

15.6 ± 18.3% (0–74) and 18.8 ± 21.7% (0–92) respectively. Since about 50% of boars showed in some extent clinical and/or spermiogramme deviations compromising their potential breeding/fertility ability, emphasizes the importance of periodical andrological testing in pig farms in order to minimize economic losses caused by unsound sires.

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## AB20

### Testicular dimensions in boars

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Testicular volume is related with semen quality/fertility in most species. Although, selection of boar sires by this parameter is scarce in tropical areas. Furthermore, literature reports of testicular dimensions are rarely found in this specie. This paper aims to reference testicular measurements to support boar selection. Testis length (L) and width (W) were measured (cm) using a pelvimeter in 431 breeding boars (Duroc  $n=146$ ; Dalland  $n=108$ ; Yorkshire  $n=64$ ; Landrace  $n=50$ ; Segher  $n=40$ ; Pietrain  $n=23$ ) aged 24 ± 21 months. Boars were free of any clinical abnormalities in their reproductive organs including testicular asymmetry. The effect of age (categorized as: A=6–11; B=12–17; C=18–23; D=24–29; E=30–35; F=≥36 months), breed and breedage on L and W was assessed using a general linear model. Length/width ratio was 2.01. Breed and age had a significant effect on L and W while breedage had a significant effect on L. Length (LSM ± SEM) was higher in Dalland (13.6 ± 0.2) and Segher (13.2 ± 0.3) compared to Duroc (11.5 ± 0.2), Landrace (11.1 ± 0.3) and Yorkshire (11.7 ± 0.3) ( $P<0.0001$ ), but it was not significantly different in Pietrain (12.3 ± 0.5) compared to Segher, Duroc and Yorkshire. Width was lower ( $P<0.01$ ) in Landrace (5.6 ± 0.2) compared to Dalland (6.4 ± 0.1) and Segher (6.4 ± 0.2), but it was similar ( $P>0.05$ ) compared to Duroc (6.0 ± 0.1), Yorkshire (5.9 ± 0.2) and Pietrain (5.9 ± 0.3). Length was lower in A (11.0 ± 0.3) compared to D (12.5 ± 0.3), E (13.2 ± 0.3) and F (13.0 ± 0.3) ( $P<0.0001$ ) but similar ( $P>0.05$ ) compared to B (11.8 ± 0.3) and C (11.8 ± 0.4). Width showed an increasing pattern according to age and seemed to stabilize around 24 months of age. In A–F, it was (5.3 ± 0.2, 5.7 ± 0.2, 5.8 ± 0.2, 6.2 ± 0.2, 6.6 ± 0.2, 6.6 ± 0.2) respectively. Length and Width disclosed by breed and age (A–F) were: Duroc (10.9 ± 0.5 × 5.6 ± 0.3, 11.6 ± 0.4 × 6.0 ± 0.2, 11.1 ± 0.3 × 5.8 ± 0.2, 11.0 ± 0.5 × 5.7 ± 0.3, 12.6 ± 0.4 × 6.6 ± 0.2, 11.9 ± 0.3 × 6.2 ± 0.2). Dalland (11.9 ± 0.5 × 5.7 ± 0.3, 12.6 ± 0.4 × 5.7 ± 0.2, 13.1 ± 0.5 × 5.8 ± 0.3, 13.9 ± 0.4 × 6.7 ± 0.2, 15.1 ± 0.6 × 7.3 ± 0.4, 15.4 ± 0.3 × 7.0 ± 0.2). Yorkshire (9.6 ± 0.4 × 5.0 ± 0.2, 12.6 ± 0.5 × 6.0 ± 0.3, 12.3 ± 0.6 × 6.4 ± 0.3, 11.5 ± 0.9 × 5.9 ± 0.6, 11.7 ± 1.3 × 6.0 ± 0.8, 12.2 ± 0.6 × 6.2 ± 0.4). Landrace

(10.9 ± 0.8 × 5.2 ± 0.5, 10.8 ± 0.4 × 5.4 ± 0.2, 11.3 ± 0.9 × 5.7 ± 0.6, 11.2 ± 0.9 × 5.7 ± 0.6, 11.1 ± 0.8 × 5.8 ± 0.5, 11.1 ± 0.8 × 5.7 ± 0.5). Segher (11.8 ± 0.8 × 5.3 ± 0.5, 12.9 ± 0.5 × 6.2 ± 0.3, 12.2 ± 0.6 × 5.8 ± 0.4, 14.5 ± 0.8 × 7.2 ± 0.5, 14.2 ± 0.8 × 7.0 ± 0.5, 13.5 ± 1.3 × 7.0 ± 0.8). Pietrain (11.2 ± 1.3 × 5.0 ± 0.8, 10.5 ± 1.3 × 5.0 ± 0.8, 11.0 ± 1.9 × 5.0 ± 1.1, 13.0 ± 0.9 × 6.0 ± 0.6, 14.2 ± 0.9 × 7.1 ± 0.6, 13.9 ± 0.6 × 7.3 ± 0.3). In general, testicular length and width were lower in full-blood breeds (i.e. Landrace, Duroc, Yorkshire) compared to composite breeds such as Dalland and Segher. These findings point out the need of using measurement standards categorized by breed and age when using testicular size as a selection criterion for breeding soundness in this specie.

