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ANIMAL REPRODUCTION

Relationship of semen quality to inbreeding and gait of Colombian Creole horses

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ABSTRACT: *High consanguinity among equines has negative effects on semen quality, thus resulting in low motility and high levels of abnormality in the spermatozoa. However, such a relationship has not been studied in Colombian Creole horses, which have been subjected to particular selection practices focusing mainly on their gait. This research assessed the relationship of semen quality to inbreeding and gait of Colombian Creole horses. Semen was collected from 50 horses using the artificial vagina method. Sperm motility and kinematics were assessed with a computerized analysis system (SCA®). Sperm vitality (SV) and abnormal morphology (AM) were assessed via the eosin-nigrosin staining test. Functional membrane integrity (FMI) was assessed via the hypo-osmotic swelling test (HOST). Genealogies and consanguinity analysis was conducted using the Breeders Assistant for Horses program. An average of 3.6 ± 0.4 % was reported for the inbreeding coefficient (Ft). A decrease in sperm motility and kinematics was reported, which was associated with an increase in consanguinity ($P < 0.05$). Furthermore, differences in consanguinity were found based on gait. Similarly, a relationship between horse gait and semen quality ($P < 0.05$) was found. Authors concluded that semen quality of Colombian Creole horses has been affected by inbreeding and its relationship with genetic selection based on gait.*
Key words: *consanguinity, spermatozoa, stallion.*

Relação da qualidade do sêmen com endogamia e marcha de cavalos crioulos colombianos

RESUMO: *A alta consanguinidade entre equinos tem efeitos negativos na qualidade do sêmen, resultando em baixa motilidade e altos níveis de anormalidade nos espermatozóides. No entanto, tal relação não foi estudada em cavalos crioulos colombianos, que tem sido submetidos a práticas de seleção específicas com foco principalmente em sua marcha. O objetivo desta pesquisa foi avaliar o relação da qualidade do sêmen com endogamia e marcha de cavalos crioulos colombianos. O sêmen foi coletado de 50 cavalos pelo método da vagina artificial. A motilidade e a cinética dos espermatozoides foram avaliadas com um sistema de análise computadorizado (SCA®). A vitalidade do esperma (VE) e a morfologia anormal (MA) foram avaliadas por meio do teste de coloração com eosina-nigrosina. A integridade funcional da membrana (IFM) foi avaliada por meio do test hipo-osmótico (HOST). A análise de genealogias e consanguinidade foi conduzida usando o programa Breeders Assistant for Horses. Uma média de 3,6 ± 0,4% foi encontrada para o coeficiente de endogamia (Ft). Uma diminuição na motilidade e cinética dos espermatozoides, que foi associada a um aumento na consanguinidade ($P < 0,05$). Além disso, diferenças na consanguinidade foram encontradas com base na marcha. Da mesma forma, foi encontrada uma relação entre a marcha do cavalo e a qualidade do sêmen ($P < 0,05$). Os autores concluíram que a qualidade do sêmen de cavalos crioulos colombianos foi afetada pela endogamia e sua relação com a seleção genética baseada na marcha.*
Palavras-chave: *consanguinidade, espermatozóides, garanhão.*

INTRODUCTION

In horse husbandry, phenotype, conformation and movement capacity are particularly important traits for genetic selection and as traits of economic interest (GÓMEZ et al., 2009). Stallion selection for genetic enhancement has often been based on subjective criteria, thus resulting in crossings with some degree of inbreeding (DORNELLES et al., 2012). Inbreeding is the process of mating genetically similar animals, producing offspring with a higher level of homozygosity (DINI et al., 2020), which may result in the phenotypic expression of lethal and semi-lethal recessive genes (WOLC et al., 2008). This is often associated with the reduction of performance in quantitative traits under constant selection pressure such as racing performance (TODD et al., 2018), reproductive traits (MÜLLER-UNTERBERG et al., 2017), and health traits (SÁNCHEZ-GUERRERO et al., 2019; VOSTRY et al., 2021). Conversely, the biological potential of the more heterozygous animals may be higher than that of their inbred counterparts (AVDI & BANOS, 2008).

