Seminal characteristics of Tropical Milking bulls in two seasons in Veracruz, Mexico

Características seminales de la raza Lechero Tropical evaluadas durante dos temporadas en el estado de Veracruz, México

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Abstract

Seminal characteristics of the Tropical Milking criollo breeds are little known. The objective of this study was to characterize the Tropical Milking bull semen in two seasons, fresh (January) and hot (May) in the Sotavento region of Veracruz, Mexico. Seventy ejaculates from seven bulls of 25.6±1.7 months of age and 319.14±6.24 Kg were used, maintained in pastures of Pará grass (*Brachiaria mutica*) and commercial concentrate of 18% crude protein (4 kg d^{-1} $animal^{-1}$). Five ejaculates were collected by bull in periods of four days each in every season using artificial vagina. The variables ejaculate volume (EV), sperm concentration (SCO), individual motility (IM), live sperm cells (LS), normal sperm cells (NS), mass motility (MM), color (CL), consistency (CS) and scrotal circumference (SC) were evaluated. A mixed linear model with repeated measurements using the MIXED procedure of SAS, and the GLIMMIX procedure were used. The SC was 34.2±0.2 cm and the correlation with ejaculate volume r=0.55 (P≤0.05). There were no differences (P>0.05) between seasons for EV, LS and CS (creamy), with mean and estimated percentage higher than 4.0 mL, 80% and 82% for both periods. The variables SCO (1196.6±77.8 x 10^6 mL^{-1}; P≤0.004), IM (75.7±5.4; P≤0.066), and NS (80.8±1.5; P≤0.014) were higher in the hot season. Likewise, frequencies for MM (quick swirls 65,8%; P≤0.064) and CL (white color 82.9%; P≤0.009) were higher. It is concluded that the hot season was not detrimental to seminal characteristics of the Tropical Milking breed.

Key words: Fertility, bull, criollo bovine, sperm, ejaculate.

Resumen

Las características seminales de la raza criolla Lechero Tropical son poco conocidas. El objetivo del presente estudio fue caracterizar el semen de toros Lechero Tropical en dos épocas del año, fresca (enero) y calurosa (mayo) en la Región Sotavento de Veracruz, México. Se utilizaron 70 eyaculados de siete toros de 25,6±1,7 meses y 319,14±6,24 kg, mantenidos en potreros de pasto Pará (*Brachiaria mutica*) y con suplemento comercial de 18 % de proteína cruda (4 kg d^{-1} $animal^{-1}$). Se recolectaron cinco eyaculados por toro en periodos de 4 días cada uno en cada época del año utilizando vagina artificial. Se evaluaron las variables volumen de eyaculado (VE), concentración espermática (CE), movilidad individual (MI), espermatozoides vivos (EV), espermatozoides normales (EN), movilidad masal (MM), color (CL), consistencia (CS) y circunferencia escrotal (CES). Se utilizó un modelo lineal mixto con mediciones repetidas utilizando los procedimientos MIXED del SAS y GLIMMIX. La circunferencia escrotal fue 34,2±0,2 cm y la correlación con volumen de eyaculado r=0,55 (P≤0,05). No hubo diferencias (P>0,05) entre épocas para VE, EV y CS (cremosa), con media y porcentajes estimados superiores a 4,0 mL, 80 % y 82 %, para ambas épocas. Las variables CE (1196,6±77,8 x 10^6 mL^{-1}; P≤0,004), MI (75,7±5,4; P≤0,066) y EN (80,8±1,5; P≤0,014) fueron mayores en la época calurosa. Igualmente, las frecuencias para MM (remolinos rápidos 65,8%; P≤0,064) y CL (color blanco 82,9%; P≤0,009), fueron superiores. Se concluye que la época calurosa no tuvo efectos detrimentales sobre las características seminales de la raza Lechero Tropical.

Palabras clave: Fertilidad, toro, bovino criollo, espermatozoides, eyaculado.

