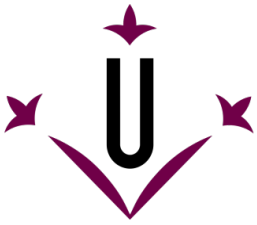

# Semen quality of Colombian Creole as compared to commercial pig breeds

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**Abstract**

Characterization of Creole breeds is still very limited, including reproductive performance. In this research, we assessed the semen quality of three Colombian Creole breeds (Zungo, Casco de Mula, and San Pedreño) relative to international breeds (Duroc, Belgian Landrace, and Pietrain). Two doses from seven boars per breed were evaluated for sperm kinetics and membrane and acrosome integrity using computer-assisted sperm analysis (CASA) and flow cytometry, respectively. The Creole pigs showed lower ($P<0.05$) volume of fluid ejaculated (185.5 mL vs 239.9 mL), sperm concentration (340.5 $x10^6$ vs 395.4 $x10^6$ sperm/mL), motility (90.9% vs 95.3%) and progressive motility (63.1% vs 67.2%) than international breeds. No relevant differences between Creole and international breeds for sperm velocity traits were observed, but Creole boars had lower ($P<0.05$) proportion of morphologic normal sperm (86.1% vs 90.6%) and of sperm with both intact plasma membrane and acrosome integrity (76.8% vs 87.5%). Mitochondrial membrane potential did not differ between breeds. Creole breeds in general produced less normal and motile sperm per ejaculate than international breeds (49.3 $x10^9$ vs 81.5 $x10^9$). Although San Pedreño had larger ejaculates than Zungo and Zungo had a greater proportion of normal and motile sperm than San Pedreño, Creole breeds did not differ for total amount of normal and motile sperm per ejaculate. The semen from Colombian Creole pigs is qualitatively acceptable being less abundant but rich in normal and motile sperm than that from commercial breeds. This should be considered when developing recommendations for semen use and conservation for AI in Creole pigs

**Keywords:** Boar**,** CASA**,** Creole pigs, Flow cytometry, Sperm.

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

**Ethical approval**

The experimental protocol was approved by the Ethical Committee on Animal Experimentation of the University of Tolima.

**Consent to participate**

Not applicable

**Consent for publication**

Not applicable

**Availability of data and materials**

Please contact author for data requests

**Authors' contributions**

RS-M and IR-B conceived, designed and performed the experiment; RS-M and JE analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

## Introduction

Colombian Creole pigs are descendants of the Iberian pigs brought by early Spanish settlers at the Colombian Caribbean coast, in the current Department of Córdoba. They were originally referred as *Lampiños*, the Spanish word for hairless, since this was one of their more notorious features (Espinosa and Ly, 2015). Over the years, they expanded throughout the country under a wide range of environments while influenced by other imported breeds. The resulting populations are currently known as Creole (Criollo, in Spanish) pigs. Besides their historical and social importance, the Creole pigs are a valuable genetic resource for supporting the economy in rural areas thanks to their adaptation to extreme environments (Ortiz and Sánchez, 2001). There are three Creole pig breeds officially recognized in Colombia: *Zungo* (ZU), located in the Atlantic coast and with a similar hairless phenotype as the Iberian *Lampiño* pigs; *Casco de Mula* (CM), which is found mainly in the eastern plains of Colombia and thus called because of its syndactyly or fused-hoof; and *San Pedreño* (SP), which is observed around the central mountain ranges of the Antioquia and Viejo Caldas regions and is characterized by their black skin and hair (Oslinger et al., 2006). Each breed has developed their own adaptation mechanisms to local ecosystems, all characterized by recurrent periods of water and food scarcity and diseases or simply poor farm management. As a result, Creole pigs show lower reproductive and growing performance but better immunocompetence and rusticity than improved commercial breeds (Linares et al., 2011). For this reason, as more intensive farming practices were introduced, Creole pigs were subsequently replaced with international improved breeds, thereby reducing dramatically their census and limiting their presence to small and disconnected nucleus in rural areas. As happened with other endangered breeds (Sierra, 2000), this led local authorities to establish specific conservation nucleus for the Creole breeds and take actions accordingly for their

phenotypic and genetic characterization. Currently, the Creole breeds are managed under the auspices of the Colombian Agricultural Research Corporation (AGROSAVIA) in three research centres, one per breed. Each breeding nucleus consists of around 70 to 140 individuals, which are distributed in family groups and subjected to a circular mating system to maintain genetic variability (Ocampo-Gallego, 2019).

A key feature to be considered for conservation purposes is the reproductive profile of the boars. Yet, no information is available on the semen quality of Creole boars. The use of advanced meaningful technologies may help to describe the semen attributes of the Creole boars in order to predict their quality and fertility potential. Flow cytometry and computer-assisted sperm analysis (CASA) are two of these techniques that have been proven to be effective to assess boar semen quality (Boe-hansen and Satake, 2019), since they provide objective data on sperm kinetics, plasma and acrosome membrane integrity and mitochondrial membrane potential ($\Delta\Psi m$), whereby sperm motility and fertilizing capacity can be estimated (Manosalva et al., 2005). These parameters are essential for an efficient use of boar ejaculates and to implement optimal conservation and production strategies (Oehler et al., 2019). Therefore, the aim of this study was to evaluate the quality of semen in the three officially registered Colombian Creole pig breeds by using the most recent techniques of flow cytometry and CASA. The results were compared with those obtained in three international commercial breeds commonly used in Colombia.