In equines, consanguinity has been reported to have negative effects on fertility

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(KJÖLLERSTRÖM et al., 2015). It has been suggested that a decrease in male fertility is one of the earliest consequences of an increased level of inbreeding because mutations occur much more frequently in the male germline (DINI et al., 2020). In particular, high inbreeding affects the sperm production capacity, produces low rates of motility, as well as high levels of sperm abnormality (COTHRAN et al., 1984). In bulls, inbreeding has a negative effect on scrotum size, sperm motility, sperm concentration, and the percentage of morphologically normal sperm (DORADO et al., 2017). In the case of ponies, it has been reported that an inbreeding coefficient above 2% for the Shetland breed is associated with lower rates of motile spermatozoa (VAN ELDIK et al., 2006). In addition, a study assessing equines from the Friesian breed reported a high level of inbreeding that produced a decreasing trend in the morphological quality of the sperm (BOER, 2007). Some breeds have higher coefficients of inbreeding than others: for example inbreeding rates of 9.9% have been reported for Standarbred (SB) stallions, while their Finnhorse (FH) counterparts only had rates of 3.6% (SAIRANEN et al., 2009).

An unbalanced contribution of ancestors can reduce genetic variation and promote inbreeding (MÜLLER-UNTERBERG; WALLMANN; DISTL, 2017). However, Colombian Creole horses have been subjected to a set of particular selection practices, which focus mainly on gait. Likewise, there is a preference for individuals with superior performance in equine fairs. Moreover, studies on the genetic diversity of domestic horses in Colombia have reported low values of genetic variability, which in turn means that these populations are closely related genetically (LUZ CORREA et al., 2015; YEPES et al., 2017). Finally, information on the degree of consanguinity of the breed and its possible impact on sperm quality and fertility is scarce. This research assessed the relationship of semen quality to inbreeding and gait of Colombian Creole horses.

MATERIALS AND METHODS

Semen collection

Semen from 50 Colombian Creole horses was collected between 2014 and 2019 in Antioquia (Colombia). The age of the horses ranged from 4 to 11 years and their fertility was confirmed by their living offspring. Only horses formally registered in the Colombian Federation of Equine Associations (Fedequinas), based on criteria of ancestry, breed and gait were selected. The animals were classified based on the following traditional types of gait: Colombian Trot and Gallop (P1), Colombian Trocha and Gallop (P2), Colombian Trocha Pura (P3) and Colombian Paso Fino (P4). The Trot gait consists of an isochronal two-beat and diagonally coupled gait, which is highly collected. There are either two or four limbs in stance phase. The Gallop gait is an asymmetric gait is a highly collected canter and it has a non-isochronal beat pattern, with at least one limb, always, in stance phase. The Trocha gait is a lateral sequence four-beat and diagonally coupled gait, in which the forelimb hit the ground before the contra lateral hind limb, and it has a non-isochronal beat pattern. The Paso Fino gait is a lateral sequence four-beat and laterally coupled gait, which has an isochronal beat pattern, and an independent limb movement (NOVOA-BRAVO et al., 2018). The animals had similar conditions in terms of food, training and reproductive management. Horses were regularly collected at least once a week and at most twice a week. The semen from each stallion was obtained within the same year and season, therefore seasonal effects were not considered. Semen was collected via the artificial vagina method with a Missouri model vagina (Minitube, Tiefenbach, Germany) lubricated with non-spermicidal gel. In addition, a mare was used to increase sexual stimulation. The gel fraction of the ejaculate was removed using a sterile filter. The semen was extended at a 1:1 ratio in an extender composed of skim milk, antibiotics and sugars (EquiPlus®, Minitube, Tiefenbach, Germany). The semen was then transported in an insulating box at 5 °C within two hours after its collection. The study complied with the Colombian legislation of ethics and experimentation with animals and the ethics committee of the Colombian Polytechnic Jaime Isaza Cadavid.

