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Abstract

The Argentine Polo Horse (AP), an autochthonous breed officially created in Argentina in the early 1980s, is globally recognized as the best equid for playing polo. Their breeding is characterized by the use of cutting-edge assisted reproductive techniques such as large-scale embryo transfer (ET) programs and cloning. The aim of this study was to determine the impact of the use of reproductive biotechnologies on the genetic structure, variability and reproductive parameters of the AP breed using genealogical data (81,633 pedigree records). In total, 18,077 animals were drawn from the last generation (2006-2015) to be employed as the reference population (WP), which was further divided into two subsets: animals produced (ET; n=13,478) and not produced by ET (NOT-ET; n=4,599). Horses produced by ET showed a significant decrease in generation interval compared with NOT-ET. Similarly, the number of stallions and broodmares, the number of foals per stallion and broodmare as well as inbreeding (F=0.89%) and average relatedness (AR=1%) were higher in ET compared with NOT-ET (F=0.6%; AR=0.54%). Our analysis also revealed that the effective number of founders and ancestors in ET showed a disproportionate gene contribution and a strong genetic bottleneck. Furthermore, the inter-herd fixation index (FST) revealed an increased genetic flow between herds and higher internal relatedness values within ET horses. In conclusion, the use of large-scale ET programs decreased genetic variability (lower effective population size and number of founders and ancestors and higher F, AR and coancestry), increased the genetic flow among herds and decreased the generation interval, thereby contributing to the higher rates of genetic progress in the AP.

Keywords	Embryo transfer; Reproductive biotechnology; Inbreeding; Pedigree information
Corresponding Author	Florencia Azcona
Corresponding Author's Institution	Instituto de Genética Veterinaria "Ing. Fernando Noel Dulout"
Order of Authors	Florencia Azcona, Mercedes Valera, Antonio Molina, Pablo Trigo, Pilar Peral García, Marina Solé, Sebastián Demyda Peyrás
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1 Highlights

- 2 ▪ More than 60% of AP were produced by embryo transfer (ET).
- 3 ▪ F , AR , and coancestry were higher in horses born by ET, while F_e and F_a were
- 4 lower.
- 5 ▪ ET also produced a greater reduction in the genetic variability of the individuals.
- 6 ▪ ET rose genetic flow and reduced the generation interval, increasing the genetic
- 7 progress.

1 **Impact of reproductive biotechnologies on genetic variability of Argentine Polo horses**

2

3 Florencia Azcona^{a*}; Mercedes Valera^b; Antonio Molina^c; Pablo Trigo^a; Pilar Peral García^a;

4 Marina Solé^d and Sebastián Demyda-Peyrás^{a*}

5

6 ^a*IGEVET – Instituto de Genética Veterinaria "Ing. Fernando N. Dulout" (UNLP - CONICET*
7 *LA PLATA). Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. Calle 60 y*
8 *118 s/n, 1900-La Plata, Argentina.*

9 ^b*Departamento de Ciencias Agroforestales. Escuela Técnica Superior de Ingeniería*
10 *Agronómica. Universidad de Sevilla. Ctra. de Utrera km 1. 41013-Sevilla, España.*

11 ^c*Departamento de Genética; Universidad de Córdoba, CN IV KM 396 Edificio Gregor Mendel,*
12 *14007; Córdoba, España*

13 ^d*Unit Animal Genomics, GIGA-R & Faculty of Veterinary Medicine, University of Liège – B34*
14 *(+1), Avenue de l'Hôpital 1, 4000-Liège, Belgium.*

15

16 *Co-corresponding authors

17 Phone: +54 221 4211799

18 Florencia Azcona: fazcona@igevet.gob.ar

19 Sebastián Demyda Peyrás: sdemyda@igevet.gob.ar

20

21 **Short title:** Genetic variability of Argentine Polo horses

22 **Abstract**

23 The Argentine Polo Horse (AP), an autochthonous breed officially created in Argentina in the
24 early 1980s, is globally recognized as the best equid for playing polo. Their breeding is
25 characterized by the use of cutting-edge assisted reproductive techniques such as large-scale
26 embryo transfer (ET) programs and cloning. The aim of this study was to determine the impact
27 of the use of reproductive biotechnologies on the genetic structure, variability and reproductive
28 parameters of the AP breed using genealogical data (81,633 pedigree records). In total, 18,077
29 animals were drawn from the last generation (2006-2015) to be employed as the reference
30 population (WP), which was further divided into two subsets: animals produced (ET; $n=13,478$)
31 and not produced by ET (NOT-ET; $n=4,599$). Horses produced by ET showed a significant
32 decrease in generation interval compared with NOT-ET. Similarly, the number of stallions and
33 broodmares, the number of foals per stallion and broodmare as well as inbreeding ($F=0.89\%$)
34 and average relatedness ($AR=1\%$) were higher in ET compared with NOT-ET ($F=0.6\%$;
35 $AR=0.54\%$). Our analysis also revealed that the effective number of founders and ancestors in
36 ET showed a disproportionate gene contribution and a strong genetic bottleneck. Furthermore,
37 the inter-herd fixation index (F_{ST}) revealed an increased genetic flow between herds and higher
38 internal relatedness values within ET horses. In conclusion, the use of large-scale ET programs
39 decreased genetic variability (lower effective population size and number of founders and
40 ancestors and higher F , AR and coancestry), increased the genetic flow among herds and
41 decreased the generation interval, thereby contributing to the higher rates of genetic progress in
42 the AP.