## Material and methods

### Animals and semen collection

Seven boars per breed from three Colombian Creole (C) breeds (ZU, CM, and SP) and from three international (I) breeds (DU: Duroc; BL: Belgian Landrace; and PI: Pietrain)

were used for this research. The C boars were all the available in the AGROSAVIA germplasm breeding nucleus of La Libertad (for CM), El Nus (for SP) and Turipana (for ZU) from April to July, 2019, while I boars were randomly sampled during the same period from a commercial stud centre (Porcigan, Cajamarca-Tolima, Colombia). Boars were maintained under standard production conditions with restricted access to feed (2 kg/d, 3340 kcal/kg DE) (Domínguez et al., 2014). All boars were 1- to 2-year-old and sexually active at sampling. Two ejaculates per boar were collected by using the gloved hand technique (vinyl gloves) and the sperm-rich fraction was retained in a sterile thermal bottle at 37 ºC (Siqueira et al., 2011). In each ejaculate, the volume of fluid ejaculated (VOL) and the sperm concentration (CO) were measured respectively by using a digital weight/volume scale (WeiHeng, Guangzhou, China) and a photometer (SMD-6 Minitube, Tiefenbach, Germany). Then, from one of the ejaculates, two doses of semen (100 mL) containing 3 $\times 10^9$ sperm were obtained by dilution with solution boar semen extender (MR-A®, kubus, Spain). The semen was diluted at 37 °C and kept for 2 hours at room temperature until reaching around 20 °C. Each dose was assessed in duplicate for sperm motility and concentration using a phase-contrast and fluorescence microscope (Labomed® Lx500, Labomed Europe, The Netherlands) and a photometer (SDM 6 minitube®, Wisconsin, USA), respectively. All doses were stored and transported at 17°C and processed for sperm quality assessments within 24 h (Torres et al., 2019).

**Sperm morphology and kinetics**

Sperm morphology and kinetics were assessed using a CASA system (Integrated Visual Optical System, IVOS II, Hamilton Thorne Inc., Beverly, MA, USA). Then, samples were incubated at 38 ºC for 3 min prior to be transferred (3 µL) to a counting chamber (Leja®, Luzernestrat, Netherlands) for morphology and kinetics evaluation of 1,000 sperm.

Regarding morphology, the percentages of sperm showing normal appearance (NO), head abnormalities, bent or coiled tails, and medial, distal or proximal cytoplasm droplets were determined. All basic sperm kinetics traits are described in Table 1. Sperm with an average path velocity (VAP) lower than 10 μm/s were considered immotile while those with a straight-line velocity (VSL) lower than 10 μm/s as progressive motile. Total motility (MOT) accounted for the percentage of motile sperm over total and progressive motility (PMOT) for the percentage of progressive motile sperm over total.

**Plasma membrane and acrosome integrity**

The integrity of the sperm plasmatic membrane (namely, viability) was assessed in samples diluted to $30x10^6$ sperm/mL stained with propidium iodide (PIO) at 12 μM. Acrosome integrity was assessed in the same samples using the double stain fluorescein isothiocyanate-conjugated peanut agglutinin (FITC-PNA) and PIO (Hernández et al., 2007). Briefly, 100 μL of each sample was mixed with 10 μL of a solution of PNA (1 μg/mL of distilled water) and PIO at 12 μM. Samples were incubated in complete darkness at 37 ºC for 10 min prior to flow cytometry analysis. Flow cytometry was performed with a high-speed fixed-alignment flow cytometer (BD FACSAriaTM II, Becton, Dickinson and Co, CA, USA), which was calibrated to exclude subcellular residues by size using a forward scatter detector. A total of 50,000 sperm were evaluated in each sample. Samples were excited at 488 nm with an argon laser running at a 220 mW and emission spectra were collected using the FL1 (505 a 545 nm, for the FITC-PNA and JC-1 green fluorescent) and FL3 (670nm, for the PIO red fluorescent) bandpass filters (Bonet et al., 2012). Data were processed with the FlowJoTM software (Ashland, OR, USA). Sperm were scored as either viable (if negative for PIO) or dead (if positive for PIO) and also as either intact (if negative for FITC-PNA) or reacted (if positive FITC-

PNA) acrosome. Moreover, sperm was grouped into four classes according to viability and acrosome integrity (viable & intact; viable & reacted; dead & intact; and dead & reacted). Each class was expressed as a percentage over total (Bonet et al., 2012).

**Mitochondrial membrane potential**

The lipophilic cationic dye 5,5′, 6,6′-tetrachloro-1,1′, 3,3′-tetraethylbenzimidazolyl carbocyanine iodide (JC-1) was used for assessing mitochondrial membrane potential (Teixeira et al., 2015). Sperm concentration was re-diluted with Dulbecco's Phosphate Buffer Saline (PBS; Sigma-Aldrich, USA) to a concentration of 1 x $10^6$ sperm/mL, from where 288 µL were taken and dispensed in a cytometry tube preheated at 37 ºC. Then, each sample was stained with 12 µL de JC-1 (153 µM, T-3168; Thermo Fisher) and incubated for 10 min at 37 °C. Finally, 300 µL of PBS were added to the mix in order to obtain a concentration of 0.5 x $10^6$ sperm/mL. Flow cytometry analysis was performed using 488 nm excitation with bandpass filters FL1 (525±30 nm) and FL2 (590±40 nm) for green and red emission, respectively. In healthy sperm, the dye is taken up by the mitochondria, where it forms aggregates that exhibit intense red/orange fluorescence. In contrast, in dysfunctional sperm, the dye remains as a monomer and the mitochondria appears fluorescent green. Consequently, the mitochondrial potential was expressed as the percentage of red ($\Delta\Psi m^{High}$) or green ($\Delta\Psi m^{Low}$) over the total (Bonet et al., 2012; Ramió et al., 2011).