Sperm assessment

The semen volume and sperm concentration of each ejaculate was assessed using a graduated cylinder and a spectrophotometer respectively (Spermacue®, Minitube, Tiefenbach, Alemania). Sperm kinematics and motility were assessed with a computer-assisted analysis system (SCA®, Microptic, Barcelona, Spain). A specific configuration was established for the software: 20 x 20 mm glass slide, optics in ph (-), drop of 7 mL, equine species, thermal plate at 37 °C and a particle size of 20 to 72 mm, capture velocity 25fps. A minimum of 500 spermatozoids were evaluated in five observation fields. Furthermore, sperm morphology and vitality were measured via eosin-nigrosin staining (BRITO et al., 2011). A droplet of semen and a droplet of eosin–nigrosin (Sigma-Aldrich, Eugene, OR, USA) were placed on a microscope slide, mixed, smeared and placed on a warming plate at 37 °C. Subsequently, 200 spermatozoa were assessed individually using an Eclipse E200 phase-contrast microscope (Nikon Inc., Tokyo, Japan). Functional membrane integrity (FMI) of the spermatozoa was assessed with the hypo-osmotic swelling test (HOST) (NEILD et al., 1999). To provide a concentration of 100 mOsmol/L, 20 µL of semen were added to 200 µL of a hypo-osmotic 5.4% sucrose solution. This mixture was incubated at 38.5 °C for 30 min. Then, 200 spermatozoa were evaluated using an Eclipse E200 phase-contrast microscope (Nikon Inc., Tokyo, Japan).

Inbreeding coefficients

Individual inbreeding coefficients (*Ft*) were estimated for each of the horses included in this study via the Breeders Assistant for Horses program, version 5.21 (Tenset Technologies Ltd, United Kingdom). The basic procedure used by this program defines F as the probability that an individual has two identical alleles by descent. For this, an ideal population of size *N* is considered to have 2*N* distinct alleles in its initial generation; thus the probability of homozygosis is 1 / 2*N*. This procedure is repeated for each generation, and it becomes necessary to take into account the results from previous generations; thus, the inbreeding coefficient for generation *t* can be expressed as:

$$F_t = \frac{1}{2N} + \left(1 - \frac{1}{2N}\right) F_{t-1} \quad (1)$$

Where the first term (1 / 2*N*) represents the increase in the degree of consanguinity resulting from the identity by descent of two copies of one gene that come from an individual from generation *t*-1 (new-generation inbreeding). The second term, represents the consanguinity produced up to the previous generation; it is kept in the 1 - 1/2*N* remaining zygotes in which no further inbreeding is observed (old inbreeding). Thus, the increase in the rate of inbreeding or consanguinity (Δ*F*) was calculated for each generation using the following:

$$\Delta F = \frac{F_t - F_{t-1}}{1 - F_{t-1}} \quad (2)$$

Where Ft is the mean consanguinity for a given generation. Based on this, the rate of inbreeding and loss of heterozygosity were estimated for 8 and 16 generations.

Statistical analysis

A descriptive statistical analysis was conducted for the various semen quality and inbreeding parameters., a quartile analysis was conducted and three categories for the inbreeding coefficient were established (low, medium and high) to assess the relationship between inbreeding and semen quality. Likewise, a generalized linear model (GLM) was fit for each dependent semen quality variable. The relationship between the gait of Colombian Creole horses, semen quality, and inbreeding were assessed by fitting generalized linear models (GLM) for each dependent variable. Data normality was assessed using the Shapiro-Wilk test and the means were compared with Tukey's test (HSD). All statistical analyses were conducted using the SAS version 9.2 software (SAS Institute Inc., Cary, NC, USA).

RESULTS

A genealogical analysis of 681 equines which were ancestors of the 50 Colombian Creole horses from which the semen samples were collected. Table 1 shows the results of the analysis of consanguinity and table 2 shows the results of the semen quality assessment.

Table 3 shows the results of the comparison of the means for the various semen quality parameters based on the inbreeding coefficient. The results showed an association between inbreeding and semen quality in Colombian Creole horses. This was evidenced by the decrease observed in the sperm's total and progressive motility values when the inbreeding coefficient was high, which contrasts with those observed with a low inbreeding coefficient ($P < 0.05$). A decrease in curvilinear velocity (VCL) was also observed with high and medium rates of inbreeding ($P < 0.05$). In addition, it was observed that higher rates of inbreeding coincided with a numerical trend towards an increase in sperm concentration and a reduction in ejaculate volume; however, these associations were very far from statistical significance ($P = 0.5$ and $P = 0.7$, respectively). (Table 3).

