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REPRODUCTIVE FUNCTIONALITY OF BULLS FROM THREE CREOLE BREEDS BY SEMINAL EVALUATION OF FRESH, FROZEN, AND POST-THAW SEMEN

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ABSTRACT

Creole cattle are an important zoogenetic resource that has been historically overlooked and marginalized. Consequently, their conservation is vital for preserving genetic biodiversity. The objective of this study was to evaluate the reproductive functionality of bulls from three Creole breeds through the seminal analysis of fresh, frozen, and post-thaw semen. Semen was collected using an artificial vagina from 60 bulls belonging to the Rodeo Creole, Longhorn, and Senepol breeds. The evaluated characteristics in fresh semen included body weight (BW), scrotal circumference (SCi), ejaculate volume (EV), sperm concentration (SCo), mass sperm motility (MSM), individual sperm motility (ISM), pH, viability, and total abnormal sperm (TA). In addition, semen straws were frozen, and the post-thaw individual sperm motility (PISM) was evaluated. Senepol bulls exhibited higher BW, SCi, and PISM, as well as lower TA (567.3 kg, 35.95 cm, 64.90% and 8.8%, respectively) compared to Rodeo Creole and Longhorn bulls. SCo and sperm viability were similar between Senepol and Longhorn bulls (P>0.05). No significant differences were found among the three Creole breeds for EV and MSM. BW and pH were significantly correlated (P<0.05) with sperm viability and TA percentage; SCi was correlated with EV; MSM with ISM, viability, pH and TA; and ISM was correlated with viability, TA and PISM. Additionally, sperm viability was correlated with both TA and PISM, and TA was also correlated with PISM. In conclusion, the quality parameters of fresh semen in these Creole bulls fall within the established reference values for the bovine species. Therefore, their semen can be cryopreserved for conservation and genetic dissemination purposes.

Keywords: Bulls, semen cryopreservation, andrological evaluation, animal genetic resources.

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INTRODUCTION

The first cattle to arrive in Mexico were introduced approximately 500 years ago to different ecological areas by Spanish conquerors (Villalobos et al., 2009; De Alba, 2011). Commonly referred as "Creole" cattle, they are descendants of animals originally brought from Spain. Creole cattle have been maintained in small populations and subjected to natural selection (De Alba, 2011; Vilaboa-Arroniz et al., 2012a). As a result, they have adapted to the adverse environmental conditions of the regions in which they are raised, developing tolerance to forage seasonality, longevity, and resistance to diseases (De Alba, 2011; Florio et al., 2011) and ectoparasites (González-Cerón et al., 2009), all desirable traits that could enhance their use in genetic improvement programs. Unfortunately, most Creole cattle populations in Mexico are at risk of extinction, mainly because these breeds are maintained in small herds (≤20 animals), managed under extensive grazing within family-based production systems (De Alba, 2011; Florio et al., 2011; Vilaboa-Arroniz et al., 2013). In addition, Creole animals are gradually being replaced by improved commercial breeds specialized in meat or milk production, which is further exacerbated by the lack of information and limited dissemination of Creole breeds (Vilaboa-Arroniz et al., 2012b; Vilaboa-Arroniz et al., 2013).

Examples of these cattle are the Rodeo Creole, Longhorn, and Senepol breeds. Although Senepol was not introduced to Mexico by Spaniards, it is considered as a *Bos taurus* Creole breed due to its development in Santa Cruz Island (US Virgin Islands), where, at the beginning of the 1900s, N'Dama cattle (breed originating from Fouta-Djallon, Guinea, in West Africa) were crossed with Red Poll cattle (breed developed in England at the beginning of the 19th century), selecting them for heat tolerance of the tropics and their ability to graze one the rough forages typical of those latitudes (Godfrey and Dodson, 2005).

