Journal of Equine Veterinary Science 92 (2020) 103170

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Short Communication

Post-thawing Sperm Quality in Chilean Purebred Stallions: Effect of Age and Seasonality

Rodrigo Castro [a, *], Pamela Morales [a], Victor H. Parraguez [b, c]

[a] Escuela de Medicina Veterinaria, Facultad de Recursos Naturales y Medicina Veterinaria, Universidad Santo Tomás, Chile
[b] Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile
[c] Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, Chile

ARTICLE INFO

Article history:
Received 29 February 2020
Received in revised form
13 June 2020
Accepted 15 June 2020
Available online 20 June 2020

Keywords:
Chilean Purebred Stallion
Age
Seasonality
Frozen

ABSTRACT

In this work, we investigated the influence of age and seasonality on sperm motility and DNA fragmentation in post-thawing semen from Chilean Purebred Stallions (CPS), a horse breed presenting the oldest genealogy record in South America with an interesting reproductive industry. Despite that semen from aged CPS is frozen all year round, there is a lack of studies characterizing the breed semen freezability in accordance with age and seasonality. Twenty fertile CPS were grouped into the young group, the middle group, and the aged group. Ten ejaculates from each stallion were obtained by using an artificial vagina during summer (December) and winter (July) and directly frozen. Subsequently, the frozen semen was thawed and analyzed by a computer-assisted semen analysis and flow cytometer assessing progressive motility, mean velocity, and DNA fragmentation spermatozoa. Kruskal–Wallis test and Pearson's correlation were used to determine statistical differences among groups and correlation among variables ($P \leq .05$). Both spermatozoa motility traits decreased progressively in accordance with age and seasonality, showing the lowest values in the aged group during winter and the highest values in the young group during summer. Deoxyribonucleic acid fragmentation increased significantly in accordance with age and seasonality being highest in the aged group during winter and lowest in the young group during summer. Post-thawing sperm quality showed a negative correlation with the age of the stallions and a positive correlation with the normal sperm morphology before freezing. These results allow the conclusion that age and seasonality are important factors that need to be considered during the selection of CPS for reproductive programs.

1. Introduction

Seasonality and age have been related to alterations in the post-thaw semen quality for most breed stallions, mainly regarding sperm motility traits and DNA fragmentation [1]. Thus, the decreased efficiency of the semen cryopreservation observed during winter in aged stallions is associated to yearly changes in photoperiod, testicular function, and hormone profile, as well as

Animal welfare/ethical statement: All procedures performed using animals were revised and approved by the Institutional Animal Care and Use Committee of the Universidad Santo Tomás (authorization N° 02-2017).

Conflict of interest statement: We wish to confirm that there are no conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property. We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript. We understand that the corresponding author is the sole contact for the editorial process (including editorial manager and direct communications with the office). He is responsible for communicating with the other authors about progress, submissions of revisions, and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the corresponding author and which has been configured to accept email from rodrigocastro@santotomas.cl.

* Corresponding author at: Rodrigo Castro, Escuela de Medicina Veterinaria, Facultad de Recursos Naturales y Medicina Veterinaria, Universidad Santo Tomás, Carlos Schorr 255, Talca, Chile.
E-mail address: rodrigocastro@santotomas.cl (R. Castro).

https://doi.org/10.1016/j.jevs.2020.103170
0737-0806/

Table 1
Mean and standard deviations of fresh seminal traits in purebred Chilean stallions in accordance with age and seasonality.

Group	Free Gel Seminal Volume (mL)	Sperm Concentration (10^6/mL)	Sperm Progressive Motility (%)	Normal Sperm Morphology (%)
Young/summer	38.67 ± 9.54^{a}	264.67 ± 48.33^{ab}	77.33 ± 15.68^{a}	87.67 ± 3.20^{a}
Middle/summer	43.75 ± 10.25^{ab}	181.08 ± 37.87^{cd}	72.08 ± 11.96^{ab}	85.42 ± 3.96^{a}
Aged/summer	52.85 ± 13.43^{b}	168.66 ± 40.01^{c}	62.60 ± 14.79^{bc}	79.72 ± 4.99^{bc}
Young/winter	34.33 ± 7.29^{a}	270.07 ± 36.35^{a}	66.33 ± 15.17^{ac}	84.00 ± 4.71^{ac}
Middle/winter	40.08 ± 12.32^{ab}	221.25 ± 31.00^{bd}	63.33 ± 10.30^{ac}	83.75 ± 3.77^{ac}
Aged/winter	43.61 ± 13.37^{ab}	196.06 ± 47.65^{cd}	59.72 ± 11.44^{bc}	75.83 ± 3.54^{b}

Different superscript letters denote statistical difference in each seminal trait.

morphofunctional changes in spermatozoa, which leads to a deterioration of post-thawing kinetic traits, DNA integrity, and mitochondrial membrane potential in the sperms [2–4]. The evaluation of fresh and post-thawing semen is necessary to predict the potential fertility of a seminal sample. Traditionally, the progressive motility has been the most important trait, but today an appropriate combination of techniques like computer-assisted semen analysis (CASA), microscopic observation, and flow cytometry may provide a better prediction of fertility than solely progressive motility analysis [5].