An analysis of consanguinity was conducted because gait is considered one of the most important selection and crossing criteria for Colombian Creole horses (Table 4). Table 5 shows the analysis of the semen quality of Colombian Creole horses based on gait. Colombian Paso Fino (P4) had the highest inbreeding coefficient (Table 4) and was also the gait with the lowest values for total motility and average path velocity (VAP) ($P < 0.05$). The schematic association between the significant parameters of semen quality ($P < 0.05$), in relation to consanguinity and gait are shown in figure 1.

Table 1 - Analysis of consanguinity from the pedigrees of 50 Colombian Creole horses.

Variable	Mean	SD	CV	SEM	Min.	Max
Generations found	11.0	2.5	23.0	0.4	5.0	18.0
Complete generations	3.0	0.7	24.6	0.1	1.0	4.0
Total ancestors 32G	136.2	72.9	53.5	10.3	32.0	355.0
Inbreeding coefficient 16G (%)	3.6	2.9	81.4	0.4	0	12.9
Rate of inbreeding 8G (%)	99.5	0.4	0.4	0.1	98.3	100.0
Rate of inbreeding 16G (%)	99.8	0.2	0.2	0.0	99.1	100.0
Loss of heterozygosity 8G (%)	0.5	0.4	82.2	0.1	0	1.7
Loss of heterozygosity 16G (%)	0.2	0.2	85.9	0.0	0	0.9

SD: standard deviation. CV: coefficient of variation. SEM: standard error of the mean. Min: minimum. Max: maximum. 8G: 8 generations. 16G: 16 generations. 32G: 32 generations.

DISCUSSION

In the current study, a high variability for the inbreeding coefficient and for loss of heterozygosity (as shown by the coefficient of variation), was observed. This could be due to a lack of data for many genealogies, where only a small quantity of the generations reported was complete (Table 1). The reason behind this could be that, in Colombia, specimens that are not intended for exhibition are not usually registered, but they are still included in reproduction programs. Likewise, using criteria that are not strictly based on genetics to cross specimens of this breed might have led to inbreeding, either accidentally or intentionally. Given the importance of maintaining genetic diversity in the equine species, several studies have been conducted in order to analyze diversity patterns among various horse breeds (VAN DE GOOR et al., 2011). However, studies on the genetic diversity of domestic horses in Colombia have reported low values of genetic variability and a high degree of gene flow, thus demonstrating that these populations are highly related in terms of genetics and therefore behave as a metapopulation (LUZ CORREA et al., 2015; YEPES et al., 2017).

Conversely, it is important to consider that the sample size in this study is small compared to that of other studies conducted in countries with a much longer tradition of crossing registered individuals. A study conducted with 1146 Friesian stallions with genealogical information from 1937 found an average inbreeding coefficient of 3.16 ± 1.64% for

Table 2 - Analysis of semen quality of Colombian Creole horses.

Variable	Mean	SD	CV	SEM	Min	Max
Concentration (10^6/mL)	184.5	88.5	48.0	14.0	22.0	364.0
Volume (mL)	56.2	55.8	99.2	9.0	12.0	309.0
TM (%)	78.6	16.2	20.6	2.3	33.0	98.3
PM (%)	47.3	19.7	41.6	2.8	7.6	82.8
VCL (µm/s)	93.1	24.5	26.3	3.5	46.7	142.9
VSL (µm/s)	37.6	11.8	31.4	1.7	15.1	62.0
VAP (µm/s)	67.8	21.2	31.3	3.0	33.4	109.1
SV (%)	77.1	12.7	16.5	1.9	44.0	96.0
FMI (%)	60.0	16.4	27.3	2.5	12.0	89.0
AM (%)	29.5	14.9	50.6	2.3	8.0	78.0

SD: standard deviation CV: coefficient of variation SEM: standard error of the mean Min: minimum. Max: maximum. TM: total motility. PM: progressive motility VCL: curvilinear velocity VSL: straight line velocity VAP: average path velocity. SV: sperm vitality. FMI: functional membrane integrity. AM: abnormal morphology.

Table 3 - Semen quality based on the inbreeding coefficient of Colombian Creole horses.