The conservation of animal genetic resources, aimed at preserving biodiversity and genetic diversity in harmony with the agro-ecological conditions and management systems of specific regions, holds economic, scientific, and social importance (Vejarano et al., 2005; Florio et al., 2011). Hence, it is essential to generate data and establish germplasm banks of the Creole cattle to support sustainable conservation programs (Vejarano et al., 2005). However, the breeding soundness of Creole bulls has been poorly characterized. In this sense, andrological studies are of great importance to assess the reproductive status of bulls that may serve as germplasm disseminators of Creole breeds, being used in artificial insemination (AI) programs. The objective of this study was to evaluate the reproductive functionality of bulls from three Creole breeds in Mexico through the seminal analysis of fresh, frozen, and post-thaw semen.

MATERIALS AND METHODS

Geographical location of the production units

The study was carried out in three production units located in Mexico: two in the state of Tabasco (Villahermosa and Emiliano Zapata), and one in the state of Jalisco (Autlán de Navarro). Specific information regarding the geographic location of each production unit and its climatic characteristics is presented in Table 1.

Characteristics of experimental animals

A total of sixty bulls were evaluated, including Rodeo Creole (n = 20), Longhorn (n = 20), and Senepol (n = 20) breeds. The mean age, body weight, and body condition score (BCS; on a scale of 1 to 9, as described by Richards et al., 1989) were 3.72±1.37 years, 519.33±57.38 kg, and 4.5±0.5, respectively. One month prior to semen

Table 1. Locality, geographic location, climate, average temperature, and annual rainfall by production unit.

Locality	Geographic location	Climate	Temperature (°C)	Rainfall (mm)
1) Villahermosa	17°59'13" NL y 92°55'10" WL, 9 masl	Hot humid	26.0	2,250
2) Emiliano Zapata	17°43'59" NL y 91°40'01" WL, 6 masl	Hot humid	27.0	2,250
3) Autlán de Navarro	19°46'00" NL 104°22'00" WL, 878 masl	Semi-dry and semi-warm	23.5	997.5

Source: INEGI (2025).

collection, all bulls were separated from cows, and dewormed internally (Levamisole hydrochloride 15%, Levamisol®, Lab. Genfar, dose 1 mL/20 kg of weight, i.m. via) and externally (Flumetrina, Bayticol® Pour-on 1%, Lab. Elanco, dose 10 mL/100 kg body weight, topical route). In addition, the bulls received intramuscular supplementation with vitamins A, D, E (Vigantol® ADE, Lab. Elanco, dose 5 ml, i.m. via). Vaccination protocols included immunization against rabies (Nobivac® Rabia, Lab. Intervet, dose 2 ml, i.m. via), anthrax (anti-carbonate vaccine, Lab. MSD, 1 mL dose, s.c. via), and blackleg (triple CES® bacterium, Lab. MSD, 5 ml dose, i.m. via). All bulls were identified and subjected to a thorough visual and physical examination to rule out pathologies or physical defects.

Study variables and processing

Body weight (BW)

The BW of each bull was determined using a mechanical scale (REVUELTA®, Model RGI-15C-DVZ, Torreón, Coahuila, Mexico), prior to semen collection.

Scrotal circumference (SCi)

SCi was measured following the methodology provided by the American Society of Veterinary Theriogenology (ASVT) for the Study of Reproductive Evaluation. Measurements were taken using a scrotal-testicular tape (Implegan® Model Coulter, Colombia).

Semen collection

Prior to semen collection, the bulls underwent external cleaning with water and neutral soap, followed by an internal preputial wash using a gentamicin-tylosin solution in 0.9% NaCl. Foreskin hairs were trimmed, and a transrectal massage of the accessory glands was carried out for 1 minute. Semen was collected once from all bulls using an artificial vagina (IMV Technologies® France), with a cow in estrus used as a teaser. Seminal samples that exhibited abnormal coloration (yellowish, reddish or brown) or an unusual odor, indicating possible contamination with urine or the presence of a genitourinary secretions, were discarded. Immediately after collection, semen samples were transported to the laboratory in a water bath at 37°C, where macroscopic and microscopic evaluations were performed.

Ejaculate volume (EV)

EV was measured in milliliters (mL) by direct observation of the graduated collection tube used during semen collection.

Semen pH

Semen pH was evaluated by placing 5µL of ejaculate onto a pH test strip (Macherey-Nagel® pH-Fix 0-14, Germany), followed by immediate visual reading.

