housing positively affected herd productivity and reduced the risks for metabolic and locomotor problems. Besides affecting other reproductive parameters, the breeding housing system may influence estrus expression. Estrus expression and detection may be reduced when housing sows adjacent to boars or weaned into groups. Therefore, housing a large number of females in groups post weaning may exacerbate existing reproductive problems and may be challenging to use intact males to detect estrus. As a consequence, reproductive management needs to be adapted to reduce reproductive failures.

Among the strategies to improve reproductive management is the use of intact, sterile males, or teasers. Teaser males are often used in other animal species, such as sheep and cattle, to improve estrus detection, and their use in swine farms is already implemented. Furthermore, teaser animals are known to stimulate estrus in females and induce early puberty since teasers are surgically modified males with the natural production and expression of male hormones and behavior, but without sperm release in seminal fluid. Techniques for creating teaser animals mostly use significant surgical interventions, limiting their use in pig farms. The objective of this study was to evaluate three surgical techniques for the preparation of intact, sterile pigs for their use to detect estrus of the females in swine farms.

Materials and methods
Animals
A total of 39 males of a composite breed Embrapa MS115 (Large White × Duroc × Pietrain) with a mean (SD) age of 63 days and weight of 32 (3) kg were used. Animals were randomly allocated to four different treatment groups and housed in individual pens. The 4 treatment groups were: no surgery (control; n = 9), epididymectomy by removal of the epididymis tail (TE; n = 10), vasectomy via scrotal access (VS; n = 10), and vasectomy via inguinal access (VI; n = 10). According to their age, they received a complete daily diet based on corn and soybean meal to achieve the nutritional requirements proposed by Rostagno et al. Animals had free access to water using an automatic nipple drinker designed for pigs.

Animals were clinically examined before the beginning of the study to assess animal health status. The procedures were always performed on four animals, one from each experimental group: 3 surgical techniques and 1 control group. The animals remained on the farm until 7 months of age when they underwent a breeding soundness examination and blood sample collection for hormone evaluation.

Preoperative examinations and procedures
In the preoperative period, water and food was withheld for 12 hours. Blood samples were collected following the clinical examination and evaluation of physiological parameters. The animals were then anesthetized with Tiletamine + Zolazepam (Virbac; 5 mg/kg, intramuscular) and Azaperone (Janssen Animal Health; 2 mg/kg, intramuscular) and transferred to the operating room. To remove dirt, sweat, epithelial cells, and transient skin bacteria, thus reducing contamination, the surgical site was cleaned with 20 mL of 2% chlorhexidine solution in water. Skin antisepsis in and around the incision site was performed with a 10% dilution of iodine using a sterile compress. Further, local anesthesia was carried out by infiltration with lidocaine without epinephrine (Bravet; 1.5 mg/kg).

Body temperature was measured, and blood samples were collected in all animals at three different time points: 1) day 0 (D0), 20 minutes before sedation.
and surgical procedure; 2) day 2 (D2), 48 hours after the procedure; and 3) day seven (D7) post surgery. These collections were always conducted between 8 AM and 8:30 AM. For all procedures, the surgery duration was recorded for later comparison between the techniques.

**Surgical Procedures**

**TE procedure.** The epididymis tails were located by exerting pressure at the scrotum base, as previously described by Althouse and Evans.14 Constant pressure was applied to stabilize the testis and to better visualize the location of the epididymal tail. An incision was made on the scrotum skin 1 to 2 cm directly over the greater curvature of the epididymal tail, deepened through the tunica muscular dartos and parietal vaginal tunica. The epididymis tail was removed by cutting the ligament to the testis and the epididymis body using a scalpel blade. Pressure was then exerted at the scrotum base so the testis could return to its normal anatomical position. The procedure was then repeated in the other testicle. At the end of the procedure, a suture with a maximum of three single isolated stitches using a nonabsorbable nylon 3-0 thread was performed.