**Statistical analysis**

Data were analysed using a linear mixed model with the breed as a fixed effect and the boar as a random effect. The effect of the breed was tested following an F-test while multiple pairwise comparisons were done using the Tukey test as post hoc. Results were

considered statistically significant at $p<0.05$. All analysis was performed using the statistic package JMP Pro 14 (SAS Institute Inc., Cary, NC).

## Results

### Sperm kinetics and morphology

On average, C pigs, compared to I pigs, showed lower ($P<0.05$) values of VOL (185.5 mL vs 239.9 mL), CO (340.5 $x10^6$ vs to 395.4 $x10^6$ sperm/mL), MOT (90.9% vs 95.3%) and PMOT (63.1% vs 67.2%) (Table 2). Within C breeds, the main differences were between ZU and SP for VOL and PMOT, with ZU presenting lower VOL (173.6 mL) and higher PMOT (93.6%) than SP (196.8 mL and 88.9%, respectively). No differences were observed for CO and MOT between C breeds. Values for VOL, CO and MOT were always higher in I than C breeds. Interestingly, this pattern changed for PMOT, where SP (57.6%), CM (59.1%) and PI (61.3%) presented the lowest values while DU (73.2%) and ZU (72.5%), the highest. Differences between breeds for sperm kinetics traits are given in Table 2. Although no differences were found for velocity traits, the distances covered were lower in C breeds, particularly for curvilinear distance (-20.7 μm, $P<0.05$). Remarkably, wobble and linearity indexes were higher in C pigs (2.1% and 2.3%, respectively, $P<0.05$), but not beat-cross frequency, which was lower (-4.0%, $P<0.05$). The means for sperm morphology traits by breed are given in Table 3. On average, the abnormalities were higher (4.5%, $P<0.05$) in C (NO: 86.1%) than in I (NO: 90.6%) breeds. Within C breeds, ZU showed the highest proportion of normal sperm (89.0%) and the SP the lowest (83.3%). The same trend was observed for all types of sperm abnormalities (Table 3), with the exception for bent tail sperm, where ZU pigs showed the highest proportion between the three C breeds.

***Plasma membrane and acrosome integrity***

The means by breed for membrane and acrosome integrity are displayed in Fig 1. On average, the C breeds showed less viable (-9.4 ± 1.3%, P<0.05; Fig. 1A) and intact (-5.0 ± 0.9%, P<0.05, Fig. 1B) sperm than I breeds. Viability did not differ across C breeds, but CM had more intact sperm than SP (85.2% vs 74.7%). The distribution of sperm plasmatic membrane and acrosome integrity groups across breeds is given in Table 4. The proportion of viable and intact sperm was lower (10.7%, P<0.05) in C (76.8%) than I breeds (87.5%). No difference was detected between C breeds (73.6% to 79.9%), which showed a similar performance than LB (79.4%). In contrast, viable sperm with reacted acrosome or dead sperm, either intact or reacted, was higher (5.7%, 1.4%, and 3.7%, respectively, P<0.05) in C breeds. Within C breeds, the main difference was for viable but reacted sperm, which was higher for SP (17.3%) as compared with CM (9.0%).

**Mitochondrial membrane potential**

The mitochondrial membrane potential ($\Delta\Psi m^{High}$) did not differ between C and I breeds. In Fig 2, the distribution of sperm fluorescence intensity within each breed and the corresponding red/green ratio dot plot is displayed. Interestingly, the ZU pigs showed the highest $\Delta\Psi m^{High}$ among all breeds (91.8%), in line with those observed in LB and DU (90.0% and 89.3%, respectively) but higher than in CM (81.4%) and PI (84.7%).

## Discussion

There is still a lack of research concerning the phenotypic and genetic characterization of Creole pigs. Here, we have characterised the semen of the Colombian Creole boars in the AGROSAVIA germplasm network for their use and conservation for AI. The quantity and quality of semen depends on genetic and environmental factors including the breed

(López et al., 2017). In our experiment, VOL was within the normal range (Bonet et al., 2013), but CO was lower than in other studies (Banaszewka and Kondracki, 2012). Results obtained here indicate that C boars produce half the sperm per ejaculate than I boars. Differences observed between C breeds were much lower, but even so SP managed to produce 16% more sperm than ZU. However, the sperm production of C boars was higher than documented in other local breeds, particularly in Mexican Creole (Sierra et al., 2016) and Iberian (Gómez, 2007), which accounted for 22.9% to 83.5% of the sperm production of C boars. Results for Fengjing and Meishan Chinese breeds were much more variable, with values from half below (Borg et al., 1993) to almost three-fold higher (Gerfen et al., 1994) than those observed in our study. This fact stresses the difficulty in makin comparisons across breed under different environmental settings, where numerous non-genetic factors may affect the results. In the present experiment, we followed the same experimental protocol in all breeds in order to avoid any potential bias. Innovative tools such as CASA and flow cytometry allow for further insights into semen quality. Although CASA outcome should be interpreted multiparametrically (Boe-hansen and Satake, 2019), MO was shown to be positively related to pregnancy rate and litter size (Broekhuijse et al., 2012; Ruiz-Sánchez et al., 2006) and the absence of morphology abnormalities to functionality of the seminiferous epithelium and epididymal maturation (Gadea, 2005).