43

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45 **1. Introduction**

46 The analysis of the genetic and demographic structure of animal populations is a valuable
47 tool to highlight important circumstances affecting their history (Valera et al., 2005). This
48 information has also been useful to analyze the dynamic changes in gene pools during a certain
49 period. As example, these methodologies have been used to describe the genetic structure of
50 horse breeds and populations worldwide (Maccluer et al., 1983; Poncet et al., 2006; Bartolomé
51 et al., 2011; Pjontek et al., 2012; Vicente et al., 2012; Borowska and Szwaczkowski, 2014;
52 Petersen et al., 2014). Furthermore, these results are particularly interesting as an auxiliary tool
53 in breeding decisions since in this species, management and mating policies are normally
54 decided by breeders in an artisanal way (Medeiros et al., 2014; Giontella et al., 2018). However,
55 the existence of detailed and reliable pedigree information is the first and necessary step to
56 obtain reliable results as well as for the implementation of breeding programs based on more
57 scientific methodologies (Cervantes et al., 2008).

58 The Argentine Polo horse (AP) is a popular horse breed among elite polo players (Maserati
59 and Mutto, 2016). The breed was officially created in the early 1980s and is managed by the
60 Asociación Argentina de Criadores de Caballos de Polo (AACCP), which is responsible for
61 determining the policies and rules for breeding practices. Its origin was associated mainly to
62 the crossing of elite native crossbred with Thoroughbred horses; however, the incorporation of
63 individuals of any breed that met certain criteria of aptitude and type was also allowed, a policy
64 that remains until the present time. Interestingly, since its creation in 1984, the AACCP
65 permitted, and even encouraged, the use of any assisted reproductive technique available for
66 the species. Thus, AP breeding is characterized by the existence of massive embryo transfer
67 (ET) programs and the use of state-of-the-art reproductive techniques such as the production of
68 sex-selected embryos (Herrera et al., 2014), oocyte transfer (Riera et al., 2016) and cloned
69 animals (Maserati and Mutto, 2016; Gambini and Maserati, 2018). Consequently, the
70 percentage of ET-produced individuals steadily increased during the last decades, currently

71 accounting for more than half of the foals produced, and positioning the AP as a leading breed
72 in terms of reproductive methods and becoming the most exported and worldwide distributed
73 Argentinean horse, with near 1500 individuals sold abroad every year (MinAgri, 2015). This
74 fact also made AP an interesting model to analyze the effect of ET in a commercial horse
75 population. In this sense, it was demonstrated that ET produce an increase in the genetic gain
76 and selection pressure in domestic animals (Mota et al., 2013), but it was also associated to
77 decreases in terms of genetic variability among and within herds. However, and despite the
78 cultural and economic importance of ET in the horse breeding, the association between the use
79 of this assisted reproductive methodology and genetic structure and variations has not yet been
80 assessed. Therefore, the aim of this study was to evaluate the effect of the use of ET programs
81 on reproductive parameters and genetic variability in a commercial horse breed characterized
82 by the use of massive ET programs.

83

84 **2. Materials and methods**

85 *2.1. Genealogical information*

86 Pedigree information of the AP was obtained from the AACCP and the Sociedad Rural
87 Argentina public databases. Additional pedigree records were obtained from the Argentine
88 Thoroughbred Studbook in order to reconstruct the registry of founder individuals. The final
89 database included 81,633 horses registered since the AACCP foundation in 1984 until 2015.
90 Only individuals with at least three equivalent complete generations (ECG; according to
91 Maignel et al. (1996)) and belonging to the last generation (born between 2006 and 2015), were
92 used as reference population (WP; $n=18,077$). This group was further divided into two main
93 subgroups: horses born (ET; $n=13,478$) and not born (NOT-ET; $n=4,599$) by embryo transfer.

94 *2.2. Demographic analysis*

95 The initial analysis included the number of animals registered per year, the number of
96 individuals used as stallions and broodmares, and the female/male rate of births. Generation

97 interval (GI), defined as the mean age of the parents at the birth of the offspring which are
 98 destined for reproduction (James, 1977), was also determined in the four possible pathways
 99 (father-son, father-daughter, mother-son, and mother-daughter). Differences in sex ratio,
 100 number of offspring and GI between groups were analyzed using Z- and T-test.

101 2.3. Genetic variability parameters

102 Genetic variability of WP was evaluated with the following parameters: 1) Inbreeding
 103 coefficient (F), defined as the probability that an individual has two identical alleles by descent
 104 (Wright, 1931); 2) Rate of inbreeding between groups (F_R), which quantifies the proportion of
 105 F of the ET group compared with NOT-ET. In this study, this parameter was computed as:

$$106 F_R = \frac{F_{ET} - F_{NOT-ET}}{F_{NOT-ET}}$$

107 Where F_{ET} and F_{NOT-ET} were the inbreeding coefficients of ET and NOT-ET, respectively,
 108 in order to determine the dynamics of the inbreeding variation between groups. 3) Average
 109 relatedness (AR), defined as the probability that an allele randomly chosen from the whole
 110 population belongs to a given animal (Gutierrez and Goyache, 2005), to determine the
 111 representation of a given individual in the total population or the percentage in which a founder
 112 was involved in the origin of the breed; 4) Coancestry coefficient for each pair of individuals,
 113 estimated as the probability that two individuals share allele identity by descent (Malécot,
 114 1948), to determine the average relatedness between animals; and 5) Effective population size,
 115 defined as the number of animals that would maintain the current increase in inbreeding if they
 116 contributed equitably to the next generation, estimated via individual increase in both
 117 inbreeding (N_e) (Gutierrez et al., 2009) and pairwise coancestry (N_{eC}) (Cervantes et al., 2011).
 118 Differences between groups were determined using the Mann-Whitney U test since sample data
 119 were not normally distributed (Kolmogorov Smirnov test, $P < 0.01$).

