

Semen quality of Colombian Creole as compared to commercial pig breeds

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Research

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Abstract

Background

Colombian Creole pigs are a valuable resource for rural livelihood and research. There are three officially recognized Creole breeds in Colombia (Zungo, ZU; Casco de Mula, CM; and San Pedreño, SP). The phenotypic characterization of these breeds is still very limited, including the reproductive performance of their boars, which is a key issue for developing conservation and dissemination strategies. The aim of this research was to assess the semen quality of Colombian Creole breeds as compared to commonly used international breeds.

Results

Seven boars for each Colombian Creole (ZU, CM, and SP) and international (Duroc, Belgian Landrace, and Pietrain) breeds were used in the experiment. Two doses of semen per boar were assessed in duplicate for sperm kinetics and membrane and acrosome integrity using computer-assisted sperm analysis and flow cytometry, respectively. On average, the Creole pigs, as compared to international breeds, showed lower ($P < 0.05$) volume of fluid ejaculated (185.5 mL vs 239.9 mL) as well as sperm concentration (340.5 vs 395.4, in million sperm/mL), motility (90.9% vs 95.3%) and progressive motility (63.1% vs 67.2%). No relevant differences between breeds for sperm velocity traits were observed, but Creole pigs had lower ($P < 0.05$) proportion of morphologic normal sperm (86.1% vs 90.6%) and of sperm with intact mitochondria plasma membrane and acrosome (76.8% vs 87.5%). Mitochondrial membrane potential did not differ between Creole and international breeds. These results mean that Creole breeds had 60.5% less normal and motile sperm per ejaculate than international breeds. Amongst Creole breeds, SP had larger ejaculates and ZU showed greater proportion of normal and motile sperm, but they did not differ for the amount of normal and motile sperm per ejaculate.

Conclusion

The semen of Colombian Creole pigs is acceptable but less abundant and rich in normal and motile spermatozoa than that collected from commercial breeds. This fact should be considered in developing recommendations for semen processing in Creole pigs. Findings provided here can give new impetus to the conservation and insemination of Creole pigs.

Introduction

Colombian Creole pigs are descendants of the Iberian pigs brought by early Spanish settlers at the Caribbean coast of Colombia, in the current Department of Córdoba. They were originally referred as *Lampíños*, a Spanish word for hairless, since this was one of their more notorious features (1). Over the years, they expanded throughout the country under a wide range of environments and likely receiving simultaneously the influence of other imported breeds. The resulting populations are currently known as Creole (Criollo, in Spanish) pigs. Besides of their historical and social importance, the Creole pigs are considered a valuable genetic resource for supporting economy in rural areas and in the study of adaptation processes to extreme environments (2). 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 *Lampíño* pigs; *Casco de Mula* (CM), mostly found in the hot and humid Eastern plains and thus called because of its solid-hoofed syndactyly; and *San Pedreño* (SP), which can be seen around the central mountain ranges of Antioquia and Viejo Caldas regions featuring black skin and hair (3). Each

breed has developed their own adaptation mechanisms to local ecosystems, all characterized however 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 (4). 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 (5), 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 (6).

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 the techniques that have been proved to be more useful to objectively assess boar semen quality (7). They provide numeric data on sperm kinetics, cytoplasmic and acrosome membrane integrity and mitochondrial membrane potential ($\Delta\Psi_m$), whereby sperm motility and fertilizing capacity can be estimated (8). These parameters are essential for an efficient use of boar ejaculates and to implement optimal conservation and production strategies (9). 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. To put the results into context, semen quality traits were compared with values obtained in three improved 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) while I boars were randomly sampled 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) (10). All of them 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 spermatozoa-rich fraction was retained in a sterile thermal bottle at 37 °C (11). In each ejaculate, the volume of fluid ejaculated (VOL) and the sperm concentration (CO) were measured. Then, from one of the ejaculates, two doses of semen (100 mL) containing 3000×10^6 spermatozoa were obtained by dilution with solution boar semen extender (MR-A®, kubus, Spain). Each dose was assessed by 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.

