different horse populations and to identify putative deleterious mutations. Publicly available whole-genome sequence data from 317 horses of 38 breeds was mapped to the horse reference genome EquCab3.0. A total of 24,540,424 single nucleotide polymorphisms (SNPs), 2,248,828 insertions and 2,776,484 deletions could be identified. On average, one variant was called every 81 bases and 17,323,823 (58.6%) of the variants were known. The transition-to-transversion ratio for SNPs was 1.98. To detect putative lethal mutations, variant effect prediction was performed for the identified variants using SnpEff. Thereby, 31,326 variants were predicted to have a high impact on protein-coding sequences, 8,691 of which were present in the heterozygous state in at least 2 horses but were not found in the homozygous state. Those variants were annotated with 16,820 effects having a high impact on a total of 3,701 genes. The comparison of those affected genes with several genes previously reported to be essential in various cell lines showed an overlap of 305 genes, which were further classified based on KEGG pathways. For example, high impact variants in 6 genes (*BARD1*, *BRCA1*, *PALB2*, *POLD1*, *RBBP8*, *XRCC3*), which are involved in homologous recombination were found. The detection of lethal variants in genes affecting embryonic development could allow for the development of genetic tests for those mutations and thereby help to improve fertility rates in domestic horse populations.

Key Words: horses and related species, bioinformatics, genetic disorder

P268 Genomic data reveals a serious underestimation of pedigree inbreeding levels in Polo Argentino horses. F. Azcona*[1], A. Molina[3], P. Peral-García[1], and S. Demyda-Peyrás[2,4], [1]*IGEVET-CONICET-UNLP, La Plata, La Plata, Buenos Aires, Argentina,* [2]*Departamento de Producción Animal, Facultad de Veterinaria, Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina,* [3]*Departamento de Genética, Universidad de Córdoba, Córdoba, España,* [4]*CONICET-CCT La Plata, La Plata, Buenos Aires, Argentina.*

The Argentine Polo Horse (APH) is a novel breed created 40 years ago in Argentina by breeding selected native horses based on sports performance with Thoroughbreds to increase speed and reactiveness. Despite closing mating, our previous reports, based on pedigree data, do not show significant increases in inbreeding. Hereby, we aim to validate these findings using genomic data. To this end, we genotyped 49 APH using the Illumina Equine GGP array (71,805 SNPs) and determined the molecular inbreeding values (F_{ROH}) using ROH. The analysis included total F_{ROH} (using all the information available) and F_{ROH} theoretically produced during the last 3, 6, and 9 generations (minimum ROH length of 16.67Mb, 8.33Mb, and 5.55Mb respectively); and the ancient inbreeding (originated before the last 9 generations). Finally, results were compared with pedigree-based inbreeding values obtained using 112,000 birth records (F_{PED}). Average pedigree inbreeding was low (F_{PED} = 0.1%, with 23 of 49 individuals showing a F_{PED} = 0), even the acceptable pedigree completeness observed (4.4 EGC; 11.1 G_{MAX}). On the contrary, F_{ROH} values were notably higher, averaging 14.71%. Correlations between PED and SNP inbreeding values were moderate, but the analysis per generation showed that only 26.68% was explained by mating produced during the last 9 generations, and only 5.55% during the last 3 (Table 1). On the contrary, 63% of inbreeding detected is explained by ancient inbreeding ($F_{ROHANC)}$, which was not correlated with F_{PED}. Although the analysis of the robust APHpedigree revealed low inbreeding values, genomic data indicate a completely different situation. All the animals demonstrated increased levels of inbreeding (F_{ROH} ranged from 11.43% to 19.67%) mostly due to an ancient basis. These results can be explained by the strong influence of Thoroughbred, which is a very old and selected breed with a high inbreeding load, was probably inherited by the APC, without being noticed. Overall, we demonstrated that that genetic characterization using pedigree records is a valuable resource to guide breeding decisions, but it can underestimate inbreeding values. Therefore, we believe that genomic analysis are necessary to obtain a more realistic idea of the genetic variability in breeds with relatively new studbooks, but even more if they were influenced by inbred breeds such as TB