Recibido: 29/03/16 Aprobado: 11/09/18

INTRODUCTION

The Tropical Milking criollo breed (LT, by its Spanish acronym) comes from cattle populations introduced to America during the Spanish conquest (Rouse, 1977). LT cattle adapted to tropical hot climates and developed characteristics that allowed it to survive and multiply (FAO, 1981; de Alba, 2011). An extensive description of the origin, development and recent research has been published (Becerril-Pérez and Rosendo-Ponce 2015b; Rosendo-Ponce y Becerril-Pérez, 2015). Additionally, female reproductive traits of the LT breed have been described (de Alba and Kennedy, 1994; Rosendo-Ponce and Becerril-Pérez, 2002; Becerril-Pérez and Rosendo-Ponce, 2015a). Moreover, elite LT bulls, with positive genetic evaluations for milk production, are used to produce artificial insemination straws, and also for natural mating. However, the seminal characteristics of LT criollo breed bulls of Mexico, nor their response to seasonal changes are not well known.

In the hot tropics, sperm production can be reduced during the hot season (Fields *et al*., 1979; Rekwo *et al*., 1987). Heat stress, coupled with moisture, prevents the thermoregulatory mechanisms of bulls to maintain a balance that does affect semen quality. Season effects on semen characteristics of *Bos taurus* were found under temperate climatic conditions (Snoj *et al.,* 2013). It has been indicated that environmental temperature and humidity are positively related to sperm abnormalities in *Bos indicus* and *Bos taurus* crosses when temperature is higher than 31 °C (Hansen, 2009; Lozano, 2009). High environmental temperature decreases the fertility of bulls, because it interferes with the oxidative glucose metabolism in sperm cells as a result of mitochondrial dysfunction (Nichi *et al*., 2006). Metabolic alterations occur due to thermal stress and cellular energy reserves exhaustion (Baumgard and Rhoads, 2013; Rhoads *et al*., 2013). However, bulls of breeds adapted to hot climates could have better semen characteristics during the hot season (Farooq *et al.,* 2013).

In view of the above, it is a must to know the semen characteristics of LT bulls under variable and adverse conditions of temperature and relative humidity, and foresee bulls management under such conditions.

As mentioned in previous paragraphs, elite LT bulls are used for artificial insemination and natural mating. However, despite seminal examination is one of the four steps of breeding soundness evaluation to classify them (Barth, 2001; Oliveira-Menegassi *et al.,* 2012), the semen of LT bulls has not been fully characterized. Thus, the aim of this study was to characterize the semen of Tropical Milking bulls in two seasons, fresh and hot.

MATERIALS AND METHODS

Location

The study was conducted in the Veracruz Campus of the Colegio de Postgraduados, located in the community of Tepetates, Municipality of Manlio Fabio Altamirano, Veracruz state, Mexico, 19° 11' N and 96° 20' W, at an altitude of 23 masl. The climate of the region is $Aw_0(w)(i')gw''$, hot humid with summer rains distributed from May to October, average annual temperature and rainfall of 26.4 °C and 1060 mm (García, 1988). The experimental period was from November to May and the semen collection was in January and May; the former is the coolest month the year (fresh season), and later the hottest (hot season). The low, average and maximum values for temperature and average for relative humidity were, 12.5; 17.4; 23.5 oC and 85.1 % for fresh season, and 17.7; 23.0; 30.1 oC and 74.9 %, for hot season.

Data source, animal handling and ejaculates collection

Seventy ejaculates from seven LT bulls of 25.6±1.7 months and 319.1±6.2 kg were used. The bulls were maintained in pastures of Pará grass (*Brachiaria mutica*), and with 4 kg d^{-1} $animal^{-1}$ of commercial concentrate of 18% crude protein, and free access to water. Bulls exercised walking 10 min d^{-1}, and were internally and externally wormed, preputial hair removed, and given an intramuscular injection of a 15 mg kg^{-1} of a metabolic stimulant based on organic phosphorus (Catosal®, Bayer Health Care, Shawnee, KS, USA). In each experimental period five ejaculates were collected in periods of 4 d each. The semen was collected on the second mount attempt of a dummy using an artificial vagina with graduate polycarbonate tube.