120 2.4. Founder and individual gene origin

121 The probability of gene origin was determined through the effective number of founders
122 (F_e), defined as the number of founders that could produce the same genetic diversity observed
123 in the population, and the effective number of ancestors (F_a), defined as the minimum number
124 of individuals (not necessarily founders) that explains the genetic variability of the breed. This
125 dual approach was employed to determine the existence of a genetic bottleneck in the
126 population, as was proposed by Boichard et al. (1997), by calculating the F_e/F_a ratio, which is
127 higher than 1 when the contribution of ancestors is unequal. Additionally, we determined the
128 number of founder genome equivalents (F_g), defined as the number of founders that would be
129 expected to produce the same genetic diversity as in the population under study if the founders
130 were equally represented and no loss of alleles occurred (Lacy, 1989). This parameter
131 specifically accounts for the random loss of alleles during genetic bottlenecks produced by the
132 unbalanced contributions of founders. Finally, the breed composition of the ten individuals
133 which contributed the most to the genetic variability of the breed was assessed to determine the
134 influence of foreign breeds in the AP composition.

135 *2.5. Population structure*

136 The possible structuring in partially isolated subpopulations among breeders was determined
137 with the fixation index (F_{ST}), following the methodology described by Caballero and Toro
138 (2000). This parameter was calculated in herds with at least 40 registered individuals belonging
139 to both ET and NOT-ET groups (n=83 herds). Thereafter, F_{ST} was estimated among herds which
140 had individuals in both groups in order to obtain the F_{ST} Spearman's rank correlation coefficient
141 between groups (ET and NOT-ET). This parameter allowed to determine if the increase of
142 genetic similarity among herds was affected by the breeding system. To identify less evident
143 subpopulation divisions derived from partial structuring, an additional comparison was
144 performed according to the methodology described by Cervantes et al. (2011) which is based
145 on the comparison between effective population sizes calculated from individual increases in
146 inbreeding and in pairwise coancestry (N_e and N_{eC}).

147 All parameter estimations were performed using ENDOG v.4.8 software (Gutierrez and
148 Goyache, 2005). The statistical analysis was carried out with STATISTICA package v.12.
149 (StatSoft., 2012).

150 **3. Results**

151 *3.1. Demographic analysis*

152 The number of individuals born and registered annually since the AACCP creation in 1984
153 increased considerably every year until 2009 (Figure 1), with approximately 40% of the total
154 population registered during the last generation (2006-2015). In those years, the female/male
155 ratio was relatively steady (near 1.5:1), but it began to increase by 2010, reaching rates close to
156 4:1 in 2014 (Figure 1). Pedigree completeness was moderate, showing the average maximum
157 and equivalent complete generations traced equal to 11.3 and 4.9, respectively, which are robust
158 values in comparison with similar studies (Bartolomé et al., 2011; Medeiros et al., 2014).
159 Similarly, equivalent complete generation in ET and NOT-ET groups was 5.1 and 4.2,
160 respectively.

161 The number of individuals produced by ET from 1990 to 2015 showed a steady increase
162 since 2004, reaching percentages higher than 60% of the total number of horses enrolled in the
163 AACCP during the last four years studied (Figure 2). The increased female/male ratio was also
164 higher in ET as compared with WP ($P<0.01$), depicting a clear preference of breeders for
165 producing mares.

166 At the time of this study, only 10% of males and 27% of females from the whole population
167 became stallions and broodmares, producing an average of 22.7 and 3.4 foals per individual,
168 respectively. However, there was a clear imbalance in individual contribution, since only 14
169 stallions (0.6% of the total) registered more than 300 foals each, accounting for 12.5% of the
170 foal crop. Furthermore, a higher percentage of horses was used as stallions in ET (11.7% vs
171 9.84% in NOT-ET; $P<0.01$), also producing five more foals per individual (26 in ET vs 21 in
172 NOT-ET; $P<0.05$). On the contrary, the percentage of females used as breeding mares was

173 significantly lower in ET (16.4%) compared with NOT-ET (31.6%, $P<0.01$), as well as the
174 number of foals born by ET per mare per year (1.9). Average GI was 8.2 years in WP (Table
175 1), resulting longer in the maternal as compared with the parental pathway. However, the four
176 pathways were considerably shorter in ET compared with NOT-ET horses (Table 1), with a
177 difference of almost 2 years in average (7.7 vs 9.6 years, respectively; $P<0.01$).

178 3.2. Genetic variability parameters

179 In general, inbreeding values were low ($F=0.82\%$ in WP), but they showed an upward trend
180 during the last six years, with higher percentages in ET compared with NOT-ET (0.89% vs
181 0.6%, respectively; $P<0.001$; Figure 3). Although F_R showed a variable trend, it was
182 predominantly positive (Figure 3), thus demonstrating a clear effect of ET on the increase of
183 inbreeding. This was also supported by the proportion of inbred animals ($F>0$), which also
184 increased from (26% to 60%) during the last generation, with a clear spike in the last five years,
185 more specifically during the last season analyzed (66% in ET vs 43% in NOT-ET). Likewise,
186 the proportion of animals with high inbreeding in WP ($F>6.25\%$; equivalent to a first-cousin
187 mating) increased during the last generation from 5% to 8%, also showing significantly higher
188 values in ET (8%) than in NOT-ET (6%) during the last year ($P<0.01$). This influence was also
189 evidenced by the strong correlation observed between F and the percentage of animals born by
190 ET per year ($r=0.72$, $P<0.05$).

191 Average relatedness was 0.9% in WP and showed significant differences between groups
192 (ET=1% vs NOT-ET=0.54%; $P<0.001$). Even though AR was low, it also tended to increase
193 progressively over time, showing a growth from (0.8 to 1; 20%) during the last generation. A
194 similar trend was also observed in coancestry coefficient between groups, showing ET a highly
195 significant increase in comparison with those of NOT-ET (2.2% vs. 0.8%; $P<0.001$). Despite
196 the lower values observed, these results clearly demonstrate the incidence of ET programs in
197 the increased AR and coancestry among individuals.