**VS procedure.** A 3 cm incision was made in the skin parallel to the long axis of each testicle (1-2 cm lateral to the medial septum and 3-4 cm caudal to the tip of the epididymis head) as described by Althouse and Evans.15 with some modifications. The incision extended through the tunica muscular dartos and vaginal parietal tunica, exteriorizing the vas deferens. The vas deferens were then isolated from the pampiniform plexus, ligated with nonabsorbable nylon thread, and a 2 cm fragment was removed. Only the skin was sutured with a single isolated stitch pattern using a nonabsorbable nylon 3-0 suture. The same procedure was repeated on the contralateral side. After the vasectomy procedure, the tissue was sent for histological examination to confirm that the vas deferens was sectioned.

**VI procedure.** This technique was performed according to Godke et al16 with some modifications. A 3 cm midline incision was made in the skin in the space between the last pair of teats, 2 cm above the scrotum. The vas deferens was then separated from the other tissues with blunt surgical scissors. After the isolation, two ligatures were made approximately 0.5 cm apart with nonabsorbable nylon thread and the cord between the two ligatures was removed. The skin was approximated with a nonabsorbable nylon thread, with a pattern of single isolated spots. The same procedure was repeated in the other cord.

**Control.** The animals in this group underwent 12-hour fasting without water and food. All the parameters were investigated. Animals were submitted to the anesthesia, but not to any surgical procedure.

**Assessments of physiological parameters**

**Blood tests.** Blood samples were collected from the vena cava with a 5 mL syringe and a 40 x 12mm needle and placed in 4-mL glass tubes containing EDTA. The blood was sent to a commercial laboratory (Santa Catarina, Brazil) for a complete hematological profile, which was performed using a Neubauer chamber under a microscope. Lactate concentration was measured immediately after collection in the lactometer (Accutrend Plus; Roche Diagnostics) using a drop of whole blood on the test strip.

**Cortisol.** Cortisol concentrations were measured in the plasma by radioimmunoassay using a commercial kit (MP Biologicals). Cortisol was not measured at D7 because of the long interval post procedure and the results may be influenced by other factors, such as environment and management. The cortisol assay had a 44% capacity of ligation and 0.17% of nonspecific ligation and 1.09% of nonspecific ligation with 90% sensitivity. The intra- and inter-assay variation was 6.88% and 9.62%, respectively.

**Testosterone.** When the males reached 7 months of age, blood was collected from the vena cava. Testosterone concentration was measured in the plasma by radioimmunoassay using a commercial kit (MP Biologicals). The testosterone assay had 1,520 counts per minute (CPM), with a 44% capacity of ligation and 0.17% of non-specific ligation with 90% sensitivity. The intra- and inter-assay variation was 6.88% and 9.62%, respectively.

**Andrological examination.** After the animals reached puberty, at approximately 7 months of age, they were submitted to andrological examinations. The genitalia were thoroughly evaluated by palpation of the testicles for pain, adhesion, or enlargement. Internal genitalia lesions, mucosal color, and penile pruritus were evaluated. Measurements of the testicular perimeter were measured at the largest diameters using a manual caliper. The formula of the sphere/cylinder model17 was used to calculate the testicular volume: \[ VT = \pi \left( \frac{D_t}{2} \right)^2 \left( 3C_t - D_t \right) / 12 \].

After clinical examinations, males were taken to an individual area with a “dummy sow” for semen collection using the gloved-hand method. A drop of ejaculate was examined through light microscopy, using magnification ×400, to determine presence of spermatozoa. Sperm morphology and motility were also assessed in the control animals. Libido evaluations were subjective, based only on the interest of the male to copulate.

**Statistical analysis**

All data were evaluated by Statistical Analysis System (SAS, 2012). The Kolmogorov-Smirnov test assessed the normal distribution. Comparisons between variables and the interactions were performed for hematological parameters, rectal temperature, lactate, and cortisol. Comparisons only between variables were performed for testosterone concentrations and testicular parameters. All analyses were performed using PROC MIXED. The least squares means was used to calculate the adjusted means for each treatment, with comparisons using the Tukey test with 5% significance. Data are presented as least squares means of the percentages (SEM).