Mass sperm motility (MSM)

To evaluate MSM, 10µL of the collected semen was placed on a slide at 37°C. MSM was determined subjectively under an optical microscope at 100X magnification % (Primo Star®, Carl Zeiss, Germany), assigning a value from 0 to 100% based on the collective movement of the sperm population, as described by Crespo and Quintero-Moreno (2014).

Individual sperm motility (ISM)

ISM was evaluated subjectively on a scale from 0 to100%. A 5µL aliquot of semen was placed on a pre-warned slide (37°C), and the percentage of cells exhibiting progressive, rectilinear movement was assessed. A total of 100 sperm cells were observed under four microscopic fields at 400X magnification (Crespo and Quintero-Moreno, 2014).

Sperm concentration (SCo)

SCo was calculated using a Neubaüer cell counting chamber (Boeco®, Germany), performing a semen dilution at a ratio of 1:200. To prepare the dilution, 30 µL of semen was mixed with 6 mL of a previously prepared spermicidal solution composed of 9 g of sodium chloride and 3 mL of formalin in 1,000 mL of distilled water. Once the dilution was made, the mixture was allowed to stand for 3 minutes to ensure proper staining of the sperm cells. Subsequently, 10µL of the diluted semen was placed in Neubaüer chamber, and sperm cells were counted using a 400X light microscope (Crespo and Quintero-Moreno, 2014). The sperm heads were counted in five tables (corner and central tables), applying the formula: Sperm / mL = (counted sperm / counted area (mm^2) × chamber depth (mm) × dilution) × 1,000.

Viability and total sperm abnormalities (TA)

To estimate viability and TA, 10µL of fresh semen was placed on a slide at 37°C and mixed with an equal amount of Eosin-Nigrosine dye. The mixture was homogenized, and a smear was prepared, which was left to dry for 30 min. The stained sample was observed under a microscope at 1000X magnification. A total of 100 spermatozoa were counted and classified as either live (white sperm) or dead sperm (red sperm). Subsequently, from the same smear, the number of spermatozoa exhibiting abnormalities

(primary and secondary) was also determined (Crespo and Quintero-Moreno, 2014).

Processing, dilution, and freezing of semen

To estimate the number of viable sperms in the ejaculate, the formula: Viable sperm = SCo × EV × ISM × normal cells was used. Then, the number of potential doses was calculated, considering a concentration of 30 × 10^6 sperm/straw; the formula used for this calculation was: Number of doses = Viability / Number of cells per dose. The amount of diluent was calculated as the number of doses × straw volume - EV. The semen was diluted at a 1:1 ratio, using a commercial Optidyl® diluent (Cryo-Vet, France) and heated in a water bath at 37°C. Subsequently, the semen was packed into 0.5 mL straws (Cassou IMV® Technologies straws, France), sealing them with polyvinyl alcohol and stored in a refrigerator at 5°C for 5 h. The freezing of the semen already in straws was carried out with nitrogen vapors for 10 min until reaching a temperature of -140°C (Chenoweth et al., 1993; Barszcz et al., 2012), which was monitored and controlled using a digital thermometer (Multilogger Thermometer, Model HH506RA, Omega Engineering, Nuevo León, Mexico). After this period, the straws were placed in goblets and mounted on sticks, before being placed into liquid nitrogen at -196 °C for storage.

Post-thaw individual sperm motility (PISM)

Twenty-four hours after the semen was cryopreserved, 10 straws per bull of each breed were randomly selected for evaluation. Straws were thawed at 37 °C for 30 seconds. Subsequently, the semen was extracted, and ISM was assessed using the same methodology described for ISM of the fresh semen.

Statistical analysis

Breed differences for BW, SCi, EV, SCo, MSM, ISM, pH, viability, TA and PISM were evaluated by the PROC GLM procedure. Means comparisons were performed with Tukey's test ($P<0.05$).

The statistical model describing the dependent variables was:

$$y = \mu + Bi + e_{ij}$$

Where y is a response variable; μ is the overall mean; Bi is the ith breed effect (Rodeo Creole, Longhorn, Senepol); e_{ij} is the residual term, NID $(0, \sigma^2)$.