198 Finally, the analysis of the effective population size showed high values in WP ($N_{eC}= 158.2$
199 and $N_e=307.1$), which agree with the open origin of the breed, as well in ET ($N_{eC}= 118.8$; $N_e=$
200 293) and NOT-ET ($N_{eC}= 266$; $N_e= 356$).

201 3.3. Founder and individual gene origin

202 As expected, a large number of individuals ($n=3818$) were considered as founders in WP,
203 since there was no pedigree limitation to include an animal within the breeding records of the
204 breed (Table 2). However, F_e was only 326. On the other hand, clear differences were observed
205 between groups, since the F_e and F_a were reduced in ET compared with NOT-ET. Similarly,
206 the F_e/F_a ratio as well as F_g were also higher in the ET group (Table 2).

207 The genetic variability explained by the 10 most important contributing ancestors in WP,
208 ET, and NOT-ET is presented in Table 3, showing that most of them were full or half-breed
209 Thoroughbred horses. Interestingly, similar results were obtained when the analysis was
210 expanded to the 50 most contributing animals (data not shown), revealing a high percentage of
211 Thoroughbred bloodlines involved in the origin of AP. It was noteworthy that the same
212 individuals were the most representative ancestors in WP and ET, even though they showed an
213 increased percentage of marginal contribution in the latter group (Table 3). On the other hand,
214 NOT-ET showed a lower influence of the most representative founders and an ample genetic
215 diversity.

216 3.4. Population structure

217 The average F_{ST} index (among breeders) showed lower values in ET than in NOT-ET (0.019
218 vs. 0.034, respectively; $P<0.001$). The Spearman's rank correlation coefficient between F_{ST}
219 index by subpopulation was very low ($r= 0.09$). These data depict an increased genetic distance
220 between herds in NOT-ET as compared to breeders who employed embryo transfer. Similarly,
221 there were clear differences in effective population size and N_{eC}/N_e ratio, which was higher in
222 NOT-ET than in ET (0.74 vs. 0.4, respectively), also evidencing a slightly larger substructuring
223 of NOT-ET in comparison with ET group.

224 **4. Discussion**

225 Genetic characterization through genealogical data plays a major role in monitoring the
226 possible loss of variability in populations. This analysis is particularly valuable in specialized
227 breeds where mating strategies and selection decisions are usually based on phenotypical traits
228 (Sánchez-Guerrero et al., 2017) but also when the use of large-scale programs of assisted
229 reproductive techniques increase the selection intensity among individuals (Marinho et al.,
230 2015). Both conditions are met in AP horses, which are strongly selected based on sportive
231 performance and produced using a reduced number of breeding animals and massive ET
232 programs (Losinno et al., 2001). Therefore, our study could be an interesting approach to
233 determine the genetic effects of different mating choices and assisted reproductive techniques
234 on a renowned commercial horse population, particularly in a species in which this kind of
235 studies is scarce.

236 In equines, the decision to produce a particular mate is usually made without following any
237 breeding scheme based on the existence of breeding values (EVB), since they are not available
238 in most breeds (Gómez et al., 2010). However, breeders are used to developing their own
239 breeding schemes by selecting maternal or paternal lineages of their interest along with a
240 subjective analysis (not standardized) of the offspring performance. In small breeds, this could
241 lead to a disproportionate use of a reduced number of stallions (and mares), with the consequent
242 appearance of genetic bottlenecks and the loss of valuable unique alleles after a few generations
243 (Janova et al., 2013). According to our results, this has been slowly but steadily occurring in
244 AP during the last decade, during which the number of stallions employed was reduced. But
245 we also observed that this phenomenon was more intense in ET bred individuals. Although AP
246 genetic variability is still high, probably because it is a novel breed created from a
247 heterogeneously-based population which also remains with an open registration policy, our
248 results suggest the existence of a genetic trend towards the loss of diversity and the increase of

249 relatedness among individuals, mostly during the last generation, that should be taken into
250 account in a medium to long-term horizon.

251 Despite its recent creation, the AP breed census is one of the largest among Argentinean
252 horses, with near 10,000 individuals registered during the last five years (Figure 1). The
253 increased number of registrations since 2004 matched with the eruption of large-scale ET
254 programs, which accounted for almost 70% of born products since that date. The use of this
255 technique could produce an increased genetic gain in a given population, as was demonstrated
256 on several species, due to an increased distribution of superior germplasm and a reduction in
257 the *GI*, particularly in the mare lineages (Gengler and Druet, 2001). Despite that genetic
258 progress cannot be determined properly in the AP because of the lack of breeding values and
259 phenotypic characterization, there is a consensus among breeders and polo players regarding
260 the improvement of the sportive performance of the breed which cannot be ignored. On the
261 other hand, such breeding policy is causing a loss of genetic variability, which is clearly
262 depicted by the steady decrease in the percentage of individuals, particularly mares, employed
263 as breeders. This effect was also described by Sitzenstock et al. (2013) in a simulation study in
264 a German horse population, showing that the implementation of large ET programs and the use
265 of few selected mares as breeders would cause a major reduction in genetic variance and
266 effective population size. In this sense, our study is the first one to validate this situation in a
267 sport horse breed, in which ET-produced horses account for more than half of the whole
268 population, utilizing populational data of more than 80,000 horses.