Continuous response variables

Ejaculate volume (EV, mL). It was measured directly from the graduate polycarbonate tube of the artificial vagina.

Sperm concentration (SCO, x 10^6 mL^{-1}). Sperm cells were counted in five quadrants (corners and middle) and in the superior and left marginal lines of a Newbauer Chamber. Five µL of sperm were diluted in 101 µL of saline formalin solution in red blood Thoma pipette (Hafez y Hafez, 2002); microscope was used with the 40X objective.

Individual motility (IM, %). Rectilinear progressive sperm motility was observed from a drop of semen diluted in a drop of sodium citrate solution in a 35 °C tempered slide; microscope was used with the 40X objective. IM was subjectively classified as very good 80-100, good 60-79, fair 40-59 and poor under 40%.

Live sperm cells (LS, %). Live and dead sperm cells were counted. A drop of sperm was mixed homogeneously with an eosin-nigrosine staining; the mix was put on the edge of the slide leaving a very fine smear and dried close to a flame. Dead and live sperm appeared pink and white colored; microscope was used with the 40X objective.

Normal sperm cells (NS, %). Normal and abnormal sperm cells were counted in the microscope with the 40X objective. Abnormalities of the head and tail of sperm cells were detected, though no differentiation was made among them.

Hydrogen potential (pH). Test strips were used, in which integers are marked (Hafez y Hafez, 2002).

Discrete response variables

Mass motility (MM). It was classified in four categories; quick swirls, slow swirls, no swirls, and motionless. A drop of semen on the slide tempered at 37° C was placed and observed on microscope with the 10X objective.

Color (CL). It was classified in five categories; white, yellowish, pink, grayish and greenish; the last three colors are considered pathological.

Consistency (CS). It was classified in five categories; creamy, creamy light, milky, opalescent and aqueous. Consistency is directly related to sperm concentration. The most favorable is creamy and the less aqueous.

Scrotal circumference (SC). This covariate was measured with a tape (cm), firmly taking the neck of the scrotum by lowering the testicles, and taken as measure the midpoint of the greater circumference.

Statistical analysis

Ejaculate volume, and the microscopic variables spermatic concentration, motility, and alive and normal sperm cells were analyzed with a linear mixed model with repeated measures:

$$Y_{ijk} = \mu + E_i + M_{j(i)} + S_k + \beta_1(X_{1ijk} - \bar{X}_{1...}) + \beta_2(X_{2ijk} - \bar{X}_{2...}) + \varepsilon_{ijk}$$

Where: Y_{ijk}= observation of the i-th season, j-th sampling period nested in the i-th season, of the k-th bull; μ = constant that characterizes the population; E_i= fixed effect of the i-th season, i = 1, fresh; 2, hot; $M_{j(i)}$ =fixed effect of the j-th sampling period nested in the i-th season, j = 1, 2,.., 5; S_k = random effect of the k-th bull, k = 1, 2,…, 6, 7 $S_k \sim IIN(0,\sigma^2_S)$; β_1 = regresion coefficient of scrotal circumference on the response variable; X_{1ijk}= scrotal circumference associated with the response variable; $\bar{X}_{1...}$ = scrotal circumference mean; β_2= regression coefficient of bull age on the response variable; X_{2ijk}= age associated with the response variable; $\bar{X}_{2...}$ = bull age mean; ε_{ijk} = random error. $\varepsilon_{ijk} \sim IIN(0,\sigma^2)$.