The partial correlations were estimated using the MANOVA option of the GLM procedure. All statistical analyses were performed with SAS software, version 9.4 (2010, SAS Institute Inc., Cary, NC, USA).

RESULTS

Means and standard deviations by breed for the bull breeding soundness variables are shown in Table 2. Differences ($P<0.05$) were found between breeds for most variables, except for EV and semen pH. Senepol bulls exhibited higher BW, Sci, and PISM, as well as lower percentage of TA (567.3 kg, 35.95 cm, 64.9% and 8.8%, respectively), compared to Rodeo Creole (502.6 kg, 34.19 cm, 33.4% and 12.9%) and Longhorn bulls (488.0 kg, 33.30 cm, 48.5% and 10.2%). SCo, ISM and sperm viability were significantly lower ($P<0.05$) in Rodeo Creole bulls compared

Table 2. Means and standard deviations by breed for body weight, scrotal circumference and semen characteristics of bulls from three Creole breeds.

	Breed		
Variable	**Rodeo Creole**	**Longhorn**	**Senepol**
Body weight (kg)	502.6±57.1a	488.0±47.2a	567.3±31.2b
Scrotal circumference (cm)	34.10±1.21a	33.30±1.42a	35.95±1.15b
Ejaculate volume (mL)	10.80±1.36a	10.20±1.47a	10.50±1.10a
Sperm concentration (×10^6/mL)	702±192a	815±107b	811±160b
Mass sperm motility (%)	92.35±2.43a	93.10±2.92a	93.40±2.14a
Individual sperm motility (%)	86.15±3.59a	88.75±3.19b	89.75±1.12b
Semen pH	6.40±0.16a	6.43±0.17a	6.49±0.19a
Sperm viability (%)	85.2±2.5a	87.5±2.7b	88.6±1.8b
Abnormal sperms (%)	12.9±3.59a	10.2±3.19b	8.8±1.12c
Post-thaw individual sperm motility (%)	33.4±2.69a	48.5±2.40b	64.9±1.72c

[a,b,c] Different letters between rows indicate significant differences ($P<0.05$).

to Longhorn and Senepol bulls, which showed similar values. Regarding PISM, 50% (100/200), 85% (170/200), and 100% (200/200) of the Rodeo Creole, Longhorn and Senepol bulls, respectively, had PISM ≥30%. The difference between ISM of fresh semen and post-thaw semen (PISM) was notably greater in Rodeo Creole bulls (52.74%) compared to Longhorn (40.30) and Senepol bulls (24.85%).

Simple correlations between BW, SCi, and seminal characteristics are shown in Table 3. Significant positive correlations were found between BW and both SCi and viability, and between SCi and EV. However, BW was negatively correlated with TA. Among the semen quality traits, MSM had a positive correlation with ISM, viability, and pH, while it was negatively correlated with TA. Additionally, ISM was positively correlated with both viability and PISM, but negatively correlated with TA. Viability itself was negatively correlated with TA and positively correlated with PISM. TA showed a significant negative correlation with PISM.

DISCUSSION

Body weight

The higher BW observed in Senepol bulls compared to the Longhorn and Rodeo Creole bulls could be attributed to breed-specific characteristics, as well as environmental and management differences between farms that could influence animal size, growth, and physical conformation (Russell et al., 2000; Espinoza-Villavicencio et al., 2009; Contreras et al., 2011). The BW values reported in the present study are higher than those documented for other local breeds in Latin America, such as Encerado Creole cattle in Ecuador (354 kg; Aguirre et al., 2012), Limonero in Venezuela (441 kg; Crespo and Quintero-Moreno, 2014), and Chinampo (347 kg; Espinoza-Villavicencio et al., 2009) and Criollo Lechero Tropical in Mexico (319 kg; Villatoro-Salinas et al., 2016). These differences could be attributed to the aforementioned factors, as well as variation on breed purity and age of the animals at the time of weighing. In this sense, it is well established that Creole cattle tend to have medium to small adult body size.