269 Generation interval, which could contribute to accelerating the genetic progress per year in
270 a given population, was lower than previously reported in several horse breeds such as
271 Thoroughbred ($GI=10.6$, Moureaux et al. (1996) and $GI=10.62$, Bokor et al. (2013)),
272 Hanoverian (GI ranging from 8.9 to 11.1, Hamann and Distl (2008)), American Quarter Horse
273 ($GI=10.5$, Petersen et al. (2014)), Spanish Sport Horse ($GI=10.8$, Bartolomé et al. (2011)),
274 French sport horse ($GI=12$; Dubois and Ricard (2007)), Selle Français ($GI=11.7$, Moureaux et

275 al. (1996)) and Pura Raza Español ($GI=10.1$, Valera et al. (2005)). It well known that mares
276 that participate in sport and recreational activities are usually treated to prevent pregnancy, thus
277 decreasing their effective breeding lifespan and increasing the GI (Valera et al., 2005). This
278 situation could be exacerbated by the relatively extended sporting life of AP compared with
279 other sports breeds, such as the Thoroughbred, considering that only the training process of a
280 polo horse demands at least five years before they start to play in high-handicap tournaments.
281 Therefore, mares can only be included in natural breeding programs at very advanced ages,
282 which are associated with lower fertility (Marinone et al., 2015), thus leading to lower
283 pregnancy rates and few foals per individual. In this study, we demonstrated that such limitation
284 could be overcome by ET because gestations are carried out by a recipient mare; therefore,
285 broodmares can be used as embryo donors at younger ages (even before 4 years old, according
286 to the AP pedigree records). This fact is particularly important in polo horses, since the
287 tournament season of elite individuals is usually restricted to only six months per year, allowing
288 the use of the mares as embryo donors or the horses as stallions during the rest of the year. This
289 hypothesis is also supported by the fact that the higher reduction in GI was observed in the
290 maternal pathways (mother-son and mother-daughter) of the ET group.

291 The reproductive effect of ET was already associated with improved genetic gains through
292 three possible mechanisms in cattle: 1) an increased selection intensity (Nicholas, 2006), by
293 increasing the number of offspring per cow; 2) an increase in the accuracy of EBV estimations,
294 by including ET data in the genetic models (Junqueira et al., 2018); and 3) a reduction of the
295 interval between generations (Mota et al., 2013). In our case, two of these mechanisms were
296 observed in the ET group (short GI and increased number of foals per mare), lending support
297 to the general consensus among breeders and players that progress in AP performance during
298 the last two decades was due to the genetic improvement of the breed as a result of the use of
299 ET. However, this fact could not be determined in this study due to the lack of a systematic
300 evaluation program and the phenotypic characterization of the AP.

301 One of the possible disadvantages of the intensive use of reproduction biotechnologies is the
302 reduction of genetic variability. In the present study, average inbreeding in AP (0.82% in WP)
303 was still lower than in other populations such as Hanoverian (1.33%, Hamann and Distl (2008)),
304 Pura Raza Español (8.48%, Valera et al. (2005)), Lipizzan (10.8%, Zechner et al. (2002)),
305 Italian Haflinger (6.59%, Gandini et al. (1992)) and Arabian (7%, Moureaux et al. (1996)).
306 However, F was slightly higher than reported on Spanish (Bartolomé et al., 2011) and Brazilian
307 (Medeiros et al., 2014) sports horses ($F \approx 0.6\%$), which have a recently created studbook and
308 sustain an open enrolment policy. In the case of AP, the increasing trend in average inbreeding
309 was observed mostly during the last 10 years, which was when ET programs were set up
310 massively in the breed. In addition, F_R was higher in the ET group during the last generation,
311 as can be seen in Figure 3. Despite that the short lifespan of the breed, along with its current
312 policy of open registry, can partially explain the low average F values detected we hypothesize
313 that the relativeness and inbreeding increase observed, particularly in ET individuals, could
314 have been even more spiked if AP was managed as a close breed. But the fact that a growing
315 number of animals registered in the studbook are being used for breeding instead of new
316 “founder” individuals during the last few years (data not shown), could derive in the long-term
317 reduction of genetic diversity of the AP.

318 Both F and AR increased abruptly (more than two-fold in inbred individuals) only during the
319 last generation. Such increasing trend was more marked in the ET group since this technology
320 allows breeders to heavily use few relevant ancestors to obtain more homogenous horses as
321 well as the most prominent individuals as breeders due to commercial interests. In a recent
322 study, Todd et al. (2018) determined a strong negative effect of inbreeding and racing
323 performance on Australian racing horses analyzing more than 150,000 race results. Similarly,
324 Gómez et al. (2009) determined the presence of inbreeding depression on several morphological
325 traits in Andalusian horses and recommended the inclusion of inbreeding measures in genetic
326 evaluation models in order to improve the accuracy of the EBV obtained. Similarly, Hansen

327 (2000) described that individuals with $F > 6.25\%$ (which its number it is also increasing in AP
328 and particularly in ET) could induce inbreeding depression in dairy cattle. Even though average
329 inbreeding is still low in the breed, the increasing trend observed during the last years,
330 particularly in ET-produced horses, is a parameter that could be considered in future mating
331 decisions among the breeders in order to avoid the appearance of inbreeding depression among
332 individuals.

333 The effects produced using ET were also reflected in the average coancestry of each group
334 (significantly higher in ET), mostly due to an increased selection intensity over the mares,
335 which are producing more foals during his reproductive life thus increasing their
336 representativeness in the pedigree, producing an increase in the AR values and further limiting
337 genetic diversity. In order to cope with this situation, the Andalusian Horse Breeder Association
338 has successfully developed and implemented systematical approaches to monitor inbred mating
339 using an ad-hoc software to determine the relationships among all available breeders in a herd
340 (Melgarejo et al., 2000), which decreased substantially the average inbreeding rate in a
341 relatively short lapse of time (Valera et al., 2005). Since the use of ET techniques increases
342 mating possibilities by using stallions or mares which are in different herds and locations, the
343 implementation of this type of technologies could help to avoid the mating highly related
344 animals.