Values over 70% for MOT, and 80% for NO, are expected and acceptable in healthy boars (Wu et al., 2018). On average, all breeds meet this requirement, although individually, there were one CM boar (78.6%), for MOT, and two CM (76.9% and 78.6%) and four SP boars (from 74.3% to 79.70%), for NO, that had lower values. As compared to I breeds, MOT and NO were only around 5% lower in C boars, which indicates that in Creole breeds, quantity rather than quality would limit production of seminal doses. Results

275 reported for MOT (80.4%) and NO (94.5%) in the Mexican Creole *Pelón de Yucatán*
276 breed point towards the same conclusion (Sierra et al., 2016).

277 Morphological abnormalities as well as velocity and distance traits fall above
278 threshold values (Kondracki et al., 2012). Interestingly, CM and SP showed lower values
279 of PMOT than ZU, which also had less abnormal sperm, particularly relative to SP.
280 Mitochondrial membrane potential was on the high side of reported values, which ranged
281 from 66.9% to 93.5% (Bryła and Trzcińska, 2015; Guo et al., 2017). The $\Delta\Psi m^{High}$
282 expresses the capacity of the mitochondria to produce the energy needed by the axonemal
283 dynein system to fuel sperm motility (Guo et al., 2017). The sperm membrane integrity
284 has shown a closer relationship to litter size than traditionally estimated sperm motility
285 (Jung et al., 2015). Thus, $\Delta\Psi m^{High}$ has been associated with functionally intact
286 mitochondria and ultimately to MOT (Agnihotri et al., 2016), PMOT (Johnson et al.,
287 2000) and litter size (Jung et al., 2015), while $\Delta\Psi m^{Low}$ with decreased acrosome reaction
288 and fertilization capacity (Zhao et al., 2014). We did not detect a clear distribution pattern
289 of $\Delta\Psi m^{High}$ across breeds, although both mitochondrial membrane and acrosome integrity
290 were higher in I breeds. However, the within breed correlation of ΔΨmHigh with
291 membrane mitochondrial and acrosome integrity were positive (ranging from 0.25, for
292 acrosome integrity in SP, to 0.93, for membrane integrity in ZU) as well as with MOT
293 (ranging from 0.14 for CM to 0.88 in SP). The more variable behaviour of these
294 parameters among Creole breeds could be explained by a combination of historical
295 differences, unequal past influences of foreign breeds, nucleus foundational effects and
296 genetic drift associated to limited population size (Burgos et al., 2013). To avoid
297 additional sampling variability, C boars were sampled from all available lineages within
298 each breed.

Boar semen traits are important indicators for predicting boar fertility and hence also for artificial insemination. Widespread use of refrigerated semen for artificial insemination has benefitted commercial pig units from higher genetic gains at lower economic costs. However, so far Colombian Creole pigs are only produced under natural mating, thereby limiting their use. The development of an artificial insemination programme for Creole pigs would favour their dissemination and therefore their conservation. A programme like this requires setting the scale capacity and conditions under which seminal doses should be prepared and stored. Seminal doses with poor motility and morphology are the main screening criteria for diagnosis of infertility and subfertility in boars (Arsenakis et al., 2017). Although these two features show a lower profile in C than in I breeds, they are still within the acceptable range. However, the reproductive efficiency of a boar is also given by the ability to produce a large amount of sperm. In this study, C pigs produced between 48.4 $x10^9$ (ZU) and 50.0 $x10^9$ (SP) normal and motile sperm per ejaculate (VOL x CO x NO x MOT), which results in a potential production capacity of 16-17 doses of 3 x $10^9$ normal and motile sperm (Schulze et al., 2019), around 10 doses less than in I boars. Thus, sperm dosage must take into account the lower performance of Creole boars in terms of the number of viable sperm per ejaculate. Unless boars are heavily used, this should not be a limitation for using AI in Creole pigs. However, this situation may occur in conservation programmes, where in order to maintain genetic variability, many AI-doses of unique boars are cryopreserved from only a few ejaculates. There is no need to differentiate between Colombian Creole breeds since all three produce a similar amount of motile sperm per ejaculate. The greater capacity of SP to produce sperm is offset by the greater sperm quality of ZU pigs.

This is the first documented study describing the semen characteristics of Colombian Creole breeds. The semen of the Creole pigs is within the acceptable range

for quality standards used in artificial insemination, but less rich in normal and motile sperm than the collected from improved commercial breeds. However, semen quantity rather than quality can be the limiting factor for an efficient production of insemination doses. Findings provided here can guide nucleus herds in developing the standards of semen processing in Creole pigs and thus give new impetus to their conservation.