Scrotal circumference

SCi is an important trait, since it is a predictor of seminal characteristics and fertility in bulls. In this study, a positive and significant correlation was found between SCi and both EV and SCo (Table 3), highlighting the influence of testicular size and volume on the physical characteristics of the ejaculate (Delgado et al., 2000; Martínez et al., 2000; Crespo and Quintero-Moreno, 2014). Senepol bulls exhibited the largest SCi (35.95 cm), followed by Rodeo Creole and Longhorn bulls (34.10 and 33.30 cm, respectively). This difference may be attributed to the higher BW of Senepol bulls (Table 2). However, no significant differences in EV were found between breeds. SCo differed only between Rodeo Creole bulls and Senepol and Longhorn bulls. Despite the observed differences in SCi among breeds, the SCi values obtained are considered acceptable according to the standards established by the ASVT, which recommends a minimum SCi ≥30 cm for a bull to be classified as a mature animal, regardless of breed (Hopkins and Spitzer, 1997). All breeds evaluated in this study recorded SCi values ≥30 cm (Table 2), which are higher than those reported by Quezada-Casasola et al. (2016),

Table 3. Partial correlations between body weight, scrotal circumference, and semen characteristics of Creole bulls in Mexico.

Variables	SCi	EV	SCo	MSM	ISM	VI	pH	TA	PISM
BW	0.354**	0.029	0.021	0.045	0.127	0.263*	0.010	-0.258*	0.144
SCi		0.270*	0.082	-0.077	-0.181	-0.089	-0.179	0.073	0.036
EV			0.029	0.042	-0.102	-0.174	-0.180	0.151	-0.061
SCo				0.129	0.036	0.218	0.239	-0.178	0.030
MSM					0.589**	0.566**	0.259*	-0.593**	-0.018
ISM						0.976**	0.136	-0.969**	0.302*
VI							0.233	-0.951**	0.293*
pH								-0.209	-0.016
A									-0.327*

BW= Body weight; SCi= Scrotal circumference; EV= Ejaculate volume; SCo= Sperm concentration; MSM= Masal motility; ISM= Individual sperm motility; VI= Sperm viability; TA= Total sperm abnormalities; PISM= Post-thaw individual sperm motility; *(P<0.05), **(P<0.01).

who found an average of 27.7 cm in Rodeo Creole bulls compared to European breeds in Chihuahua, Mexico, across different seasons. Conversely, similar SCi values to those observed in our study have been reported in other Creole breeds, such as the Colombian Creole Costeño con Cuernos (CCC, 34 cm) and Romosinuano (33.0 cm) in Colombia (Palmieri et al., 2004). Differences or similarities in SCi among Creole breeds could be explained by environmental conditions, available food, BW, breed characteristics, and age-related variation within breeds.

Ejaculated volume

The absence of significant differences in EV among the breeds evaluated in this study aligns with the findings of Quezada-Casasola et al. (2016), who reported similar EV values ranging from 3.2 to 3.5 mL for Rodeo Creole and European bulls, but lower than the means observed in the present study (10.2 to 10.8 mL). In other breeds such as Encerado of Ecuador, CCC and Romosinuano, mean EV values ranged from 3.3 to 4.67 mL/ejaculate (Palmieri et al., 2004; Aguirre et al., 2012; Villatoro-Salinas et al., 2016), also lower than those reported here. These differences could be attributed to breed, management practices, and the semen collection method used (artificial vagina or electro ejaculator). EV varies both within and between individuals due to multiple factors, such as breed group, age, season, collection method and frequency, quality of feeding, and the duration of sexual arousal prior to ejaculation (Kommisrud and Andersen, 1996; Páez-Barón and Corredor-Camargo, 2014; Crespo and Quintero-Moreno, 2014). In addition, unlike SCo and motility, EV has been shown to be influenced by the technician performing the seminal collection, and the type of ejaculatory device used (Mathevon et al., 1998). It should be noted that, in this study, the seminal collection conditions were standardized and carried out by the same technician. Lastly, the EV values observed here fall within the range of values reported in the literature for bulls, which spans from 2 to 12 ml per ejaculate (Barszcz et al., 2012; Páez-Barón and Corredor-Camargo, 2014).