**Results**

No interaction was identified between the time of evaluation and treatment groups for rectal temperature \( \left( P = .52 \right) \), hematocrit \( \left( P = .91 \right) \), platelets \( \left( P = .19 \right) \), leukocytes \( \left( P = .85 \right) \), lymphocytes \( \left( P = .67 \right) \), monocytes \( \left( P = .34 \right) \), serum lactate \( \left( P = .98 \right) \), and cortisol \( \left( P = .37 \right) \). Therefore, the classifying variables (surgical procedure and time) were evaluated separately for each variable response.

There was no difference in the body temperature between the treatment groups \( \left( P = .40 \right) \) or day \( \left( P = .14 \right) \); Table 1).

For hematocrit, leukocytes, and monocytes, there were no differences between the treatment groups \( \left( P = .06 \right) \), and \( P = .25 \) respectively) and the time of evaluation \( \left( P = .39 \right) \), \( P = .88 \), and \( P = .92 \), respectively; Table 1).

While there were no differences observed in platelet count among the surgical groups when compared with the control group (TE: 366,226 [29,477] cells/mm³, \( P = .059 \); VS: 421,945 [27,610] cells/mm³, \( P = .40 \) or day \( \left( P = .67 \right)."
For lymphocytes, none of the surgical treatment groups (TE: 9837.54 [605.13] cells/mm³, D2: 459,862 [26,530] cells/mm³), D0 (429,594 [33,213] cells/mm³, D1: 430,913 [23,970] cells/mm³), or D7 (413,372 [27,174] cells/mm³, P = .31).

There was no difference in cortisol concentration between the treatment groups (TE: 3.87 [0.35] µg/dL; VS: 3.85 [0.31] µg/dL; VI: 3.27 [0.33] µg/dL; and control: 3.42 [0.37] µg/dL; P = .45). However, a difference in cortisol concentration was identified between D0 (3.97 [0.15] µg/dL) and D2 (3.24 [0.24] µg/dL; P = .02; Figure 1D).

No differences in testicular measurements (Table 1) were observed between the treatment groups (testicular length P = .10, width P = .33, and volume P = .12). There was also no difference between the testosterone concentrations among treatment groups at 7 months of age (P = .98; Table 1).

A difference in surgical duration was observed between the surgical techniques performed (P < .001). The fastest surgical technique was TE (18.16 [1.32] minutes), followed by VI (25.78 [1.25] minutes), and VS the most time consuming (32.53 [1.19] minutes) as described in Figure 2. All boars in the 3 surgical procedure groups presented healthy libido without sperm cells in the ejaculate.

**Discussion**

All surgical procedures used in the experiment effectively produced intact, sterile boars to be used for estrus detection in sows. Estrus detection is the process of identifying which females are receptive to mating. In the swine industry, sexually mature sows should cycle every 3 weeks and estrus can last for 48 to 64 hours. The most common external estrus signal is standing estrus, a physical sign of oxytocin release, increased estrogen levels, state of ovulation, and receptivity to mating. Estrus expression and duration can be affected by several factors including age, parity, season or temperature, genetic composition, body condition, nutrition, and previous boar exposure.

**Sus scrofa** has a large number of functional olfactory receptors. Once stimulated, the olfactory signals can alter the brain, changing the physiology and behavior of sows. Thus, the ideal boar exposure would involve physical contact where the boar is allowed to nudge, sniff, and fully stimulate the female to help with gilt development and identify sows in estrus. In individual sow crate housing systems, the boar walks in front of the females while the worker checks the sow for estrus. Ideally, estrus must be detected twice a day and performed 8 to 12 hours apart to identify the onset accurately. However, many farms struggle with sow longevity in the herd due to a decreased ability to detect estrus and complete successful mating. Low-quality estrus detection, stimulation, and mating are reflected in the breeding herd records as reproductive failures, negative pregnancy checks, or a low number of piglets born alive. Sows not accurately detected to be in estrus and subsequently inseminated are subject to a

**Table 1:** Least squares means (SEM) of hematological and endocrine parameters and testicular measurements of intact, sterile boars created using three different surgical techniques and control males