## References


Agnihotri, S.K., Agrawal, A.K., Hakim, B.A., Vishwakarma, A.L., Narender, T., Sachan, R., Sachdev, M., 2016. Mitochondrial membrane potential ( MMP ) regulates sperm motility. In Vitro. Cell. Dev. Biol. - Animal. 52, 953–960. https://doi.org/10.1007/s11626-016-0061-x

Arsenakis, I., Appeltant, R., Sarrazin, S., Rijsselaere, T., Van Soom, A., Maes, D., 2017. Relationship between semen quality and meat quality traits in Belgian Piétrain boars. Livest. Sci. 205, 36–42. https://doi.org/10.1016/j.livsci.2017.09.009

Banaszewka, D., Kondracki, S., 2012. An Assessment of the Breeding Maturity of Insemination Boars Based on Ejaculate Quality Changes. Folia Biol. 60, 151–162. https://doi.org/10.3409/fb60

Boe-hansen, G., Satake, N., 2019. An update on boar semen assessments by fl ow cytometry and CASA. Theriogenology 137, 93–103. https://doi.org/10.1016/j.theriogenology.2019.05.043

Bonet, S., Briz, M., Yeste, M., 2012. A proper assessment of boar sperm function may not only require conventional analyses but also others focused on molecular markers of epididymal maturation. Reprod. Domest. Anim. 47, 52–64. https://doi.org/10.1111/j.1439-0531.2012.02033.x

Bonet, S., Casas, I., Holt, W., Yeste, M., 2013. Boar Reproduction, Springer. ed. Berlin. https://doi.org/10.1007/978-3-642-35049-8

Borg, K.E., Lunstra, D.D., Christenson, R.K., 1993. Semen Characteristics , Testicular Size , and Reproductive Hormone Concentrations in Mature Duroc , Meishan , Fengjing , and Minzhu Boars '. Biol. Reprod. 49, 515–521. https://doi.org/10.1095/biolreprod49.3.515

Broekhuijse, M., Šoštarić, E., Feitsma, H., Gadella, B., 2012. Application of computer-assisted semen analysis to explain variations in pig fertility. J. Anim. Sci. 90, 779–789. https://doi.org/10.2527/jas.2011-4311

Bryła, M., Trzcińska, M., 2015. Quality and fertilizing capacity of boar spermatozoa during liquid storage in extender supplemented with different antibiotics. Anim. Reprod. Sci. 163, 157–163. https://doi.org/10.1016/j.anireprosci.2015.11.005

Burgos, W., Souza, C., Megens, H., Ramayo, Y., Melo, M., Lemús, C., Caal, E., Soto, H., Martínez, R., Álvarez, L., Aguirre, L., Iñiguez, V., Revidatti, M., Martínez, O., Llambi, S., Esteve, A., Rodríguez, M., Crooijmans, R., Paiva, S., Schook, L., Groenen, M., Pérez, M., 2013. Porcine colonization of the Americas : a 60k SNP story. Heredity (Edinb). 321–330. https://doi.org/10.1038/hdy.2012.109

Domínguez, I., Sánchez, J., Bórquez, J., 2014. Manual de balanceo de dietas por computadora: serie porcinos, Dirección. ed, Universidad Autónoma del Estado de México. Universidad Autónoma del Estado de México, Toluca, México.

Espinosa, C., Ly, J., 2015. Cerdos criollos colombianos y agricultura sostenible. Rev. Comput. Prod. Porc. 22, 1–9.

Gadea, J., 2005. Sperm factors related to in vitro and in vivo porcine fertility, in: Theriogenology. pp. 431–444. https://doi.org/10.1016/j.theriogenology.2004.09.023

374 Gerfen, R., White, B., Cotta, M., Wheeler, M., 1994. Comparison of the semen
375 characteristics of Fengjing, Meishan and Yorkshire Boars. Theriogenology 41, 461–
376 469.
377 Gómez, M., 2007. Contrastación seminal de cinco variedades de cerdo Ibérico
378 (Manchado de Jabugo, Retinto, Torbiscal, Entrepelado y Lampiño) y estudio de la
379 fertilidad y prolificidad utilizando semen refrigerado de la variedad Torbiscal.
380 Escuela Superior Politecnica de Chimborazo.
381 Guo, H., Gong, Y., He, B., Zhao, R., 2017. Relationships between mitochondrial DNA
382 content, mitochondrial activity, and boar sperm motility. Theriogenology 87, 276–
383 283. https://doi.org/10.1016/j.theriogenology.2016.09.005
384 Hernández, M., Roca, J., Gil, M., Vázquez, J., Martínez, E., 2007. Adjustments on the
385 cryopreservation conditions reduce the incidence of boar ejaculates with poor
386 sperm freezability. Theriogenology 67, 1436–1445.
387 https://doi.org/10.1016/j.theriogenology.2007.02.012
388 Johnson, L., Weitze, K., Fiser, P., Maxwell, W., 2000. Storage of boar semen. Anim.
389 Reprod. Sci. 62, 143–172. https://doi.org/10.1016/S0378-4320(00)00157-3
390 Jung, M., Rudiger, K., Schulze, M., 2015. Review Article In Vitro Measures for
391 Assessing Boar Semen Fertility Impact of Sperm Motility on Boar Fertility. Reprod
392 Dom Anim 50, 20–24. https://doi.org/10.1111/rda.12533
393 Kondracki, S., Iwanina, M., Wysokińska, A., Huszno, M., 2012. Comparative analysis of
394 Duroc and Pietrain boar sperm morphology Comparative analysis of Duroc and
395 Pietrain boar sperm morphology. Acta Vet. Brno 81, 195–199.
396 https://doi.org/10.2754/avb201281020195
397 Linares, V., Linares, L., Mendoza, G., 2011. Caracterización etnozootécnica y potencial
398 carnicero de Sus scrofa “cerdo criollo” en Latinoamérica. Sci. Agropecu. 2, 97–110.