Sperm concentration

The mean SCo of Rodeo Creole bulls was lower ($P<0.05$) than that of Longhorn and Senepol bulls (Table 2). SCo is an important parameter in semen evaluation, since several authors have reported a high correlation between sperm count and bull fertility (Hidalgo-Ordoñez et al., 2005; Páez-Barón and Corredor-Camargo, 2014). The mean SCo observed in the present study (702 to 815 × 10^6/mL) was lower than those found by Quezada-Casasola et al. (2016) in Rodeo Creole bulls; and by Palmieri et al. (2004) in CCC and Romosinuano bulls, with values ranging from 945 to 1440 x10^6/mL. However, the values obtained in the present study agree with those reported by Aguirre et al. (2012) for Encerado Creole (745 × 10^6/mL) and Crespo and Quintero-Moreno (2014) for Limonero bulls (839.5 × 10^6/mL). These latter authors noted that SCo does not negatively affect bull fertility as long as it remains above the lower threshold established for the species and a high percentage of morphologically normal sperm is present.

Mass sperm motility

MSM is a subjective estimate of overall sperm motility, based on wave vigor, and is associated with ISM, viability, semen pH and TA (Table 3). In the present study, no significant differences in MSM were observed among the breeds studied ($P>0.05$). According to Quintero-Moreno et al. (2017), MSM values above 60% are considered good or satisfactory. In the resent study, the overall MSM mean was 92.92±2.49%.

Individual sperm motility

ISM is an important characteristic of semen due to its positive correlation with the progressive rectilinear movement of the sperm and fertility (Christensen et al., 2004; Quintero-Moreno et al., 2017). The ISM found in the present study was significantly higher in Senepol and Longhorn compared to Rodeo Creole bulls. Furthermore, all three Creole breeds evaluated here demonstrated higher ISM values than those reported in previous studies for similar breeds: Rodeo Creole bulls (83%; Quezada-Casasola et al. (2016), Encerado Creole bulls (82.7%; Aguirre et al., 2012), CCC and Romosinuano cattle (66 and 67%, respectively; Palmieri et al., 2004). The ISM percentages reported in the current study indicate that the Creole bulls possess good sperm quality fertility (Palmieri et al., 2004), as ISM is frequently associated with fertility outcomes. Crespo and Quintero-Moreno (2014), in their study on Creole Limonero (70.5%), noted that variation in motility values may be influenced the subjective nature of semen evaluations, as well as potential differences in evaluation criteria between technicians.

pH

pH is an indicator of changes in semen quality due to genitourinary tract disease. In this study, no differences in semen pH were found between the bulls of the three breeds evaluated ($P>0.05$). However, the values obtained are within the range of pH values (6.2 to 7.0) reported in the literature (Barszcz et al., 2012; Cabrera and Pantoja, 2012).

Sperm viability

Senepol and Longhorn bulls had better sperm viability than Rodeo Creole bulls (85.2%). However, the sperm viabilities found in this study are acceptable. Sperm viability is of great importance for cryopreserve semen, since there is a rapid and marked decrease in the number of live sperm after ejaculation (Crespo and Quintero-Moreno, 2014; Páez-Barón and Corredor-Camargo, 2014). Similar sperm viability values to those found in the present study have been reported by Argudo et al. (2019) in Ecuadorian Highland Creole cattle, collected with both artificial vagina and electroejaculator (86.5 and 87.4%, respectively). However, lower values (81.9 and 72.77%) were reported for Criollo Lechero Tropical (Villatoro-Salinas et al., 2016) and Criollo Limonero (Crespo and Quintero-Moreno, 2014). In addition, Quezada-Casasola et al. (2016) found a higher sperm viability (93.0%) in Rodeo Creole bulls. Despite the similarities and differences between Creole breeds, all authors agree that the values found are satisfactory for use in AI protocols. Sperm viability is directly linked to bull fertility, as well as ISM, and thus these characteristics could help determine semen quality, especially when cryopreserving semen for AI.

