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Effect of seasonality on the seminal quality of Quarter horse stallions

Efecto de la estacionalidad sobre la calidad seminal de sementales Cuarto de Milla

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ABSTRACT

The objective was to evaluate the influence of the year seasons on the seminal quality of Quarter Horses. 12 stallions aged 9 to 12 years were used, a Colorado artificial vagina was used for semen collection, motility, concentration and volume were immediately evaluated, it was considered as spring (March 20-June 21); summer (June 22-September 23); autumn (September 24-December 21) and winter (December 22-March 19). The parameters showed no significant difference in volume (P>0.05) between stations. However, there was a difference between the months of May (81.1±15.2) vs February (46.6±2.8), in sperm motility it was higher (P<0.05) in summer (85.6±2.6) vs winter (71.5±19.0), the sperm concentration $10x^6$ was significantly higher (P<0.05) in summer (269.8±51.4) vs winter (207.9±80.4). These results us to conclude that the season of the year has an influence on semen quality. Therefore, when implementing reproductive biotechnologies in the north of México, we can use the equine semen of Quarter horse stallions, preferably obtained at the time with the highest production and seminal quality.

Keywords: time of year, stallions, seminal quality.

RESUMEN

El objetivo fue evaluar la influencia de las épocas del año sobre la calidad seminal de caballos Cuarto de Milla. Se utilizaron 12 sementales con edad de 9 a 12 años, se utilizó una vagina artificial Colorado para la recolección de semen, inmediatamente se evaluó la motilidad, concentración y volumen, se consideró como primavera (20 de marzo- 21 de junio); verano (22 de junio- 23 de septiembre); otoño (24 de septiembre- 21 de diciembre); invierno (22 de diciembre- 19 de marzo). Los parámetros no mostraron diferencia significativa en volumen (P>0.05) entre estaciones. Sin embargo, hubo diferencia entre los meses de mayo (81.1±15.2) vs febrero (46.6±2.8), en motilidad espermática fue mayor (P<0.05) en verano (85.6±2.6) vs invierno (71.5±19.0), la concentración espermática $10x^6$ fue significativamente mayor (P<0.05) en verano (269.8±51.4) vs invierno (207.9±80.4). Estos resultados permiten concluir que la estación del año tiene influencia sobre la calidad seminal. Por lo cual al implementar biotecnologías reproductivas en el Norte de México podemos utilizar el semen equino de sementales Cuarto de Milla, preferentemente obtenido en la época con mayor producción y calidad seminal.

Palabras clave*:* época del año, sementales, calidad seminal.

INTRODUCTION

In recent years, equine production has increased worldwide, leading to the implementation of reproductive biotechnologies, which demands the use of high quality semen. Domestic and wild animal species use reproductive seasonality as a strategy to achieve the birth of their offspring, when environmental conditions are favorable (Bustos & Torres, 2012; Aurich, 2016). In such sense, equines in the northern hemisphere initiate their reproductive activity in early spring and summer, during this time semen production and endocrine testicular function increase, while in autumn and winter they may decrease. Other factors influence sperm production such as photoperiod, latitude and breed (Johnson, 1991; Bronson, 2009). These changes are mainly due to the relationship between daylight hours and the hypothalamus-pituitary-gonads axis (Monteiro *et al.,* 2011; Das *et al.,* 2013). This stimulus is captured by the retina, which acts as a photoreceptor that registers the presence or absence of light, and then passes to the suprachiasmatic nucleus (SCN) and paraventricular nucleus in the hypothalamus that operates as a biological clock, which regulates the endogenous circadian rhythm. The signal is transferred to the superior cervical ganglion (SCG), at this point the electrical signal is transformed into chemical signal releasing noradrenalin (Barrell *et al.,* 2000: Cortés-Vidauri *et al.,* 2018), which is taken up by pinealocytes through alpha and beta adrenergic receptors and induces the synthesis of transferase for melatonin synthesis in the pineal gland (PG) (Bustos & Torres, 2012). Some studies have evaluated the effect of season on seminal production and quality (Janett *et al.,* 2003a; Aurich, 2016). Similarly, testicular size, sperm production, seminal volume, sperm morphology, testosterone levels, serum levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) have been evaluated in equine stallions, showing that these parameters change during the breeding season (Pereira *et al.,* 2012; Brabender *et al.,* 2016; Gautier *et al.,* 2018). In a study that was conducted with three stallions at a latitude of 22°53′09″S reported that no significant differences were found between seasons in seminal parameters (Pereira *et al.,* 2012). However, despite the importance of this species, in Mexico, the reproductive behavior of stallions specifically of Quarter horse breed is still unknown. Therefore, the objective of this study was to evaluate the influence that the time of the year has on the production and seminal quality in these Quarter horse stallions under the conditions of northern Mexico at 26°N Latitude.