<table>
<thead>
<tr>
<th></th>
<th>Rectal temperature, °C</th>
<th>Hematocrit, %</th>
<th>Leukocytes, cells/mm³</th>
<th>Testicular length, cm</th>
<th>Testicular width, cm</th>
<th>Testicular volume, cm³</th>
<th>Testosterone, ng/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE (n = 10)</td>
<td>37.80 (0.47)</td>
<td>41.17 (0.76)</td>
<td>18713 (1353.66)</td>
<td>12.80 (0.63)</td>
<td>6.33 (0.23)</td>
<td>343.52 (35.29)</td>
<td>6.25 (1.92)</td>
</tr>
<tr>
<td>VS (n = 10)</td>
<td>38.77 (0.33)</td>
<td>42.28 (0.71)</td>
<td>19472 (1267.66)</td>
<td>14.63 (0.61)</td>
<td>6.86 (0.22)</td>
<td>463.05 (33.65)</td>
<td>5.92 (1.74)</td>
</tr>
<tr>
<td>VI (n = 10)</td>
<td>37.98 (0.46)</td>
<td>41.29 (0.75)</td>
<td>22927 (1334.28)</td>
<td>14.48 (0.63)</td>
<td>6.34 (0.23)</td>
<td>422.76 (35.29)</td>
<td>6.14 (1.82)</td>
</tr>
<tr>
<td>CONTROL (n = 9)</td>
<td>37.96 (0.46)</td>
<td>43.53 (0.76)</td>
<td>21025 (1352.78)</td>
<td>14.91 (0.67)</td>
<td>6.42 (0.25)</td>
<td>423.70 (37.20)</td>
<td>5.29 (2.02)</td>
</tr>
<tr>
<td>DAY 0</td>
<td>38.27 (0.39)</td>
<td>41.49 (0.62)</td>
<td>20110 (1113.89)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>DAY 2</td>
<td>38.59 (0.39)</td>
<td>42.11 (0.68)</td>
<td>21014 (1218.33)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>DAY 7</td>
<td>37.53 (0.39)</td>
<td>42.60 (0.72)</td>
<td>20515 (1247.91)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

TE = tail epididymectomy; VS = vasectomy via scrotal access; VI = vasectomy via inguinal access; NA = not applicable.
reduced farrowing rate and litter size.\textsuperscript{20} In addition, several studies have shown numerous poor-quality matings result in considerable variation in the number of times sows are mated and overworked boars. Only 35\% of all copulations lasted 2 minutes or more, and 63\% of all copulations were disrupted, mainly by competitor boars.\textsuperscript{21} This scenario is changing with the shift from individual crates to the use of group pen housing of sows.\textsuperscript{22} This will impact the success of estrus detection due to several risk factors associated with using an intact boar to detect estrus in a group of sows. An alternative is the use of a teaser animal. Teaser animals are males that have had their reproductive system surgically altered to render them sterile.\textsuperscript{23} The primary purpose of these animals is to assist in estrus detection to better manage when artificial insemination occurs. The procedure to create intact, sterile males is commonly used on bulls and rams; however, there is limited information on the best technique to produce an intact, sterile boar. Vasectomy is one of the procedures used to produce a teaser boar and has been recognized as a useful tool in manipulating estrus in sows.\textsuperscript{24} To produce an intact, sterile animal, sedation is necessary. In our study, the sedation protocol used proved to successfully immobilize the animals and help with muscle relaxation and sedation. Lower doses were required in comparison with other drugs, with more desirable results.\textsuperscript{25} This sedation protocol was used in all the animals for all the surgical groups (TE, VI, and VS) and very efficient according to the evaluated parameters. The hematological measures are of great importance for evaluating changes in blood cells as a result of the procedures. Our experiment showed no difference in hematocrit between the treatment groups because none of the surgical techniques used caused an incision capable of leading to significant blood loss. The VI group presented higher values for platelets than the TE group, which is probably related to the incision site, a region with a thicker lipid layer and a higher number of capillaries. This region bleeds more when disrupted, is a site of constant movement due to walking, and is often in direct contact with the floor when the animal is lying down. However, the higher platelet value in the VI group was not significantly different from the platelet value in the control group. There were no identified
differences in the number of leukocytes or monocytes among the treatment groups or time from surgery.

The VI group showed a significant increase in lymphocytes as compared to the other groups. Lymphocytes are markers of the inflammatory process and defense system with the role of presenting antigens. The lymphocyte increase was possibly associated with the surgical location and manipulation during the procedure due to the difficulty of cleaning the inguinal region, therefore increasing the chance of infection.