Chilean purebred is a rustic and adaptable horse breed presenting the oldest genealogy record in South America as shown in data administered by the National Agriculture Society since 1893 [6] and constitutes an interesting breeding industry. In Chile, purebred stallions are mainly used for rodeo practice, an equestrian sport closely related to Chilean country culture and folklore [7,8]. For this reason, this horse has been declared a natural monument by the Chilean government [6]. In spite of its social, economic, and cultural importance, up to the present date, there are no systematic studies reporting about the characterization of the breed semen freezability in accordance with age and seasonality. The acquisition of this information is of high importance because aged stallions with high genetic value are frequently used for artificial insemination around the year. The aim of the present study is to evaluate whether age and seasonality affect sperm motility and DNA fragmentation in post-thawing semen from Chilean Purebred Stallions (CPS).

2. Materials and Methods

All procedures performed in animals were revised and approved by the Institutional Animal Care and Use Committee of the Universidad Santo Tomás (authorization N° 02–2017). Twenty healthy and proven fertility CPS were used. All the animals were registered in the Chilean Horse Genealogy Registry (National Agriculture Society) and were in a range of 5- to 24-year-old, with per cycle pregnancy rate of 92.7 ± 3.2% during the last breeding season. The stallions were separated into three groups: the young group (5- to 11-year-old; n = 9), the middle group (12- to 18-year-old; n = 7), and the aged group (19- to 24-year-old; n = 4). The study was carried out in Santiago, Chile (latitude: 33°27′ S. longitude: 70°38′ W). All stallions were kept clinically healthy in individual boxes bedded with straw, were exercised regularly and individually, and had daily access for approximately 1 hour to a paddock without any direct mare contact. They were fed three times daily with hay, oat, and pellets supplemented with minerals and had ad libitum access to water at all times. During the study, the stallions were kept under daily sperm output condition, with the purpose of standardizing the sperm emissions of each stallion. Ten semen samples from each stallion were obtained three times a week in summer (December; 14.8 hours of daylight: 9.2 hours of darkness, average temperature 20°C) and winter (July, 10.4 hours of daylight: 13.6 hours of darkness, average temperature 9°C), with the aid of an artificial vagina (Missouri model). The volume of gel-free ejaculate (mL) was evaluated in a calibrated measuring cylinder, and the sperm concentration (10^6/mL) was measured using a SpermaCue photometer 12300/0500 (Minitube, Tiefenbach, Germany). The ejaculates were diluted 1:1 (v/v) with a prewarmed (37°C) skim milk–based extender (Botusemen, Botupharma, Botucatu, Brazil), and percentage of progressively motile sperms was analyzed under a phase-contrast light microscope with a heated stage (37°C) at 200× magnification (Olympus CH-II, Olympus Optical, Hamburg, Germany). All studied ejaculates had normal sperm morphology over 70%; eosin-nigrosin–stained smear was analyzed under a phase-contrast light microscope at 1000× magnification [9]. All analyses were carried out by the same person. Seminal samples were frozen as follows: fresh semen was extended 1:1 (v/v) with Botusemen and then centrifuged at 400 × *g* for 10 minutes at room temperature (Eppendorf; Hamburg, Germany). The fluid fraction was removed and the sperm pellet resuspended in a freezing egg yolk–based extender (Botucrio, Botupharma, Botucatu, Brazil) to a final concentration of 400 × 10^6 sperm/mL. In a next step, sperms were packaged into 0.5 mL straws and cooled for 20 minutes at 5°C before starting the freezing process. The straws were placed on a Styrofoam float and held 2 cm above liquid nitrogen for 10 minutes. Afterward, the straws were plunged into the liquid nitrogen and stored for two months. After post-thawing (60 seconds at 37°C), semen samples were analyzed for progressive motile spermatozoa (%) and mean velocity (μm/seg) [10] in a CASA system (ISAS-V, Proiser, Valencia, Spain) and further evaluated to determine post-thawing DNA fragmentation [11] in a flow cytometry (Beckman Coulter Gallios, NJ), using acridine orange staining (A6014-10G Acridine orange, Sigma Aldrich, Saint Louis, MI). The flow cytometer was equipped with a 488 nm argon-ion laser with an output power of 15 mW for photon excitation and a 530/30 nm band-pass filter, as well as a 650 nm long-pass filter for detection of green and red fluorescence emission, respectively. Acridine orange stain shows green fluorescence when intact double-stranded DNA is present, whereas denatured single-stranded DNA shows red fluorescence. The DNA fragmentation percentage was calculated from sperm fractions with single- and double-stranded DNA.