Inbreeding coefficient	Low < 1.2%	Medium 1.2 - 2.9%	High > 2.9%
Concentration (10^6/mL)	169.9 ± 30.2	176.0 ± 21.5	196.5 ± 22.5
Volume (ml)	68.7 ± 15.8	52.1 ± 16.5	56.6 ± 12.6
TM (%)	85.5 ± 3.9 ^{a}	79.7 ± 3.8^{ab}	75.7 ± 3.4^{b}
PM (%)	57.5 ± 7.3^{a}	48.0 ± 4.9^{ab}	43.4 ± 3.6^{b}
VCL (µm/s)	109.9 ± 9.2^{a}	90.9 ± 5.0^{b}	89.5 ± 5.3^{b}
VSL (µm/s)	43.5 ± 4.8	37.0 ± 2.2	36.2 ± 2.7
VAP (µm/s)	79.7 ± 8.3	66.6 ± 4.3	65.1 ± 4.6
SV (%)	83.3 ± 3.5	77.3 ± 2.5	74.8 ± 3.4
FMI (%)	63.9 ± 3.7	63.4 ± 2.7	55.6 ± 4.4
AM (%)	24.7 ± 5.9	30.3 ± 3.0	30.2 ± 3.9

Results are expressed as mean ± standard error of the mean (SEM). Different letters in the rows indicate statistically significant differences ($P < 0.05$).TM: total motility. PM: progressive motility. VCL: curvilinear velocity. VSL: straight line velocity. VAP: average path velocity. SV: sperm vitality. FMI: functional membrane integrity AM: abnormal morphology.

five generations, and 15.17 ± 1.75% for the complete pedigree (BOER, 2007). Considering that the average number of complete generations in this study was only 3.0 ± 0.7, and the inbreeding coefficient found was 3.6 ± 2.9% (Table 1), it could be considered higher than the value reported in the above-mentioned study for five complete generations. However, inbreeding coefficients are higher for some breeds than for others. For instance, a study conducted in Finland with Standardbred and Finnhorse stallions reported inbreeding levels of 9.9% for the former and only 3.6% for the latter (SAIRANEN et al., 2009).

The variability observed in the results for semen quality (Table 2) may be considered a trait of the equines, since different authors have attributed this effect to factors such as breed, age, and environment (DOWSETT & KNOTT, 1996; HOOGEWIJS et al., 2011; JANETT et al., 2003). Similar results have been obtained in previous studies for semen quality of Colombian Creole horses (RESTREPO et al., 2019); while for different Dutch horse breeds with different levels of inbreeding, lower semen quality means have been reported (DINI et al., 2020).

Conversely, regarding the relationship reported between inbreeding and semen quality in horses, other researchers found increased inbreeding coefficients in Shetland ponies to be correlated with lower progressive motility rates (VAN ELDIK et al., 2006). Similarly, DINI et al. (2020) indicated that Shetland ponies, a breed with a small population

Table 4 - Analysis of consanguinity of Colombian Creole horses according to their gait.

Variable / Gait	P1	P2	P3	P4
Complete generations	2.8 ± 0.2	2.7 ± 0.3	3.0 ± 0.2	3.3 ± 0.2
Generations found	11.4 ± 0.8	10.7 ± 0.6	12.0 ± 0.4	9.3 ± 0.8
Total ancestors 32 G	137.2 ± 26.1	98.7 ± 26.5	153.9 ± 16.2	124.5 ± 15.9
Inbreeding coefficient 16G (%)	3.7 ± 0.6^{ab}	3.1 ± 1.4^{ab}	2.8 ± 0.6^{b}	5.0 ± 0.1^{a}
Rate of inbreeding 8G (%)	99.5 ± 0.1	99.6 ± 0.2	99.6 ± 0.1	99.4 ± 0.1
Rate of inbreeding G (%)	99.8 ± 0.0^{ab}	99.8 ± 0.1^{ab}	99.8 ± 0.0^{ab}	99.7 ± 0.1^{b}
Loss of heterozygosity 8G (%)	0.5 ± 0.1	0.4 ± 0.2	0.4 ± 0.1	0.6 ± 0.1
Loss of heterozygosity 16G (%)	0.2 ± 0.0^{ab}	0.2 ± 0.1^{ab}	0.2 ± 0.0^{ab}	0.3 ± 0.1^{a}

Results are expressed as mean ± standard error of the mean (SEM). Different letters in the rows indicate statistically significant differences ($P < 0.05$). P1: Colombian Trot and Gallop. P2: Colombian Trocha and Gallop. P3: Colombian Trocha. P4: Colombian Paso Fino. 8G:8 generations. 16G: 16 generations. 32G: 32 generations.