MATERIAL AND METHODS

General Management

All methods and handling of the stallions used in this study were in strict accordance with the guidelines for the ethical use, care and welfare of animals in research (FASS, 2010) with institutional approval reference number UAAAN-UL/ 037/22-CA-MV-LN.

Location

The experiment was conducted from March 2019 to February 2020, in the Comarca Lagunera (northern Mexico), (Latitude 26° 23'N and Longitude 104° 47' W) under natural photoperiod conditions. The mean annual rainfall of the study area is 230 mm and the temperature is 24° C, with a maximum temperature of 41 °C and a minimum of -1 °C (in summer and winter, respectively). The day length is 13 hours, 44 minutes in the summer solstice and 10 hours, 33 minutes in the winter solstice (INIFAP, 2020).

Stallion management

Twelve Quarter Horse stallions were used, 9-12 years old and a body condition of 5 (scale 1-9) according to the classification proposed by Carroll & Huntington (1988), these animals were housed in stables (4 m x 4 m) with floor and sawdust bedding, having controlled access to a sun area (6 m x 4 m), with a dirt floor. The stallions were fed 1.5 % DM of their live weight based on hay, alfalfa and 1 kg of commercial concentrate with 14 % crude protein and free access to water.

Semen collection

To evaluate the four seasons of the year were considered as spring (March 20-June 21); summer (June 22-September 23); fall (September 24-December 21); winter (December 22-March 19). The stallions were divided into two groups, semen was collected twice to each group on 23-24-25-26 of each month for one year, for semen collection a Colorado model artificial vagina (The 22" CSU Model™ Animal Reproduction Systems, Inc) was used.

Variables evaluated

All variables were determined immediately after each ejaculate.

Ejaculate volume (mL) was evaluated with a 50 mL conical tube graduated in 1 mL measurements. It was filtered before being evaluated to eliminate the gel portion of the ejaculate. To measure total sperm motility, the slides were tempered previously by placing them on a slide heating plate (Animal Reproduction Systems). The same technician throughout the year determined *total sperm motility* (%) subjectively, by placing a drop (20

µl) of pure semen on a slide. It was covered with a cover slip and then observed under a microscope (axiostar plus serien-Nr: 3108027112), with a 10X objective, according to the observed movement, a percentage of total motility of the sample was assigned. *Sperm concentration* (x10^6). It was determined by placing a drop (20 µl) of semen using a SpermaCue spectrophotometer (Minitube, Qro, Mexico), (Rodríguez *et al.*, 2008).

Statistical analysis

All data were analyzed using the StatView 5.0 software program (SAS® OnDemand for Academics Dashboard). Multivariate analysis of variance (ANOVA) was performed. Means for each season of the year were compared using Duncan's post hoc test, with significance values of ($P<0.05$).

RESULTS

The results of the effect of the different seasons on semen production and semen quality in Quarter horses are shown in Table 1. These results show that there is an effect of summer season ($P<0.05$) on total motility (85.6±2.6) and sperm concentration (269.8±51.4) vs. winter (71.5±19.0)-(207.9±80.4).

However, the results of the effect of month on semen quality showed that there was a significant difference in the different months of the year (Figure 1). A higher semen volume ($P<0.05$) was observed during the months of May and June compared to February. Sperm motility was higher in August ($P<0.05$) compared to December and February. On the other hand, sperm concentration was higher in May ($P<0.05$) compared to January.