Total abnormal sperms

Senepol had the lowest percentage of TA compared to Longhorn and Rodeo Creole bulls ($P<0.05$). The presence of significant defects in the sperm suggest altered spermatogenesis (Koivisto et al., 2009). The TA found by Quezada-Casasola et al. (2016) in Rodeo Creole is higher (20.2%) than that of the bulls in this study. Other authors in Latin America report values of abnormalities for Creole bulls in the range of 11.7 to 21.5% (Palmieri et al., 2004; Aguirre et al., 2012; Crespo and Quintero-Moreno, 2014; Villatoro-Salinas et al., 2016; Argudo et al., 2019). Several authors point out that a good quality fresh semen must have ≤30% TA (Páez-Barón and Corredor-Camargo, 2014; Crespo and Quintero-Moreno, 2014).

Post-thaw individual sperm motility

PISM was lower for Rodeo Creole bulls than for Longhorn and Senepol bulls. ISM is a key indicator of sperm activity after thawing, and it is critical for its use in biotechnologies such as AI, oocyte maturation and embryo transfer (OMTE) and in vitro fertilization (IVF) (Hafez and Hafez, 2000; Beran et al., 2012). A significant reduction in PISM, similar to that observed in Rodeo Creole and Longhorn bulls, has been reported by Teixeira et al. (2011) in Creole Curraleiro bulls, where the ISM of fresh semen was 84.5% and the PISM was 33.1%, attributing this decrease to the cryopreservation process. Aguirre et al. (2012) found an ISM of 82.7% and PISM of 43.0%. while Rubio-Guillén et al. (2009) reported an ISM of 62.91% and PISM of 40.0%. In all cases, the researchers considered semen with an PISM ≥30% to be viable, whereas, in the present study, only 50% of the Rodeo Creole bulls and 85% of the Longhorn had PISM ≥30%. Unfortunately, there is little information available on semen characteristics of Creole breeds, particularly regarding physiological and reproductive traits (Nichi et al., 2006; Teixeira et al., 2011). Therefore, more studies are required to expand the inventory and dissemination of Creole germplasm, particularly for the breeds evaluated in this study. In general, good ISM of fresh semen is not a guarantee of the quality of post-thaw semen.

Correlations among the characteristics studied

Partial correlations

BW had a positive correlation with SCi and a negative correlation with TA, while SCi was positively correlated with EV, indicating that heavier animals tend have larger testicles and produce higher semen volume with more viable sperm and a lower percentage of TA. According to some authors, SCi in bulls is a predictor of seminal characteristics, as there is a positive correlation between SCi, EV, and SCo, indicating that the size and volume of the testicles influence the physical parameters of the ejaculate (Delgado et al., 2000; Martínez et al., 2000; Crespo and Quintero-Moreno, 2014). However, in the present study, no significant correlations between BW and SCi were observed with the seminal characteristics of fresh or frozen semen (PISM). Similar results were found in Creole Curraleiro bulls by Teixeira et al. (2011), who noted that testicular measurements were correlated with EV and that the bulls with higher BW had larger testicles, producing better quality semen with a higher percentage of normal sperm. However, Martínez et al. (2000) found a negative correlation between SCi and sperm viability.

CONCLUSIONS

Senepol bulls had higher body weight (BW), scrotal circumference (Sci), and lower total abnormalities (TA) compared to Rodeo Creole and Longhorn bulls. Senepol and Longhorn bulls had higher sperm concentration (SCo), individual sperm motility (ISM), and sperm viability than Rodeo Creole bulls. However, all the values obtained for the semen quality characteristics evaluated here are within the established values

for the bovine species. Therefore, the semen of Creole bulls could be cryopreserved for the conservation and dissemination of Creole breeds in Mexico. In addition, an association was found between BW and SCi, as well as between mass sperm motility (MSM), ISM, and TA. The drastic reduction in post-thaw individual sperm motility (PISM) observed in Rodeo Creole bulls requires further investigation.

Author Contributions

Victor Hugo Severino Lendechy and Víctor Fernando Torres Aburto participated actively in the literature review. Victor Hugo Severino Lendechy and Jorge Alonso Peralta Torres participated actively in the methodology. Victor Hugo Severino Lendechy, Víctor Fernando Torres, Mateo Itzá Ortiz, and Jorge Alonso Peralta Torres participated actively in the discussion of the results, as well as in the review and approval of the final version of the article.

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