Lactate has been used to evaluate stress responses in animals. Serum lactate concentrations differed with surgical techniques, but there was no time difference. All surgical treatment groups showed increased values due to the surgery and healing process. However, the inflammatory response was minimal. Tissue hypoxia occurs around the incision injury, increasing lactate in the body. The only group that did not differ from the control group was the TE group, which, had a shorter surgical time and less tissue manipulation compared to the other techniques, resulting in reduced cell injury and faster healing process. This could indicate that increased animal manipulation was possibly responsible for increased lactate levels among the surgical groups.

As expected, there was an effect of time in relation to plasma cortisol concentrations. Cortisol was higher on the day of the surgery than 48 hours after the procedure. This was possibly related to physical restraint of the animals for blood collection and other parameter evaluations. In our study, no differences in cortisol were observed between treatment group, suggesting that the cortisol increase observed on D0 was due to restraint and sample collection.

The use of vasectomized boars is an efficient tool to improve gilt reproduction parameters. Faster gilt response to boar stimulation is indicative of a more developed hypothalamic-pituitary-ovarian axis. van Wettere et al. demonstrated that gilts mated at first estrus with a vasectomized boar had a higher farrowing rate and a larger first litter size than gilts not mated by a vasectomized boar on the first estrus. Our data show that all the vasectomy procedures were efficient for producing intact, sterile boars. However, the VS technique was the most invasive and time-consuming procedure compared with the others.

The VS procedure allowed for greater ease of reaching the sperm duct than the VI approach because there was less adipose tissue. On the other hand, it was observed that manipulation of tissues to expose the spermatic duct for the VS technique generated edema that momentarily made it difficult to visualize and differentiate structures. The VS procedure was the most time consuming, lasting approximately 35 minutes, in contrast with the VI procedure, which lasted approximately 25 minutes.

Vasectomized bulls have been reported to show increased teasing and mounting behaviors, but less aggressive behaviors compared with nonvasectomized bulls. In addition, libido of these vasectomized animals varied. In our study, we did not observe any differences among the testosterone levels, libido, or sexual interest during the boar soundness exam among the different surgical methods. This was also confirmed in other species. The epididymectomy procedure is an efficient and easy technique already used in domestic pigs, and is efficient to maintain the libido.

Studies in humans have shown that epididymectomy following a vasectomy reduced scrotal pain. In our study, this technique was the easiest to perform, mainly due to the position of the anatomical structures. The epididymis tail was easily accessible and resulted in less tissue manipulation and a smaller skin incision. Removing the epididymis tail was also the most straightforward and economical procedure for creating a teaser bull. Some complications have been identified after surgery, such as infection, reconnection of the spermatic duct, and removal or partial ligation of the artery along with the duct. To reduce the risk of contamination, all the animals in the present work were housed in a clean pen during the postoperative period to reduce the risk of infections.

The VI procedure should be chosen for use in animals with lower weights. Boars with thicker lipid layers make it challenging to locate the structures and require increased tissue manipulation, surgical time, and capillary damage leading to more significant bleeding and alteration of some hematological parameters as seen in the current study. Each male was observed an average of 10 to 15 minutes for the andrological exam performed at 7 months of age. Some males in this study did not perform mating, which is common and has no relation to the surgical techniques. All pigs had testosterone concentrations typical for their age. The testicular measurements and volume did not differ among animals from different treatment groups.

In conclusion, all the surgical techniques evaluated were efficient in producing intact, sterile boars with no alteration of the physiological parameters to prevent their use. The TE procedure was the fastest and least invasive procedure to produce intact, sterile boars.

**Implications**

Under the conditions of this study:

- Intact, sterile boars can be used for estrus detection in group housing.
- The 3 techniques used in this study effectively produced intact, sterile boars.
- The TE procedure was the most practical for producing intact, sterile boars.

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**Figure 2**: Duration of the different surgical procedures. Lower case letters represent a significant difference ($P < .05$). TE = tail epididymectomy; VS = vasectomy via scrotal access; VI = vasectomy via inguinal access.
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Conflict of interest
None reported.

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