Repeated measures were made to follow the semen production of bulls; sampling units were not many, as it is the case frequently in studies with endangered small criollo populations or local breeds (Palmieri *et al.,* 2004; FAO, 2013; Farooq *et al.,* 2013); the data were processed with the MIXED procedure of SAS (SAS Institute, 2010), using a compound symmetry covariance structure. The arcsine transformation was used for response variables measured as percentages, although no change was observed in effect significances when using the untransformed original variables. Mass motility and the discrete macroscopic variables, color and consistency were analyzed with a generalized linear mixed model, using a multinomial distribution with accumulated logistic link function for mass motility, and a binary probability function with logistic link function for color and consistency; the data were processed using the GLIMMIX procedure. In order to facilitate the interpretation of results, response frequencies were estimated with the FREQ procedure. A mean comparison test was done for some continues variables.

RESULTS AND DISCUSSION

Estimated means of the continuous response variables are presented in Table 1. At the age of 25.6±1.68 months and 319.14±6.24 kg, the LT bulls had a SC of 34.2±0.24 cm; except for SC on ejaculate volume, neither SC nor age were significant (P>0.05) on other response variables. The LT bulls SC was higher than 33.2±0.5 cm of the Venezuelan Criollo Limonero of 30.6±38 months, and under a hot climate with annual temperature and precipitation of 27.4 °C and 920 mm (Madrid-Bury *et al.,* 2011), and the 30.2±0.8 cm of Brown Swiss x Cebu bulls (Madrid-Bury *et al.,* 1997). Crossed bulls (*Bos indicus* 75% x *Bos taurus* 25%) under 30 months of age had SC of 34.2 cm in winter and 36.1 cm in summer (Prieto *et al.,* 2007). SC of LT bulls was consistent with established specifications for different genotypes (Irons *et al.*, 2007). EV was not different between seasons; however, SC affected EV $\hat{\beta}$ 0.37±0.17 (P≤0.03). Despite differences in mean temperature and relative humidity of the two seasons, bulls produced a similar EV of 4.04±0.4 mL. However, individual variation among bulls was also manifested by the minimum and maximum values observed (1.5 - 8.0). In the subtropical weather of Pakistan, Holstein bulls had overall superior EV than Jersey bulls with 4.05±0.03 and 2.92±0.03 mL, though the summer season deteriorated semen quality in both breeds (Fiaz *et al*., 2010); opposite results were observed in adapted Pakistan Cholistani bulls, that had EV superior to 5 mL in summer (Farooq *et al.,* 2013). Besides, in Venezuela under a hot climate, and two season variables in temperature (dry 30.3 °C; rainy 26.8 °C) and relative humidity, (dry 62.8%; rainy 85.5%), Holstein and Brown Swizz bulls had EV of 3.6±1.2 mL in the dry season and 4.8±2.1 mL in the rainy season (Valle *et al.,* 2005). The LT bulls, unlike the Europeans not adapted to hot climates, had similar EV in both seasons, fresh and hot. Similar results were obtained with three year old Sahiwal bulls in four seasons in Pakistan (Ahmad *et al.*, 2003). Well managed and fed bulls of very well adapted breeds can have stable EV through seasons. The estimated correlation between EV and SC was r=0.55 (P≤0.05), higher than r=0,40 estimated in Brown Swiss x Cebu bulls (Madrid-Bury *et al.,* 1997). SCO was different between seasons, the mean estimated over 1100 x 10^6 mL^{-1} for the hot season can be considered satisfactory, though the mean estimated for the fresh season was lower than 800 x 10^6 mL^{-1}. The SCO interval was 170 to 2180 x 10^6 mL^{-1}, with 25% of samples ≥ 1250 x 10^6 mL^{-1}. In the hot season IM was of category good and close to 77%, in the fresh it remained in the same category, but it was lower (P≤0.066). The SCO is an indicator of the LT bulls capability to produce good enough ejaculates for sperm dilution. Costeño con Cuernos and Romosinuano bulls of 37 months had between them similar SCO of 1.009±0.6 x 10^9 mL^{-1} (Palmieri *et al.,* 2004), though less than 1196.6±77.8 x 10^6 mL^{-1} in the hot season and greater than 776.0±77.8 x 10^6 mL^{-1} in the fresh season for LT bulls; note that the Colombian Romosinuano is renowned by its reproductive performance. On the other hand,

Table 1. Semen characteristics of Tropical Milking bulls in two seasons.