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## AB21

### Lipid analysis of seminal plasma from Nellore bulls (*Bos taurus indicus*) with high and low resistance to cryopreservation

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The objective of this study was to compare the lipid pattern of seminal plasma of Nellore bulls sensitive and resistant to cryopreservation. Semen samples were collected from 15 animals using electronic tiles. Computerized analysis of sperm kinetics (CASA) and plasma membrane integrity (IPM, epifluorescence microscopy and flow cytometry) in semen fresh and post-thawing were carried out in the samples. The animals were separated into two groups according to their spermatoc qualities: group A, bulls with low resistance to semen cryopreservation ( $n=6$ ), which presented total motility (TM) and IPM <30% after thawing; group B, bulls with high resistance to semen cryopreservation ( $n=9$ ), presenting TM and IPM >50% after thawing. Semen samples were thawed in a 37 °C water bath for 2 min for lipid extraction performance and centrifuged for seminal plasma separation. The lipid pattern of the seminal plasma was performed by mass spectrometry analysis (MALDI-MS) and presented in mass-load ( $m/z$ ). The spermatoc kinetics and IPM data were submitted to statistical analysis by PROC MIXED (SAS INST., INC, Cary, NC), as

averages and compared by the Tukey test, are already available in the MetaboAnalyst Software 2.0. In the MALDI-MS analysis, a difference was observed with no lipid profile of the seminal plasma between the groups, group B presented a higher relative abundance of 34 lipid ions in relation to group A, most lipids with triglycerides ( $m/z$  805.59, 893.74, 793.60, 861.68, 745.57, 947.79, 891.74, 747.59, 795.61) and phosphatidylcholines ( $m/z$  816.60, 804.59, 782.58, 828.54, 746.59, 754.55, 706.55). Therefore, bulls with high sperm resistance to cryopreservation showed greater abundance of specific lipids in the seminal plasma that may be related to the greater resistance of this group to cryopreservation. It was concluded that there was difference in the seminal plasma lipid patterns between bulls in breed Nellore with high and low sperm resistance to cryopreservation.

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## AB22

### Imaging flow cytometry assessment of viability and mitochondrial membrane potential during cryopreservation of alpaca spermatozoa

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Sperm cryopreservation is a technique that allows and facilitates the use of high-value male genetic material. Raw alpaca semen is known to have high viscosity, low motility and moderate morphology anomalies. In other domestic mammals, there is well known how the cryopreservation process affects sperm motility, sperm viability, mitochondrial membrane potential (MMP), acrosome status, sperm capacitation, DNA fragmentation, etc; however, in alpacas there are very few studies that quantify how the cryopreservation process affects these variables. The objective of this study was to quantify by imaging flow cytometry how the cryopreservation process affects sperm viability and MMP, and additionally sperm motility. Thirty alpaca testicles obtained from a local slaughterhouse in Peruvian Highlands were transported overnight to Lima. Spermatozoa were recovered from the tail of the epididymis with 1 mL of an extender based on skim milk, egg yolk, fructose and dimethylacetamide. Only samples with a minimum of 30% motility and  $50 \times 10^6$  sperm/mL concentration were cryopreserved using a controlled-rate freeze machine. Sperm function variables were assessed before and after the cryopreservation process. Viability and MMP were assessed by imaging flow cytometry (FlowSight, Amnis) using SYBR14 (100 nM) with propidium iodide (12  $\mu$ M) and MitoTracker Deep Red FM (100 nM), respectively. Sperm motility was assessed using light microscopy. The effect of cryopreservation on the percentages of viability, MMP and sperm motility were evaluated using paired *t*-tests. Percentages of spermatozoa with viability, MMP

and motility obtained in samples before the cryopreservation ( $49.45 \pm 12.50\%$ ,  $50.95 \pm 12.29\%$ , and  $46.67 \pm 8.64\%$ , respectively) were significantly ( $p < 0.05$ ) higher than in thawed samples ( $33.54 \pm 10.46\%$ ,  $36.11 \pm 10.85\%$ , and  $24.63 \pm 7.12\%$ , respectively). It is concluded that cryopreservation of alpaca spermatozoa affects negatively viability, MMP and sperm motility.

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## AB23

### Identification of six genes related to fertility in alpacas

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Conventional semen analysis is a common used technique to test fertility in alpacas, where it is necessary the use of semen recently collected. Absence of adequate protocols for semen collection and elimination of semen viscosity in alpacas, are important difficulties for massive conventional semen analysis implementation. Even if it was possible, the use of conventional semen analysis does not exactly reveal fertility potential in males. For reproductive selection in male alpacas, it is necessary to find more specific methods for fertility testing, such as semen analysis by flow cytometry and fertility biomarkers. In humans there are approximately 101 genes related to spermatogenesis and fertility, where 38 are homologous in mice. Due to the variation on nucleotide sequences of these genes, that generates different phenotypic expressions, there have been defined biomarkers that express increased or decreased fertility in human and mice. Therefore, the aim of this study was to identify 6 genes with an important role in spermatogenesis and fertility in alpaca. The selected genes were: PMR-1 related to condensation of the sperm nucleus into compact hydrodynamic shape; Acrosin, ADAM2 and Calmegin involved in sperm-egg interaction; AKAP4 required for motility and BRD2 with an important role on spermatogenesis regulation. Nucleotide sequences were searched on open-access National Center for Biotechnology Information (NCBI), to align phylogenetically related species to alpacas. These multiple sequences were aligned *in silico* using CLUSTAL-W program with the purpose of finding the most conserved regions of each gene. Then, 2 pair of primers for each gene were designed using PERL PRIMER software. Melting temperature ( $T_m$ ) was calculated using OligoAnalyzer 3.1 software from Integrated DNA Technologies (IDT). The validation *in silico* of these primers was carried out using PRIMER-BLAST tool (NCBI) against whole genome-shotgun in alpaca. It gave us the efficiency and specificity of the primers. We concluded that