Table 1. Means and Standard Deviation of the mean of fresh semen parameters of ejaculates collected monthly during spring, summer, fall and winter from Quarter horse stallions (n=12)

Parameters	Spring	Summer	Autumn	Winter
Volume (mL)	72.3±43.2^{a}	70.3±35.6^{a}	60.0±17.4^{a}	53.8±9.8^{a}
Total motility (%)	82.9±5.8^{ab}	85.6±2.6^{a}	79.4±17.9^{ab}	71.5±19.0^{b}
Concentration (10x^6)	265.3±42.1^{ab}	269.8±51.4^{a}	231.7±82.7^{ab}	207.9±80.4^{b}

Unequal superscripts (a, b) indicate statistically significant difference (P≤0.05)

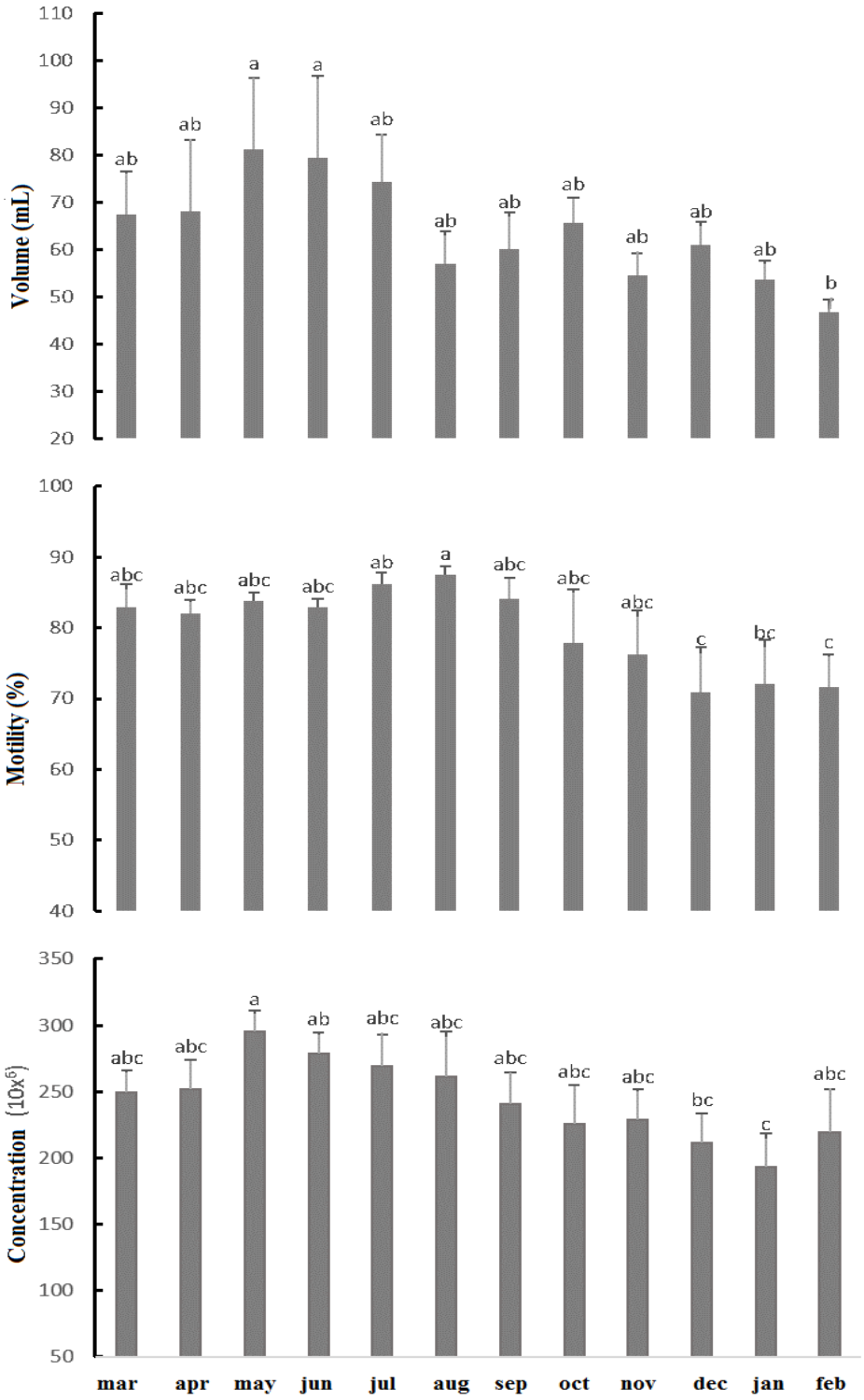

Figure 1. Mean and standard error of the mean of seminal parameters evaluated during one year in. Unequal superscripts (a, b, c) indicate statistically significant difference (P≤0.05)

DISCUSSION

According to the results obtained in this study, there is no marked reproductive seasonality in volume (P>0.05). However, there is a significant difference in total motility (P<0.05) and sperm concentration (P<0.05) in Quarter horse stallions at 26° N Latitude, which at a practical level gives a better understanding of the reproductive behavior of stallions in this zone. The results agree with those reported by Pereira *et al.* (2012) in the study conducted at Latitude 22° 53' 092" S, who found no significant difference in volume, but contrast with the results obtained in studies conducted on Franches Montagnes stallions (Janett *et al.,* 2003a) and Quarter horse stallions (Clay *et al.,* 1987) that show higher volume in spring. On the other hand other researchers reported that the best season of the year was summer in Warmblood stallions (Janett *et al.,* 2003b), Mangalarga Marchador stallions (Waddington *et al.,* 2017), Andalusian stallions (Crespo *et al.,* 2020), Arabian stallions (Kandiel & Khawagah, 2018).

Another important parameter for the evaluation of seminal quality and which, unlike volume, directly influences the fertility of the stallion is the percentage of motile spermatozoa whose ability to move conditions the possibility of successful fertilization. Total motility, was significantly different (P<0.05) between seasons, showing to be better during summer vs. winter, which contrasts with that reported by Deichsel *et al.