Morphology, sperm kinetics and motility

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 °C for 3 min prior to be transferred (3 µL) to a counting chamber (Leja®, Luzernestrat, Netherlands) for morphology and kinetics evaluation of 1,000 spermatozoa. Regarding morphology, the percentages of spermatozoa 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. Spermatozoa with an average path velocity (VAP) lower than 10 µm/s were considered immotile while those with a lineal velocity (VSL) lower than 10 µm/s as progressive motile. Total motility (MOT) accounted for the percentage of mobile spermatozoa over total and progressive motility (PMOT) for the percentage of progressive mobile spermatozoa over total.

Plasma membrane and acrosome integrity

The integrity of the sperm plasmatic membrane (namely, viability) was assessed in samples diluted to 30×10^6 spermatozoa/mL stained with propidium iodide (IP) at 12 µM. Acrosome integrity was assessed in the same samples using the double stain PNA-FITC (peanut agglutinin-fluorescein isothiocyanate) and IP (12). Briefly, 100 µL of each sample was mixed with 10 µL of a solution of PNA (1 µg/mL of distilled water) and IP at 12 µM. Samples were incubated in complete darkness at 37 °C for 10 min prior to flow cytometry analysis. Flow cytometry was performed with a high-speed fixed-alignment flow cytometer (BD FACSAria™ 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 spermatozoa 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 PNA-FITC and JC-1 green fluorescent) and FL3 (670nm, for the IP red fluorescent) bandpass filters (13). Data were processed with the FlowJo™ software (Ashland, OR, USA). Sperm were scored as either viable (if negative for IP) or dead (if positive for IP) and also as either intact (if negative for PNA-FITC) or reacted (if positive PNA-FITC). 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 (13).

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 (14). Sperm concentration was re-diluted with Dulbecco's Phosphate Buffer Saline (PBS; Sigma-Aldrich, USA) to a concentration of 1×10^6 sperm/mL, from where 288 µL were taken and dispensed in a cytometry tube preheated at 37 °C. 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 0.5×10^6 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 form aggregates that exhibit intense red/orange fluorescence. In contrast, in dysfunctional (possibly apoptotic) sperm, due to alterations in the membrane potential, the dye remains as a monomer and the mitochondria appears fluorescent green. Consequently, the mitochondrial potential was expressed as the percentage of red ($\Delta\Psi_m^{\text{High}}$) or green ($\Delta\Psi_m^{\text{Low}}$) over total(13,15).

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

Motility, 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 vs 395.4, in million 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 ($-20.7 \mu\text{m}$, $P < 0.05$). 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 ($-9.4 \pm 1.3\%$, $P < 0.05$; Fig. 1A) and intact ($-5.0 \pm 0.9\%$, $P < 0.05$, Fig. 1B) spermatozoa than I breeds. Viability did not differ across C breeds, but CM had more intact spermatozoa 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 ($\Delta\Psi^{\text{High}}$) 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 $\Delta\Psi^{\text{High}}$ 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

Animal genetic resources are a valuable input for rural development and research. As a piece of traditional animal agriculture, Creole pigs are an integral part of Colombian heritage, and as such efforts have been taken for their *conservation and dissemination* (16). *Moreover, throughout Latin America, Creole pigs were required to adapt across a range of environments, some of them extreme in terms of temperature and humidity, which, in a context of climate change, makes them a very useful material for the study of the molecular mechanisms underlying stress response* (17). *Colombian Creole pigs, like other local breeds, are facing extinction because of dramatic decreases in their census due to substitution and extensive intercrossing with improved foreign populations* (18). *However, there is still a lack of research concerning their genetic characterization, conservation and use. It is known that Creole breeds show an acceptable reproductive performance but slow growth* (4,19) which partly can be explained because they are usually produced in family or traditional farms, where feeding and management practices are far from optimal. Moreover, owing to their limited herd size, these farms do not often have at their disposal a boar available (2) or adaptable to their resource-driven production systems (20). Therefore, the key role of the AGROSAVIA germplasm network is to contribute to the genetic characterization of C breeds, while ensuring their conservation and promoting their use through artificial insemination. Assessing semen quality is crucially important to run such a programme. This is what we have done for Colombian Creole boars in light of the latest technologies (7) using the semen of worldwide disseminated breeds as control.