Table 5 - Analysis of the semen quality of Colombian Creole horses according to their gait.

Variable / Gait	P1	P2	P3	P4
Concentration (10^6/mL)	173.9 ± 27.6	196.1 ± 56.2	199.9 ± 23.7	170.0 ± 25.4
Volume (mL)	67.7 ± 25.0	53.5 ± 23.4	43.8 ± 8.5	63.7 ± 17.7
TM (%)	74.5 ± 4.2^{bc}	87.0 ± 2.8^{a}	83.0 ± 3.1^{ab}	71.1 ± 6.0^{c}
PM (%)	46.4 ± 5.8	51.9 ± 4.5	50.0 ± 4.7	40.8 ± 6.2
VCL (µm/s)	97.4 ± 7.7	96.0 ± 6.2	94.9 ± 5.4	83.1 ± 8.0
VSL (µm/s)	36.3 ± 2.7	40.3 ± 4.2	37.9 ± 2.9	36.7 ± 13.2
VAP (µm/s)	73.7 ± 3.5^{a}	68.5 ± 7.2^{ab}	67.9 ± 4.6^{ab}	60.8 ± 6.8^{b}
SV (%)	73.6 ± 4.9	81.4 ± 3.4	80.2 ± 2.2	74.0 ± 4.2
FMI (%)	60.0 ± 4.5	67.9 ± 6.1	61.1 ± 3.7	54.0 ± 6.5
AM (%)	27.6 ± 3.5	37.8 ± 12.0	27.2 ± 3.4	31.0 ± 4.0

Results are expressed as mean ± standard error of the mean (SEM). Different letters in the rows indicate statistically significant differences ($P < 0.05$). P1: Colombian Trot and Gallop. P2: Colombian Trocha and Gallop. P3: Colombian Trocha. P4: Colombian Paso Fino. TM: total motility. PM: progressive motility. VCL: curvilinear velocity. VSL: straight line velocity. VAP: average path velocity. SV: sperm vitality. FMI: functional membrane integrity. AM: abnormal morphology.

(<1000 registered horses) and a high degree of inbreeding, had the lowest semen quality among the studied breeds. In addition, they found lower semen quality in KWPN (Koninklijk Warmblood Paardenstamboek Nederland)-Harness horses than KWPN-Riding horses, potentially because of a higher coefficient of inbreeding (DINI et al., 2020). This is similar to the results reported in this study, in which seminal quality decreased when the inbreeding coefficient was higher than 2.9%. Likewise, a study

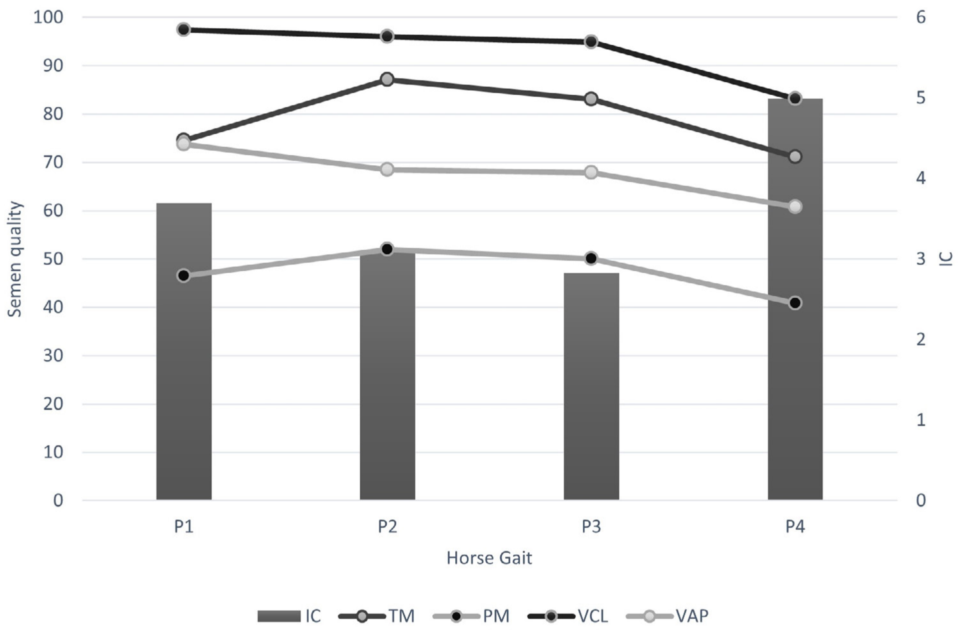

Figure 1 - Schematic association between semen quality, consanguinity and gait of Colombian Creole horses.