* (2016) in Shetland Ponies who found no significant difference between seasons. On the other hand, in a study conducted in Lusitano thoroughbred stallions (Clay *et al.,* 1987; Gamboa *et al.,* 2010) agree with the results obtained regarding the months where they reported better motility during May and June. Other studies showed to have better motility during summer (Janett *et al.,* 2003b; *Pereira et al.,* 2012; Dorado *et al.,* 2014; Kandiel & Khawagah, 2018). All these studies and our results contrast with other authors who reported having better motility during autumn (Waddington *et al.,* 2017) as well as the study performed on Warmblood stallions (Janett *et al.,* 2003b; Morrel *et al.,* 2013).

Sperm concentration ($x10^6$) is the number of sperm per milliliter, which is another parameter that directly influences seminal quality by increasing or decreasing stallion fertility. It was observed that the results are in agreement with what has been reported in other studies finding higher sperm concentration during summer (Kandiel & Khawagah, 2018). However, they contrast with other studies that reported that there is no significant difference between seasons (Pereira *et al.*, 2012; Deichsel *et al.,* 2016) similarly our results, contrast with what was reported by (Janett *et al.,* 2003b; Gamboa *et al.,* 2010; Waddington *et al.*, 2017) who reported higher sperm concentration during autumn. Thus, the effect of month on sperm concentration coincides with the results of this study and that reported by Clay *et al.* (1987) who reported higher concentration during the month of May. Díaz (2010), mentions that in equines, the physiological mechanism of reproductive seasonality delays gametogenesis from the autumn end to the spring beginning, which is

why it has been reported that seminal quality is lower during winter (Diekman *et al.,* 2002). However, it is important to consider sire age and management because they can affect seminal quality (Kandiel & Khawagah, 2018).

CONCLUSION

In conclusion, there was a seasonal effect on the production and semen quality of Quarter horse stallions in northern Mexico (latitude 26°). This suggests selecting the equine semen to be used in reproductive biotechnologies, preferably collecting it in the months where there is a higher semen quality.

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Errata Erratum

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