López, A., Van, A., Arsenakis, I., Maes, D., 2017. Boar management and semen handling factors affect the quality of boar extended semen. Porc. Heal. Manag. 3, 1–12. https://doi.org/10.1186/s40813-017-0062-5

Manosalva, I., Cortes, C., Leyva, V., Valdivia, M., De Los Reyes, M., Barros, C., Moreno, R., 2005. Efecto de la refrigeración sobre la motilidad, integridad de membrana acrosomal y reacción acrosomal en espermatozoides caninos. Rev. Investig. Vet. del Peru 16, 114–128. https://doi.org/10.15381/rivep.v16i2.1548

Ocampo-Gallego, R.J., 2019. Análisis de diversidad genética en cerdo criollo san pedreño utilizando datos de pedigrí. Ecosistemas y Recur. Agropecu. 6, 333. https://doi.org/10.19136/era.a6n17.2049

Oehler, C., Janett, F., Schmitt, S., Malama, E., Bollwein, H., 2019. Development of a flow cytometric assay to assess the bacterial count in boar semen. Theriogenology 133, 125–134. https://doi.org/10.1016/j.theriogenology.2019.04.036

Ortiz, W., Sánchez, M., 2001. Los cerdos criollos de America Latina, in: FAO (Ed.), Los Cerdos Locales En Sistemas Tradicionales de Producción. Roma, pp. 13–37.

Oslinger, A., Muñoz, J., Álvarez, L., Ariza, F., Moreno, F., Posso, A., 2006. Characterization of Colombian creole pigs by RAMs. Acta Agronómica 55, 45–50. https://www.redalyc.org/pdf/1699/169920333007.pdf

Ramió, L., Fernandez, J., Peña, A., Colas, S., Cebrian, T., Muiño, B., Ramırez, A., Concha, L., Rigau, T., Rodríguez, J., 2011. ‘ In Vitro ’ Capacitation and Acrosome Reaction are Concomitant with Specific Changes in Mitochondrial Activity in Boar Sperm : Evidence for a Nucleated Mitochondrial Activation and for the Existence of a Capacitation-Sensitive Subpopulational Structure. Reprod Dom Anim 46, 664–673. https://doi.org/10.1111/j.1439-0531.2010.01725.x

Ruiz-Sánchez, A.L., O´Donoghue, R., Novak, S., Dyck, M., Cosgrove, J., Dixon, W.,

Foxcroft, G., 2006. The predictive value of routine semen evaluation and IVF technology for determining relative boar fertility 66, 736–748. https://doi.org/10.1016/j.Theriogenology.2005.12.012

Schulze, M., Nitsche-Melkus, E., Jakop, U., Jung, M., Waberski, D., 2019. New trends in production management in European pig AI centers. Theriogenology 137, 88–92. https://doi.org/10.1016/j.theriogenology.2019.05.042

Sierra, A., 2000. Conservación genética del cerdo pelón en yucatán y su integración a un sistema de producción sostenible: primera aproximación. Arch. Zootec. 49, 415–421.

Sierra, A., Ortiz, J., Bojórquez, J., Canul, M., Tamayol, J., Rodríguez, J., Sanginés, J., Magaña, M., Montes, R., Segura, J., 2016. Conservación y uso sustentable del cerdo pelón en Yucatán. Quehacer Científico en Chiapas 11, 13–28.

Siqueira, A.P., Wallgren, M., Hossain, M.S., Johannisson, A., Sanz, L., Calvete, J.J., Rodriguez-martinez, H., 2011. Linköping University Post Print Quality of boar spermatozoa from the sperm- peak portion of the ejaculate after simplified freezing in MiniFlatpacks compared to the remaining spermatozoa of the sperm-rich fraction. Theriogenology 7, 1175–1184. https://doi.org/10.1016/j.theriogenology.2010.11.024

Teixeira, S.M.P., Chaveiro, A., Silva, F.M., 2015. The effects of three extenders on refrigerated boar semen. S. Afr. J. Anim. Sci. 45, 82–88. https://doi.org/10.4314/sajas.v45i1.10.

Torres, M., Monteiro, M., Passarelli, M., Papa, F., Dell'Aqua, J., Alvarenga, M., Martins, S., de Andrade, A., 2019. The ideal holding time for boar semen is 24 h at 17 °C prior to short-cryopreservation protocols. Cryobiology. 86, 58–64. https://doi.org/10.1016/j.cryobiol.2018.12.004.