Variables	Season	
	Fresh (January)	Hot (May)
Ejaculate volume (mL)	4.0±0.4[a]	4.1±0.4[a]
Sperm concentration (x 10^6 mL^{-1})	776.0±77.8[a]	1196.6±77.8[b]
Motility (%)	68.3±54[a]	75.7±5.4[b]
Live sperm cells (%)	80.4±3.1[a]	83.4±3.1[a]
Normal sperm cells (%)	76.2±1.5[a]	80.8±1.5[b]

a, b: Different letters in row indicate statistical difference (P≤0.05).
(least square mean ± standard error).

in Holstein x Sahiwal bulls SCO was less than 900 x 10^6 mL^{-1} (Andrabi *et al*., 2002). For SCO, evidence shows that criollo bulls had a better performance than European or crossbreed bulls under tropical conditions.

Of the total number of ejaculations, 50% had IM ≥75%. The minimum and maximum IM were 40 and 98%. High IM is related to sperm cell capacity to travel, but can be affected negatively by high environmental temperatures that cause damage to testicular tissue and seminiferous tubules (Lozano, 2009). IM over 70% is considered good according to sperm quality standards (Spitzer *et al*., 1998). IM of LT bulls was over 75% in the hot season, and above of some criollo adapted breeds as Pampa Chaqueño from Paraguay with maximums of 69.1% and 71.5% in summer and winter (Oka *et al*., 2012), Costeño con Cuernos and Romosinuano with 67.0±8.0 and 68.0±8.0% (Palmieri *et al.,* 2004), and *Bos indicus* Sahiwal 65.3±0.4 and Cholistani 63.51±1.03%, year round, the latter performing better during the dry summer (Ahmad *et al*., 2003; Farooq *et al.,* 2013). However, Holstein and Jersey bulls had IM of 71.0±0.7 and 73.5±0.8% during summer (Fiaz *et al*., 2010).

LS interval was 40 to 99%, with 25% of the samples ≥ 90%. LS percentage was over 80 in both seasons, and also indicates the adaptability of LT bulls to the tropical environment, as happened with the Cholistani in all seasons (Farooq *et al.,* 2013), compared with 72.3±22.0% of European bulls (Valle *et al*., 2005). LS were similar in both seasons and higher than 80%.

NS were over 75% in both, fresh and hot seasons. A high NS percentage, as happened to LT bulls, over 80% in summer is related positively with fertility (Spitzer *et al*., 1998; Irons *et al*., 2007), NS could be affected also by high temperatures that produce an increase in testicular temperature and abnormal sperm cells (Nichi *et al*., 2006); sperm cell abnormalities are shown two weeks after an increase in testicular temperature (Hansen, 2009). Crossbreed bulls at thirty months of age had NS of 70.9±14.7%, almost 10% less than LT bulls in summer (Prieto *et al*., 2007). The Cholistani produced also over 80% NS in summer, but reached 90% in winter (Farooq *et al.,* 2013). Abnormal sperm for LT bulls were lower than 30%, threshold that does not affect fertility (SFT, 1976).

No variability was observed for pH, with value of 7 on all measures in both seasons; pH values between 6.2 and 7.4 are appropriate for breeding bulls (Irons et al., 2007). Similarly, Valle *et al.* (2005) and Ahmad *et al.* (2003), showed low variability for pH, in European and Indi bulls (6.7±0.5 and 6.9±0.01).