345 Effective population size is an important indicator of the evolution of the genetic variability
346 of a given population. In WP N_{eC} was lower than other composite breeds, such as Brazilian
347 Sport Horse (N_{eC} 188.5, Medeiros et al. (2014)) Anglo-Arab horse (N_{eC} 291.2, Cervantes et al.
348 (2011)) and Spanish Sport Horse (N_{eC} =1046, Bartolomé et al. (2011)), suggesting a reduced
349 variability probably due to the selection strategy employed. Cervantes et al. (2011) also
350 demonstrated that N_{eC} and N_e are expected to present very similar values to each other in a non-
351 genetically structured population, since individual increase in inbreeding tend to raise by the
352 presence of subdivision in a population, while individual increase in coancestry is practically

353 not affected. In our study, the N_{ec}/N_e ratio in NOT-ET was twice than in ET, supporting the idea
354 that the individuals bred by natural gestation were more related within groups, probably due to
355 the lower connection between herds since broodmares normally remains in the same herds
356 during all his reproductive life. This was also shown by the increased F_{ST} value in this group as
357 well as by the low correlation among herds. Therefore, the joint analysis of this parameters
358 supports the idea that ET allows an increased mobility of the selected germplasm among herds,
359 mostly via female lineages, in comparison with NOT-ET, in which the homogeneity is observed
360 within rather than between groups.

361 The F_e is another important parameter to determine whether the contribution of the original
362 breeding individuals is balanced. It is expected that the total number of founders is equal to F_e
363 when all of them contribute equally (Lacy, 1989). However, this does not usually occur in
364 livestock populations, where F_e is generally smaller, indicating a loss of genetic diversity in
365 proportion to how those values differ. This fact was evident in several horse populations
366 studied, such as the Spanish Sport Horse (Bartolomé et al., 2011) and the Andalusian Horse
367 (Valera et al., 2005). In our study, F_e and F_a were substantially lower than the number of
368 founders and ancestors in WP, whereas the F_e/F_a ratio suggest a clear bottleneck in the
369 population. This trend was highly exacerbated in ET population, where F_e and F_a were lower,
370 and the bottleneck is more noticeable compared with NOT-ET. This fact could be explained by
371 the use of a reduced number of stallions and broodmares, causing a high selection pressure,
372 which is also supported by the differences observed in the F_g . Since our clustering criteria was
373 only the use of ET techniques during breeding, this study clearly demonstrate, in a realistic
374 breeding situation with than 18,000 horses analyzed, that large ET programs have a marked
375 effect on the reduction of the population genetic variability.

376 Finally, it was noteworthy that the most contributing ancestors of the breed were
377 Thoroughbred horses. Beyond the fact that the incorporation of animals of any origin was
378 allowed, this information confirmed that the AP breed was highly influenced by this breed in

379 its beginnings. However, our analysis also showed that the Thoroughbred influence was
380 produced by a scarce number of stallions extensively employed as breeders during a long period
381 of time rather than a by the massive use of Thoroughbred horses as breeders at the beginning.
382 This fact could also have affected genetic variability, as was already described in the Brazilian
383 Sports horse (Medeiros et al., 2014), the Spanish Asturcón (Álvarez et al., 2011), and three
384 Czech draught horse breeds (Vostrá-Vydrová, 2016). In this sense, it is also well known that
385 the Thoroughbred horse is a very old breed created from a few famous stallions (Binns et al.,
386 2012) with a limited genetic variability (Cunningham et al., 2001; Bokor et al., 2013). Since
387 this breed was heavily involved in the origins of polo horses, it would not be unwise to assume
388 that such scarce diversity could be dragged towards the AP, further affecting its genetic status.
389 However, this could not be assessed since there are no population studies performed in polo
390 ponies using molecular or genomic data estimations of the genetic variability of the breed.

391

392 **5. Conclusions**

393 In this study, we analyzed the genealogical data of an AP horse population, recognized
394 worldwide by its sports skills in polo game, in which horses are mainly produced by the use of
395 large-scale ET programs. Our results not only demonstrated that such reproductive
396 methodologies could enhance the distribution of superior germplasm, but also could
397 compromise the genetic variability in a long-term basis. Even though average relatedness and
398 inbreeding have been rising in this population during the last years, the situation is not yet
399 worrying, probably due to the existence of an open studbook. We also demonstrated that
400 inbreeding increase and loss of genetic variability were highly dependent on the use embryo
401 transfer programs. Therefore, their use may be complemented by practices to mitigate the
402 potential negative effect produced by the inbreeding depression on performance traits in the
403 future generations of AP horses.

404

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412

413 **Conflict of Interest Statement**

414 The authors declare that the research was conducted in the absence of any commercial or
415 financial relationships that could be construed as a potential conflict of interest.

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- 546
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548 **Fig. 1.** Number of total records in the Argentine Polo Horse Studbook from 1984 to 2015.

549

550 **Fig.2.** Percentage of horses born by embryo transfer registered in the Argentine Polo

551 Horse Studbook.

552

553 **Fig. 3.** Evolution of inbreeding coefficient in WP, ET and NOT-ET in the last generation

554 and rate of inbreeding.

555 **Table 1**

556 Generation interval in Argentine Polo Horses.

557

Generation interval (years)			
<i>Pathways</i>	WP	ET	NOT-ET
<i>Father-son</i>	7.9 ± 4.3	7.7 ± 4.4	9 ± 3.7
<i>Father-daughter</i>	7.6 ± 4.1	6.9 ± 3.8 ^a	8.7 ± 4.2 ^b
<i>Mare-son</i>	8.5 ± 3.6	8.2 ± 3.5 ^a	10.8 ± 4 ^b
<i>Mare-daughter</i>	8.5 ± 4.2	7.9 ± 3.8 ^a	10.6 ± 4.8 ^b
<i>Average</i>	8.2 ± 4.3	7.7 ± 3.8 ^a	9.6 ± 4.4 ^b

558 Values are expressed as mean ± standard deviation. All statistical comparisons correspond only to ET and
559 NOT-ET groups. Superscript letters show statistical differences (P<0.01). WP: Whole population born in
560 the last generation; ET: Horses from WP born by embryo transfer; NOT-ET: Horses from WP not born by
561 embryo transfer.