The acquired data were grouped in accordance with age and seasonality, and subsequently, the differences between the groups were analyzed by means of the nonparametric Kruskal–Wallis test. Pearson's correlation was used to determine the correlation among variables. Statistical analysis was performed using the SPSS statistics program (version 20.0, IBM-SPSS, Armonk, NY). Statistical differences were considered when $P \leq .05$. Data are presented as mean ± standard deviation.

3. Results

The average age in the young group, middle group, and aged group was 5.78 ± 1.09, 14.86 ± 2.6, and 22 ± 1.8 years, respectively.

The evaluation of fresh semen in accordance with age and seasonality showed that free gel seminal volume was significantly

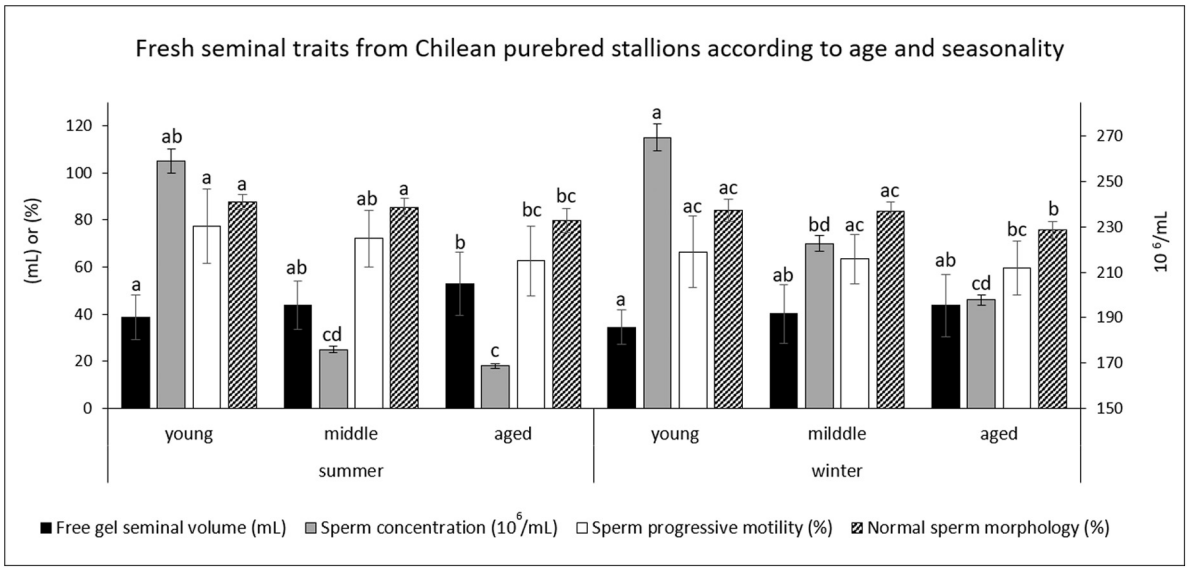

Fig. 1. Within the same trait, means with different superscripts are significantly different; $P < 0.05$.

higher in the aged group during summer ($P = .01$) but showed no differences during winter ($P = .33$). Sperm progressive motility values tended to increase during summer for all age groups, showing a value significantly highest in the young/summer group ($P = .03$), whereas motility values showed no differences during winter ($P = .99$). The sperm concentration was significantly higher in the young group during both seasons ($P = .01$). In all age groups, there were no significant difference between summer and winter ($P = .7$). Normal sperm morphology was significantly lowest in the aged group during winter and summer ($P = .01$), but there were no significant differences in accordance with season ($P = .08$) (Table 1 and Fig. 1).

Analyses of post-thawing semen samples showed that sperm progressive motility was significantly highest in the young/summer group ($P = .01$). Although the young and aged group showed a significant decrease during winter ($P = .01$), the middle group only tended to diminish its values ($P = .29$). The aged group showed the lowest value for sperm progressive motility in both seasons.