The quantity and quality of semen depends on genetic and environmental factors including the breed (21). In our experiment, VOL was within the normal range (22), but CO was lower than in other studies (23). 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 (24) and Iberian (25), which accounted for 22.9% to 83.5% of the sperm production of C boars. Results in Fengjing and Meishan Chinese breeds were much more variable, with values from half below (26) to almost three-fold higher (27) than those observed in our study. This fact stresses the difficulty in making comparisons across breeds 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 allows for further interpretation of semen quality. Although CASA outcome should be interpreted multiparametrically (7), it has been shown that MO is positively related to pregnancy rate and litter size (28,29) and the absence of morphology abnormalities to functionality of the seminiferous epithelium and epididymal maturation (30). Values over 70% for MOT, and 80% for NO, are expected and acceptable in healthy boars (31). 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 reported for MOT (80.4%) and NO (94.5%) in the Mexican Creole *Pelón de Yucatán* breed point towards the same conclusion (24).

Morphological abnormalities as well as velocity and distance traits fall above threshold values (32). Interestingly, CM and SP showed lower values of PMOT than ZU, which also had less abnormal sperm, particularly relative to SP. Mitochondrial membrane potential was on the high side of reported values, which ranged from 66.9% to 93.5% (33,34). The $\Delta\Psi_m^{\text{High}}$ expresses the capacity of the mitochondria to produce the energy needed by the axonemal dynein system to fuel sperm motility (33). The sperm membrane integrity has shown a closer

relationship to litter size than traditionally estimated sperm motility (35). Thus, $\Delta\Psi_m^{\text{High}}$ has been associated to functionally intact mitochondria and ultimately to MOT (36), PMOT (37) and litter size (35). In fact, spermatozoa with $\Delta\Psi_m^{\text{Low}}$ are less able for acrosome reaction and in the ability to fertilize (38). We did not detect a clear distribution pattern of $\Delta\Psi_m^{\text{High}}$ across breeds, although both mitochondrial membrane and acrosome integrity were higher in I breeds. However, the within breed correlation of $\Delta\Psi_m^{\text{High}}$ with membrane mitochondrial and acrosome integrity were positive (ranging from 0.25, for acrosome integrity in SP, to 0.93, for membrane integrity in ZU) as well as with MOT (ranging from 0.14 for CM to 0.88 in SP). The more variable behaviour of these parameters among Creole breeds could be explained by a combination of historical differences, unequal past influences of foreign breeds, nucleus foundational effects and genetic drift associated to limited population size (39). To avoid additional sampling variability, C boars were sampled from all available lineages within each breed.

Boar semen traits are important indicators for predicting boar fertility and hence their importance in 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 dissemination and therefore 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 (40). These two features, although show a lower profile in Colombian Creole breeds than in international breeds, 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 58.2 and 67.5 billion sperm cells, which results in a potential production capacity of 17–18 doses of 3×10^9 normal and motile sperm, around 10-fold 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 by ejaculate. 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.

Conclusions

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 spermatozoa 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.

Declarations

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Ethical approval

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

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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.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Sperm kinetics parameters

Parameter	Unit	Description
Velocity Average Path (VAP)	$\mu\text{m/s}$	Average velocity of the smoothed path of the sperm head
Velocity Straight Line (VSL)	$\mu\text{m/s}$	Average velocity measured in a straight line from the beginning to the end of a track.
Velocity Curvilinear (VCL)	$\mu\text{m/s}$	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 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

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						SEM	Difference C - I
	Creole			International				
	ZU	CM	SP	LB	DU	PI		
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: spermatozoa concentration; MOT: total motility (percentage of mobile spermatozoa); and PMOT: progressive motility (percentage of spermatozoa with at least 80% of linear movement); see Table 1 for other trait abbreviations.

^{a,b,c} Superscripts with different letters in a row represent statistical differences ($P < 0.05$).