562 **Table 2**

563 Analysis of genetic variability obtained through genealogical information analysis in
 564 Argentine Polo Horses.

565

<i>Parameter</i>	<i>WP</i>	<i>ET</i>	<i>NOT-ET</i>
Animals	18077	13478	4599
Number of founders	3818	2153	2817
Number of ancestors contributing to AP	3286	1653	2268
Ancestors explaining 50% of genetic variation	18	12	64
Effective number of founders F_e	326	246	606
Effective number of ancestors F_a	43	31	95
Founder genome equivalent F_g	30.2	22.5	62.5
F_e/F_a ratio	7.6	7.9	6.4

566

WP: Whole population born in the last generation; ET: Horses from WP born by embryo transfer; NOT-
 567 ET: Horses from WP not born by embryo transfer; AP: Argentine Polo Horse

568 **Table 3**

569 Most important contributing ancestors to the Argentine Polo Horse.

Ancestor	Sex	Birth year	Breed composition	Explained variability %		
				WP	ET	NOT-ET
SPORTIVO	M	1992	Thoroughbred	8.3	10.5	1.9
PUCARA	M	1981	Thoroughbred	7.7	9.1	3.9
EL SOL	M	1987	Thoroughbred	4.6		4.5
RAINBOW CORNER	M	1993	Thoroughbred	3.9	4.3	2.5
LA LUNA	F	≈1981	Thoroughbred	3.8		1.8
SIMPATICA	F	1986	50-100% Thoroughbred	3.4		
POLO NEVADITO*	M	1993	75% Thoroughbred	3	2.3	5
GUINDA	F	≈1980	-	2.2	2.7	
LAMBADA	F	≈1996	75-100% Thoroughbred	1.9	2.4	
CHUSMA	F	-	Thoroughbred	1.8	2.2	
TOP SECRET	M	1972	Thoroughbred		5	
GAUCHA ACHA	F	1973	Thoroughbred		4.6	
INDULTO	M	1978	Thoroughbred			2.6
POLO PUREZA	F	1988	Thoroughbred			2.7
TORNADO	M	1996	50% Thoroughbred			0.8
GAETO	M	1998	Thoroughbred			1.2
VENGO TARDE	M	1982	Thoroughbred			1.1

570 *Not founder. M, male; F, female; WP: Whole population born in the last generation; ET: Horses from WP
571 born by embryo transfer; NOT-ET: Horses from WP not born by embryo transfer.

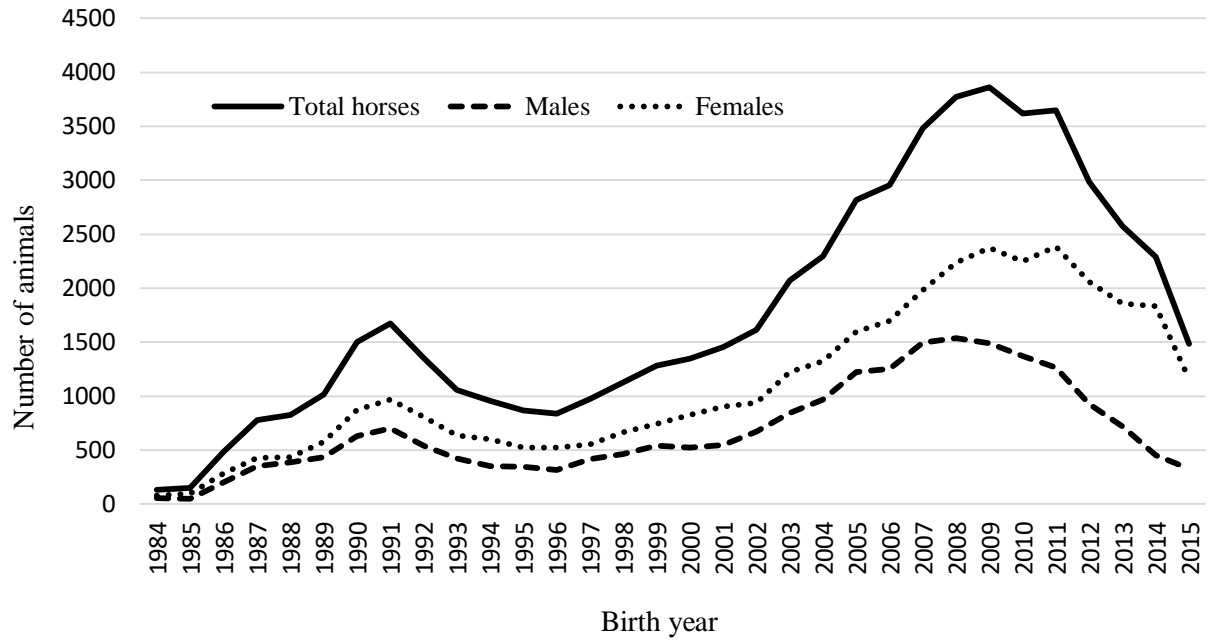


Fig. 1. Number of total records in the Argentine Polo Horse Studbook from 1984 to 2015