Key Words: horses and related species, breed/population identification, inbreeding, single nucleotide polymorphism (SNP), homozygosity

P269 Genomic improvement of the horse X chromosome and characterization of the pseudoautosomal boundary. M. Jevit*[1], B. Davis[1], C. Casanteda[1], D. Miller[2], and T. Raudsepp[1], [1]*Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX, USA,* [2]*Cornell University, Ithaca, NY, USA.*

The horse reference genome was improved with the release of *EquCab3* and the first Y chromosome reference. The most complex sequences, such as those in the sex chromosomes, are still unresolved. These include large amplicons, repeats, and the pseudoautosomal boundary (PAB). We initiated a comprehensive study specifically to improve the assembly of the horse sex chromosomes. To refine the assembly of complex regions, we are utilizing 3 new technologies: trio-binning, Hi-C and Bionano optical mapping. Trio-binning uses long-read sequences from F1 interspecific hybrids and short reads from parent species. High molecular weight blood DNA was extracted from a female hinny and sequenced on 2 PacBio Sequel cells. Paired-end short reads (150bp) for horse (*Twilight*) and donkey (*Willy*) were obtained from SRA. These sequences as well as the hinny long reads were assembled with trio-binning function of the Canu assembler program. The initial assembly was scaffolded with Hi-C data as well as a Bionano optical maps one of a Thoroughbred stallion (*Bravo*). The resulting assembly is 2.4 Gb in total separated into 162 scaffolds. Our initial goal was to use these assembly to better define the PAB - the end of the pseudoautosomal region (PAR) where X-Y recombination stops. Despite the evolutionary and biological importance of the PAB, the region has been characterized at molecular level in only a few species, and is not well-defined in horse. Previously, we identified and Sanger sequenced 4 BAC clones - 2 spanning PAB-X and PAB-Y. To identify the PAB of the horse, BAC sequences were aligned to the Y assembly, EquCab3 and a 42 Mb-size contig from trio-binning assembly which corresponds to the short arm of the X. We identified a region on both X and Y where X-Y homology drops from over 97% (PAR) to almost zero, indicative of the PAB. This region corresponds to the location of the *XKR3Y* gene in the Y but is not well-annotated in the X. We identified a duplication and an inversion in EquCab3 which was not consistent with the corresponding region in the X-BACs, or the new 42 Mb Xp contig, suggesting a mis-assembly in EquCab3. We believe that these approaches combined will also resolve other complex portions in the horse sex chromosomes.

Key Words: trio-binning, HiC, sex chromosomes, Bionano, pseudoautosomal boundary

P270 Gene expression of chondrogenic markers to assess the differentiation of equine mesenchymal stem cells in different 3D systems. A. Cequier[1], A. Romero[1,2], A. Vitoria[1,2], F. Vázquez[1,2], P. Zaragoza[1], C. Rodellar[1], and L. Barrachina*[1,2], [1]*Laboratorio de Genética Bioquímica LAGENBIO (Universidad de Zaragoza), Instituto Agroalimentario de Aragón– IA2, Universidad de Zaragoza-CITA; Instituto de Investigación Sanitaria de Aragón (IIS), Zaragoza, Spain,* [2]*Servicio de Cirugía y Medicina Equina, Hospital Veterinario, Universidad de Zaragoza, Zaragoza, Spain.*

Mesenchymal stem cells (MSCs) can be used to treat several diseases in animals and people. The horse is particularly valuable as translational model for cartilage repair. The ability of MSCs to differentiate into chondrocytes has granted them an increasing interest for this purpose. However, obtaining quality cartilage in vitro remains challenging because MSC differentiation requires specific environmental signals. A 3D configuration is usually provided by pelleting cells, but nutrients may not reach the center of the pellet and necrosis areas tend to form. The use of hydrogels to provide 3D configuration closer to that in the cartilage