Wu, Y., Lai, W., Liu, Z., Wei, H., Zhou, Y., Tan, J., Sun, H., Li, S., Peng, J., 2018. Serum and Seminal Plasma Element Concentrations in Relation to Semen Quality in Duroc Boars. Biol. Trace Elem. Res. 189, 85–94. https://doi.org/10.1007/s12011-018-1459-y

Zhao, X., Ren, J., Zhao, S., Cui, L., Hao, H., Wang, H., Du, W., Qin, T., Liu, Y., Wang, D., Zhu, H., 2014. Apoptosis-Like Events and In Vitro Fertilization Capacity of Sex-Sorted Bovine Sperm. Reprod Dom Anim 49, 543–549. https://doi.org/10.1111/rda.12305

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463 **Table 1.** Sperm kinetics parameters

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| Parameter | Unit | Description |
|---|---|---|
| Velocity Average Path (VAP) | µm/s | Average velocity of the smoothed path of the sperm head |
| Velocity Straight Line (VSL) | µm/s | Average velocity measured in a straight line from the beginning to the end of a track. |
| Velocity Curvilinear (VCL) | µm/s | Average velocity measured over the actual point-to-point track followed by the sperm |
| Distance of Average Path (DAP) | µm | Average distance traveled throughout the route |
| Distance Straight Line (DSL) | µm | Average distance traveled in a straight line |
| Distance Curvilinear (DCL) | µm | Average distance traveled curvilinear traveled |
| Straightness (STR) | % | VSL/VAP ratio, measures movement density |
| Wobble (WOB) | % | VCL/VAP ratio, measures sperm wobble |
| Linearity (LIN) | % | VSL/VCL ratio, measure the direction |
| Amplitude of Lateral Head (ALH) | µm | Amplitude of lateral head displacement turn regarding an intermediate piece |
| Beat-Cross Frequency (BCF) | Hz | Frequency with which the curvilinear path crosses the linear one as a function of time |

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**Table 2**. Means for sperm volume, concentration and kinetics traits by breed and difference between Colombian Creole (C) and international (I) breeds.

| Trait[1] | Breed | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Creole | | | International | | | | Difference |
| | ZU | CM | SP | LB | DU | PI | SEM | C - I |
| VOL (mL) | 173.6$^{d}$ | 186.1$^{cd}$ | 196.8$^{bc}$ | 255.7$^{a}$ | 207.6$^{b}$ | 256.4$^{a}$ | 3.8 | -54.4±3.1* |
| CO ($10^6$/mL) | 335.5$^{c}$ | 343.1$^{c}$ | 342.8$^{c}$ | 375.2$^{bc}$ | 419.1$^{a}$ | 391.9$^{ab}$ | 9.9 | -54.9±6.3* |
| MOT (%) | 93.6$^{ab}$ | 90.2$^{ab}$ | 88.9$^{b}$ | 95.9$^{a}$ | 95.7$^{a}$ | 94.3$^{ab}$ | 1.5 | -4.4±1.2* |
| PMOT (%) | 72.5$^{a}$ | 59.1$^{b}$ | 57.6$^{b}$ | 67.7$^{ab}$ | 73.2$^{a}$ | 61.3$^{b}$ | 2.5 | -4.2±2.0* |
| VAP (µm/s) | 88.4 | 89.0 | 94.2 | 91.0 | 85.2 | 84.1 | 3.6 | -3.8±2.9 |
| VSL (µm/s) | 63.0 | 55.0 | 57.8 | 54.6 | 56.6 | 53.3 | 2.7 | -3.8±2.2 |
| VCL (µm/s) | 169.7 | 174.7 | 180.6 | 191.0 | 166.9 | 168.9 | 7.2 | 0.4±5.9 |
| DAP (µm) | 33.0$^{c}$ | 33.3$^{c}$ | 40.1$^{bc}$ | 47.9$^{a}$ | 45.0$^{ab}$ | 39.6$^{bc}$ | 1.8 | 8.7±1.5* |
| DSL (µm) | 22.3$^{bc}$ | 18.0$^{c}$ | 20.9$^{c}$ | 26.9$^{ab}$ | 28.2$^{a}$ | 23.0$^{abc}$ | 1.3 | 5.7±1.1* |
| DCL (µm) | 65.34$^{c}$ | 67.5$^{c}$ | 79.2$^{bc}$ | 102.7$^{a}$ | 89.3$^{ab}$ | 82.2$^{b}$ | 3.4 | 20.7±2.8* |
| STR (%) | 71.8$^{a}$ | 61.9$^{b}$ | 62.5$^{b}$ | 60.2$^{b}$ | 66.9$^{ab}$ | 62.8$^{b}$ | 2.0 | -2.1±1.7 |
| WOB (%) | 53.1$^{ab}$ | 52.3$^{ab}$ | 53.3$^{b}$ | 48.4$^{a}$ | 52.9$^{ab}$ | 51.0$^{ab}$ | 1.2 | -2.1±1.0* |
| LIN (%) | 39.1$^{a}$ | 33.2$^{ab}$ | 34.4$^{ab}$ | 30.0$^{b}$ | 34.5$^{ab}$ | 33.3$^{ab}$ | 1.7 | -2.3±1.4* |
| ALH (µm) | 8.11 | 8.4 | 8.0 | 8.6 | 7.48 | 8.1 | 0.3 | -0.1±0.3 |
| BCF (Hz) | 28.8$^{b}$ | 30.3$^{b}$ | 31.4$^{ab}$ | 34.4$^{a}$ | 33.9$^{a}$ | 34.4$^{a}$ | 4.0 | 4.0±0.6* |

[1] VOL: volume of fluid ejaculated; CO: sperm concentration; MOT: total motility (percentage of motile sperm); and PMOT: progressive motility (percentage of sperm with at least 80% of linear movement); see Table 1 for other trait abbreviations.

[a,b,c,d] Superscripts with different letters in a row represent statistical differences ($P$<0.05).