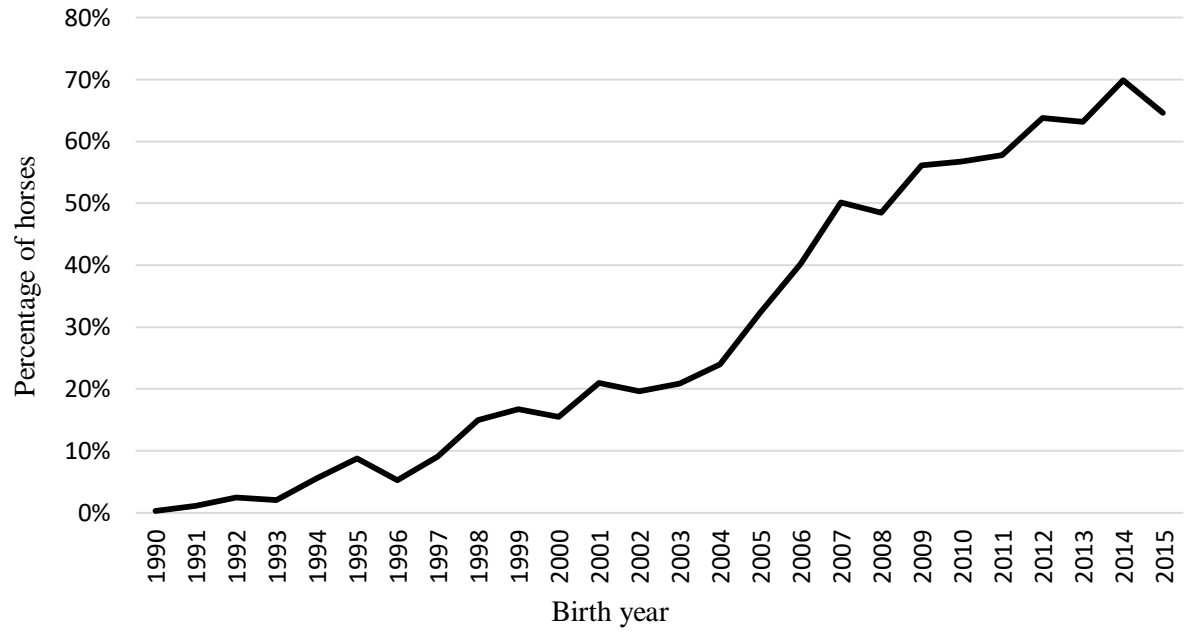


Fig.2. Percentage of horses born by embryo transfer registered in the Argentine Polo Horse Studbook.



Fig.2. Percentage of horses born by embryo transfer registered in the Argentine Polo Horse Studbook.

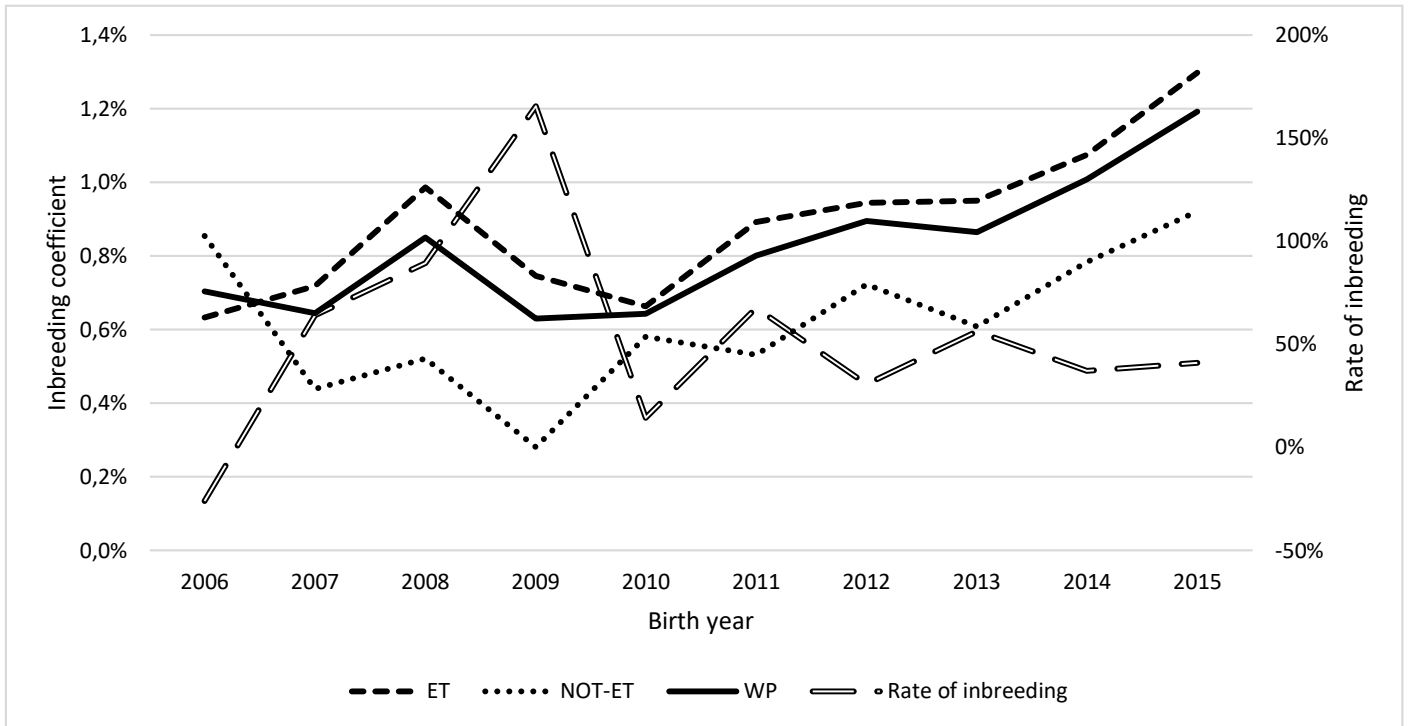


Fig. 3. Evolution of inbreeding coefficient for WP, ET and NOT-ET, in the last generation, and the rate of inbreeding.

Conflict of Interest Statement

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