Fine-tuning inbreeding analysis reveals genomic regions associated with the abundance of kinetic subpopulations in fresh and frozen stallion sperm

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Sperm quality is a major contributor to the fertility of livestock animals. This issue is even more important in stallions since this species is well-known for its moderate fertility in comparison with domestic animals. But also, stallions show a lack of sperm freezability in nearly 30% of the horses. Inbreeding depression, triggered by the mating of close relatives, was demonstrated as a cause of a decrease in sperm quality in stallions, but no studies were yet performed assessing its effect on the kinetic subpopulation patterns in stallion sperm. We determined the effect of homozygosity on sperm kinetic subpopulations in fresh and frozen/thawed samples from 18 Pura Raza Española stallions. To this, we first analyzed 148 sperm samples (8 per stallion, 72 fresh, and 72 frozen/thawed) using the motility module in a Sperm Class Analyzer (SCA™, Microptic, Spain). Three velocities (VCL, VSL, and VAP), three composite indexes (STR, LIN, and WOB), and two traits associated with sperm head movement (ALH and BCF). Thereafter, we performed a two-step clustering discriminant analysis to determine the existence of sperm kinetic subpopulations, after which each spz was allocated to one of these. All the individuals were also genotyped using an HD-SNP array (Axiom670K, Thermofisher™) to determine a runs-of-homozygosity-based inbreeding value (F_{ROH}) at the genomic and chromosomal level. Finally, we correlated the inbreeding values with the abundance of each sperm subpopulation in the fresh and frozen/thawed samples separately. The phenotypic analyses revealed the existence of three different sperm subpopulations: Sp1, including slow but progressive spz; Sp2, including fast and progressive spz and Sp3, including medium-fast but not progressive spz. Genomic analysis showed a large variability in terms of homozygosity, ranging from 1.65 to 22.21% at the genome level, but also at the chromosome level. For instance, F_{ROH} ranged between 0 and ~24% in ECA11 and between 0 and ~60% in ECA18 and ECA23. In fresh samples, most of the correlations between F_{ROH} and SP abundance were positive in Sp1 (commonly associated with moderate fertility) and negative in Sp2 (which is the sperm motility pattern most associated with fertilization success). But also, the magnitude of the association varied greatly among chromosomes, being the increase of F_{ROH} in ECA4 and ECA15 being the most associated with a decrease in Sp2. In frozen samples, FROH increase was mostly associated with an increase in SP1, but results in SP2 were much more variable and with a lesser magnitude. On the contrary, the increase in F_{ROH} in ECA14 and ECA23 was associated with a decrease in the abundance of Sp3 (associated with an increase in fertilization failure due to a lack of progress and premature capacitation triggered by the freezing process). Overall, this preliminary study demonstrated that the increase of inbreeding in certain chromosomes is associated with changes in the sperm kinetic subpopulation patterns in stallion sperm, in particular in fresh samples, where the abundance of sperm subpopulations associated with increased fertility declined with the increase of genomic inbreeding. In addition, our results suggest that inbreeding can affect differentially the

freezability of stallion sperm. Further studies on a large stallion dataset are being developed to validate these results.