* P<0.05

480 **Table 3**. Means for sperm morphology traits by breed and difference between Colombian
481 Creole (C) and international (I) breeds

| | Breed | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Creole | | | International | | | | Difference |
| Trait[1] (%) | ZU | CM | SP | LB | DU | PI | SEM | C - I |
| NO | 89.0$^{ab}$ | 86.0$^{bc}$ | 83.2$^{c}$ | 90.3$^{ab}$ | 90.1$^{ab}$ | 91.5$^{a}$ | 1.2 | -4.5±0.9* |
| HA | 0.8$^{b}$ | 0.8$^{b}$ | 1.9$^{a}$ | 2.0$^{a}$ | 1.1$^{b}$ | 0.8$^{b}$ | 0.1 | 0.1±0.1 |
| BT | 3.0$^{a}$ | 1.6$^{b}$ | 1.5$^{b}$ | 1.1$^{b}$ | 1.2$^{b}$ | 0.5$^{b}$ | 0.3 | -1.1±0.2* |
| CT | 0.6$^{ab}$ | 0.9$^{a}$ | 0.5$^{ab}$ | 0.3$^{b}$ | 0.2$^{b}$ | 0.2$^{b}$ | 0.1 | -0.5±0.1* |
| MCD | 1.6$^{abc}$ | 3.1$^{a}$ | 2.4$^{ab}$ | 2.0$^{abc}$ | 1.2$^{bc}$ | 0.8$^{c}$ | 0.4 | -1.0±0.3* |
| DCD | 3.0$^{bc}$ | 5.2$^{ab}$ | 6.1$^{a}$ | 3.3$^{bc}$ | 2.6$^{c}$ | 3.1$^{bc}$ | 0.6 | -1.7±0.5* |
| PCD | 2.0$^{bc}$ | 2.5$^{abc}$ | 4.3$^{a}$ | 0.9$^{c}$ | 3.7a$^{b}$ | 3.1$^{ab}$ | 0.5 | -0.3±0.4 |

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483 [1.] Proportion of sperm with normal appearance (NO) HA: Head abnormality, BT: Bent
484 tail, CT: Coiled tail, MCD: Medial cytoplasm droplets, DCD: Distal cytoplasm droplets,
485 PCD: Proximal cytoplasm droplets or showing head abnormalities (HA), bent tail (BT),
486 coiled tail (CT), medial cytoplasm droplets (MDC), distal cytoplasm droplets (DCD) and
487 proximal cytoplasm droplets (PCD)
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489 [a,b,c] Superscripts with different letters in a row represent statistical differences ($P$<0.05).
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491 * P<0.05
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510 **Table 4**. Means for sperm plasma membrane and acrosome integrity by breed and
511 difference between Colombian Creole (C) and international (I) breeds.
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| | Breed | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Creole | | | International | | | | Difference |
| Trait[1] (%) | ZU | CM | SP | LB | DU | PI | SEM | C - I |
| Alive & Intact | 76.8$^{b}$ | 79.9$^{b}$ | 73.6$^{b}$ | 79.4$^{b}$ | 91.2$^{a}$ | 93.0$^{a}$ | 1.8 | -10.7±1.4* |
| Alive & Reacted | 13.5$^{ab}$ | 9.0$^{bc}$ | 17.3$^{a}$ | 15.7$^{a}$ | 4.3$^{cd}$ | 2.8$^{d}$ | 1.3 | -5.7±1.0* |
| Dead & Intact | 4.2$^{ab}$ | 5.3$^{a}$ | 1.1$^{c}$ | 1.2$^{c}$ | 2.5$^{bc}$ | 2.8$^{bc}$ | 0.5 | -1.4±0.4* |
| Dead & Reacted | 5.5$^{a}$ | 5.7$^{a}$ | 8.0$^{a}$ | 4.8$^{ab}$ | 2.0$^{bc}$ | 1.4$^{c}$ | 0.8 | -3.7±0.6* |

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514 [1] Plasma membrane integrity was defined as either alive or dead while acrosome integrity
515 as either intact or reacted.
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517 [a,b,c.] Superscripts with different letters in a row represent statistical differences ($P<0.05$).
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519 * $P<0.05$
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**Fig.1. Plasma membrane (A) and acrosome membrane integrity (B) in sperm of Colombian Creole and international pig breeds**. The Creole breeds (ZU: Zungo; CM: Casco de Mula; and SP: San Pedreño) showed less viable (-9.4 ± 1.3%, $P<0.05$) and intact (-5.0 ± 0.9%, $P<0.05$) sperm than international breeds (LB: Belgian Landrace; DU: Duroc; and PI: Pietrain). $^{a,b,c,d}$. Within trait, superscripts with different letters represent statistical differences ($P<0.05$).

**Fig.2. Mitochondrial membrane potential in sperm of Colombian Creole (A-C) and international (D-E) pig breeds.** Membrane potential is represented on a logarithmic scale according to the fluorescence emission colour shift from red ($\Delta\Psi m^{High}$: High potential) to green ($\Delta\Psi m^{Low}$: Low potential). Creole breeds showed the extreme values for $\Delta\Psi m^{High}$, with Zungo (**A**) showing the highest (91.8%) and Casco de Mula (**C**) the lowest (81.4%) value. $^{a,b,c.}$ Different letters represent statistical difference for $\Delta\Psi m^{High}$ ($P<0.05$).