* $P < 0.05$

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

Trait ¹ (%)	Breed						SEM	Difference C - I
	Creole			International				
	ZU	CM	SP	LB	DU	PI		
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	3.7 ^{ab}	3.1 ^{ab}	0.5	-0.3±0.4

1. Proportion of sperm with normal appearance (NO) HA: Head abnormality, BT: Bent tail, CT: Coiled tail, MCD: Medial cytoplasm droplets, DCD: Distal cytoplasm droplets, PCD: Proximal cytoplasm droplets or showing head abnormalities (HA), bent tail (BT), coiled tail (CT), medial cytoplasm droplets (MDC), distal cytoplasm droplets (DCD) and proximal cytoplasm droplets (PCD)

^{a,b,c} Superscripts with different letters in a row represent statistical differences ($P<0.05$).

* $P<0.05$

Table 4. Means for sperm plasma membrane and acrosome integrity by breed and difference between Colombian Creole (C) and international (I) breeds.

Trait ¹ (%)	Breed						SEM	Difference C - I
	Creole			International				
	ZU	CM	SP	LB	DU	PI		
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*

¹ Plasma membrane integrity was defined as either alive or dead while acrosome integrity as either intact or reacted.

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

* $P < 0.05$

Figures

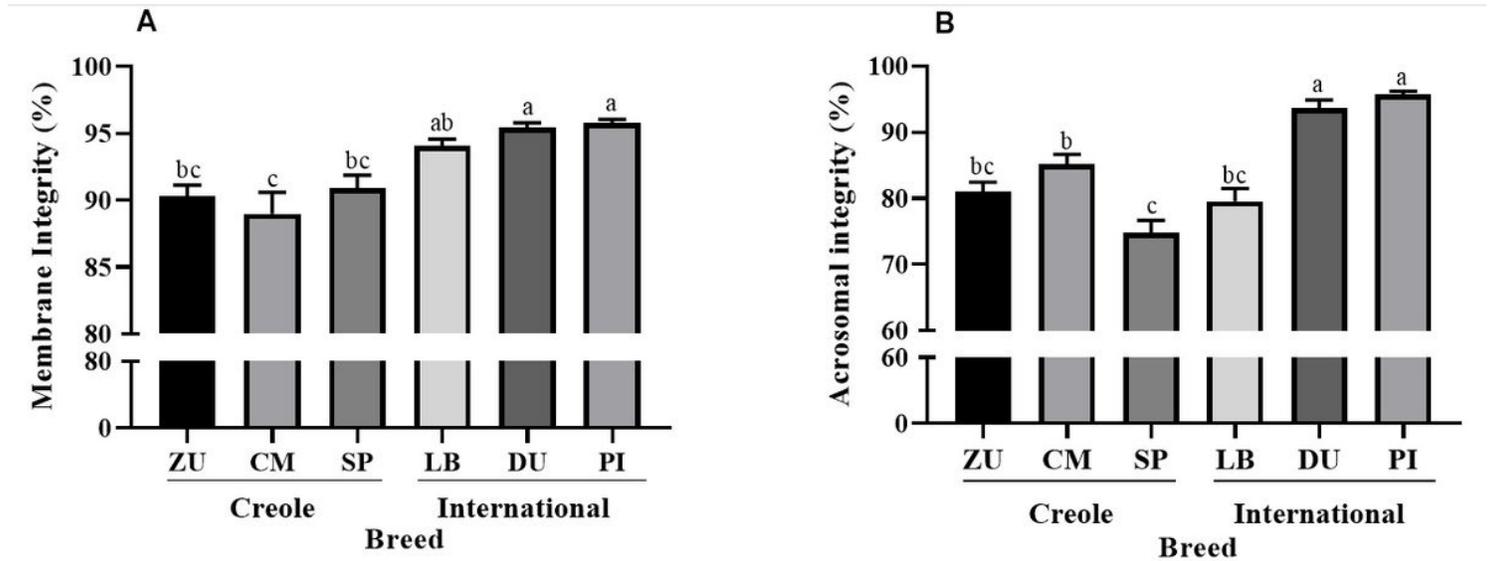


Figure 1

Plasma membrane (A) and acrosome 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 ($-9.4 \pm 1.3\%$, $P < 0.05$) and intact ($-5.0 \pm 0.9\%$, $P < 0.05$) spermatozoa than international breeds (LB: Belgian Landrace; DU: Duroc; and PI: Pietrain). a,b,c, d. Within trait, superscripts with different letters represent statistical differences ($P < 0.05$).