IC: Inbreeding coefficient (%). TM: total motility (%). PM: progressive motility (%). VCL: curvilinear velocity (µm/s). VAP: average path velocity (µm/s).

with Friesian horses reported that ejaculate volumes increased with higher inbreeding rates and; although, inbreeding was not found to have any effect on other semen quality traits, a specific analysis of inbreeding in 12 of the 26 studied ancestors did have a significant effect on one or more semen quality traits (BOER, 2007).

Having a larger population of horses with complete genealogies would likely result in more precise associations between inbreeding and other semen quality parameters such as vitality and normal morphology, which were not statistically different in this study (Table 3). Inbreeding coefficients over 2% in Shetland ponies have been associated with lower rates of morphologically normal spermatozoa (VAN ELDIK et al., 2006).

This breed's degree of consanguinity is influenced by gait. This was particularly evident when studying the differences in the rates of inbreeding between the Colombian Trocha and Paso Fino gaits (Table 4). Regarding the above, no specific reports of the association between these parameters were reported, but consanguinity is often associated with the reduction of performance in quantitative traits under constant selection pressure such as racing performance (TODD et al., 2018). In addition, progress in the study of the relationship between the genotype and the gait of the Colombian Creole horses has been made. An investigation reported that DMRT3 gene mutation seems to explain the horses' ability to perform the Paso Fino gait but not the other diagonal coupled gaits (Trocha and Colombian Trot) (NOVOA-BRAVO et al., 2018).

Nevertheless, given the similarity between the criterion defined as "airs" (Pace, Trot, and Gallop) in Hispano-Árabe horses and that of gait in the Colombian Creole horses, it is possible to highlight a study which the authors found that if the reproductive management of the Hispano-Árabe horse is correct, there will be low increases in inbreeding per generation, so there would be no danger of losing genetic variability (Gómez et al., 2011).. This might be relevant based on the results of this study, since it could serve as a projection for the development of selection schemes for Colombian Creole horses.

The results of this study show how inbreeding may affect the semen quality of Colombian Creole horses, and in turn show how consanguinity has been influenced by the selection of this breed, considering gait as a primary criterion (Table 5). Thus, for some semen quality parameters, simultaneous associations with the inbreeding coefficient and gait were observed (Figure 1). Likewise, it indicated that having gait as one of the main criteria for selection and crossing has had an impact on the inbreeding coefficient. Therefore, it is important to consider inbreeding as a relevant factor when developing future reproduction-oriented programs for Colombian Creole horses. In addition, it is necessary to establish selection and crossing programs with objective criteria whose aim is to preserve the genetic variability and traits of this breed. Higher rates of inbreeding might compromise the potential fertility of stallions. Furthermore, using gait as a selection and crossing criterion for Colombian Creole horses has had an impact on the breed's levels of inbreeding and especially on the established gaits. Therefore, the semen quality of Colombian Creole horses is influenced by gait, possibly due to the relationship observed between this trait and the existing degrees of inbreeding.

This study provides information, which could be considered a starting point for the creation of selection and crossing programs for Colombian Creole horses. Such programs aimed to decrease the rate of inbreeding while incorporating schemes that include different traits and objective criteria in their search for the preservation of the breed.

CONCLUSION

We concluded that semen quality of Colombian Creole horses has been affected by inbreeding and its relationship with genetic selection based on gait.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

BIOETHICS AND BIOSSECURITY COMMITTEE

The study complied with the Colombian legislation of ethics and experimentation with animals. While conducting this experiment, the authors fulfilled the Colombian Law 84 of December 1989, Chapter VI (Of the Use of Live Animals in Experiments and Research), Article 26 and Law 1774 of 2016, Article 1.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally to the conception and writing of this manuscript. All authors critically reviewed the manuscript and approved the final version of the manuscript.

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