Frequencies for discrete response variables are shown in Table 2. Out of the four categories considered for MM, only three were observed, motionless semen was not. The quick and slow swirls together were at least 80% in both seasons, although a highest percentage of quick swirl (over 65%) was observed during the hot season, meaning four times higher than that categories slow swirls and no swirls, and slightly higher than 61.3% for crossbreed bulls in Colombia (Prieto *et al.,* 2007). MM in LT bulls was not affected by higher temperatures of the summer. Also, out of the five CL categories, only the two best were observed, with no presence of colors associated to low quality semen. White color was the most frequent in both seasons, though the difference with respect to the yellowish was four times largest in the hot season. In this study EV was higher for white color ejaculates rather than yellowish, 4.23±0.2 *vs* 3.53±0.2 mL ($P \leq 0.05$). Palmieri *et al.* (2004) in the Costeño Con Cuernos and Romosinuano breeds defined three white color categories matt, milky and clear, the first had the highest SCO with 1600 x 10^6 mL^{-1}. Crossbreed bulls had 95.3% of ejaculates yellowish to yellow (Ruíz *et al*., 2010). The most frequent white semen color of LT bulls is related to best semen quality (Irons *et al.,* 2007). The same frequency pattern was observed for CS, with no presence of three categories associated to low quality semen; the creamy and creamy light categories are the best, but SCO was higher in the former ($P \leq 0.05$), with 1048.3±56.3 *vs* 653.6±118.2 x 10^6 mL^{-1}, respectively. Only the two better CS categories were observed in this study, showing the same frequency pattern in both seasons; the creamy CS was over 82%, associated to good quality semen (Irons *et al*., 2007). Crossbreed bulls had 94.4% of ejaculates of creamy to creamy light (Ruíz *et al*., 2010).

Estimated means of the semen characteristics per mass motility (MM) category are presented in Table 3. Clearly, the quick swirl category showed superior response in all characteristics, which surpassed thresholds of 4,0 mL and 1000

Table 2. Frequencies of the seminal characteristics of Tropical Milking bulls in two seasons.

Variables		Season	
		Fresh (January)	Hot (May)
Mass motility (%)	Quick	40.0^{a}	65.8^{b}
	Slow	40.0^{a}	17.1^{b}
	No	20.0^{a}	17.1^{b}
Color (%)	White	60.0^{a}	82.9^{b}
	Yellowish	40.0^{a}	17.1^{b}
Consistency (%)	Creamy	85.7^{a}	82.9^{a}
	Creamy light	14.3^{a}	17.1^{a}

a, b: Different letters in row indicate statistical difference (P≤0.05).

Table 3. Semen characteristics of Tropical Milking bulls grouped by mass motility.

Variables	Mass motility (swirls)		
	Quick	Slow	No
Ejaculate volume (mL)	4.4±0.2^{a}	3.5±0.2^{b}	3.9±0.3a^{b}
Sperm concentration (x 10^6 mL^{-1})	1166.8±60.7^{a}	773.0±77.2^{b}	862.3±159.2^{b}
Motility	84.4±2.0^{a}	65.2±2.6^{b}	47.1±3.0^{c}
Live sperm cells (%)	86.7±1.1^{a}	82.8±2.0^{a}	66.9±4.6^{b}
Normal sperm cells (%)	82.2±0.8^{a}	77.2±1.0^{b}	70.0±1.8^{c}

a, b, c: Different letters in row indicate statistical difference (P≤0.05).
(least square mean ± standard error).

x 10^6 mL^{-1} for EV and SCO, moreover of 80% for IM, LS and NS. Mass motility categories are also indicators of semen quality. Results show that MM is a very good characteristic for easy and fast first appraisal of semen quality. Means estimated in the quick swirl class indicate very good breeding soundness for LT bulls (Spitzer *et al.*, 1998; Irons *et al.,* 2007).

CONCLUSIONS

No detrimental effects on semen of the Tropical Milking bull were observed during the hot season, with higher mean temperature and humidity; in contrast, it was observed a better performance for some semen characteristics. Evidence show that semen characteristics of the Tropical Milking bulls young were good enough for ejaculates to be used for the making of artificial insemination straws, and for considering natural mating in fresh and hot seasons. However, the prior individual complete assessment of each bull is required to meet their reproductive soundness potential. Further research seems warranted in the Tropical Milking criollo breed for the study of reproductive performance traits bulls.

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