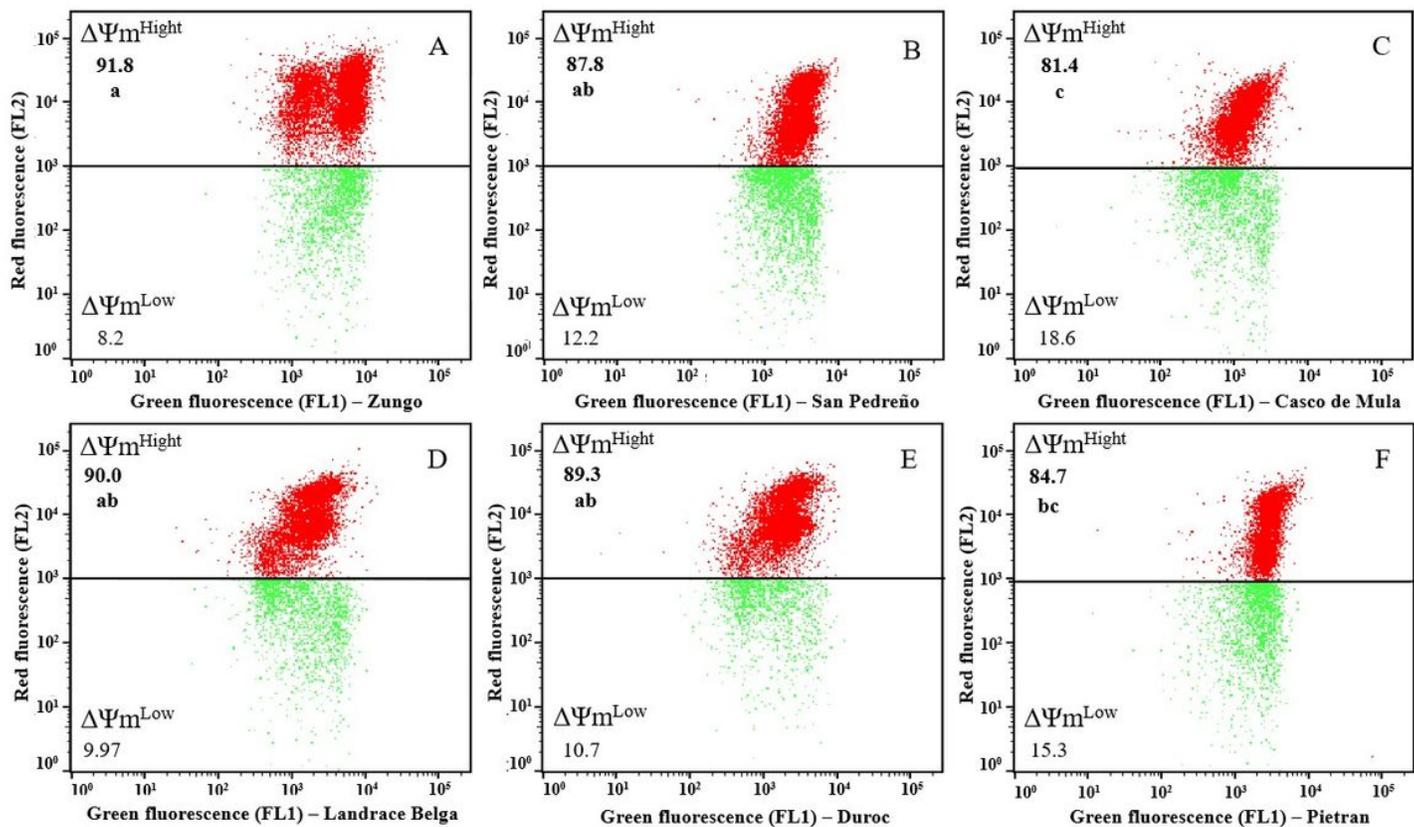


Figure 2

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 ($\Delta\Psi_m^{\text{High}}$: High potential) to green ($\Delta\Psi_m^{\text{Low}}$: Low potential). Creole breeds showed the extreme values for $\Delta\Psi_m^{\text{High}}$, 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 $\Delta\Psi_m^{\text{High}}$ ($P < 0.05$).