Mean sperm velocity showed the greatest value in the young/summer group, and it was significantly higher than in the aged/summer group ($P = .01$). There was no difference among groups during winter, but the aged group tended to show a minor value for the mean sperm velocity ($P = .06$). Velocity values tended to decrease during winter, but only in the young group, the value declined significantly ($P = .01$). DNA fragmentation increased progressively and significantly in accordance with age in both seasons. All age groups showed higher values during winter ($P = .02$), reaching the highest value for the aged stallions ($P = .01$). In both seasons, the values of the young group were significantly lower than in the aged group ($P = .01$) (Table 2 and Fig. 2).

The age of the stallions was negatively correlated with post-thawing sperm quality, showing the following values for the evaluated variables: sperm motility ($r = -0.68$), mean sperm velocity ($r = -0.56$), and DNA integrity ($r = -0.54$). Normal sperm morphology before the freezing process showed a positive correlation with post-thawing sperm motility ($r = 0.52$) and DNA fragmentation ($r = 0.57$). All the other evaluated variables only showed a weak correlation with the post-thawing sperm quality (Table 3).

4. Discussion

This is the first study aimed to determine whether age and seasonality affect the post-thawing sperm quality in the CPS, using one extender and one freezing curve; then, the results could be used as preliminary data for a proper selection of stallions, avoiding negative effects on potential fertility during artificial insemination. The most important outcome of this study is that the post-thawing sperm quality in the CPS can be modified in accordance with the age of the stallions and seasonality, where the better post-thawing sperm quality was observed in the semen samples obtained from young stallions during summer. The most correlated properties with the post-thawing sperm quality were age of the stallions and the normal sperm morphology before freezing.

In general, it is accepted that the success of sperm cryopreservation depends, in part, on the quality of fresh semen; thus, semen from young stallion obtained during summer could be the best option. However, sometimes the individual differences between stallions may overcome the effects of age and season [3]. Fresh semen obtained from CPS showed similar variations regarding age

Table 2
Mean and standard deviations of sperm kinetics and DNA fragmentation in postthawing semen from purebred Chilean stallions in accordance with age and seasonality.

Group	Progressive Motility (%)	Mean Velocity (μm/seg)	DNA Fragmentation (%)
Young/summer	45.34 ± 5.12^{a}	47.31 ± 4.07^{a}	9.33 ± 2.15^{a}
Middle/summer	39.32 ± 4.95^{b}	43.15 ± 3.52^{ab}	11.42 ± 2.11^{ab}
Aged/summer	35.14 ± 3.04^{b}	38.78 ± 4.75^{bc}	12.56 ± 2.01^{b}
Young/winter	38.93 ± 4.10^{b}	40.09 ± 4.82^{bc}	13.73 ± 2.15^{bc}
Middle/winter	35.01 ± 4.27^{bc}	38.89 ± 5.46^{bc}	15.58 ± 2.31^{c}
Aged/winter	30.12 ± 4.95^{c}	34.14 ± 6.03^{c}	19.94 ± 2.15^{d}

Different superscript letters denote statistical difference in each seminal trait.

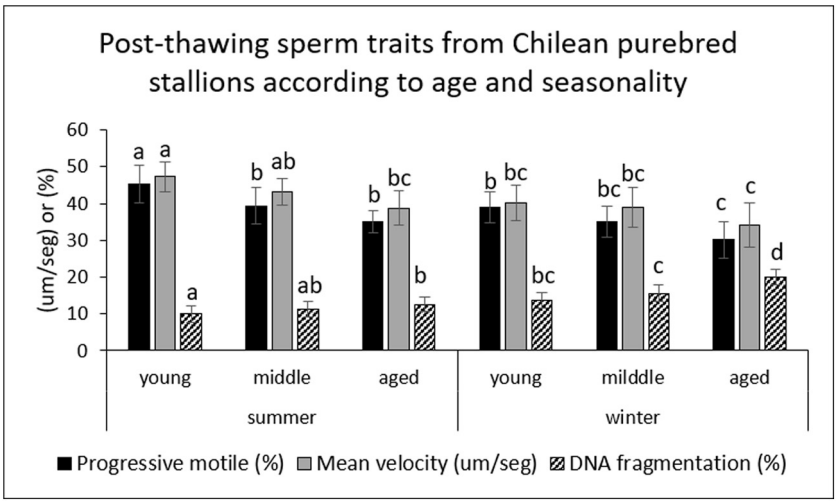

Fig. 2. Within the same trait, means with different superscripts are significantly different; $P < 0.05$.

and seasonality as those described for other stallion breeds, thereby also observing better seminal quality in young stallions during summer season [12,13]. Thus, seminal traits evaluated in fresh semen samples were in accordance with the normal parameters reported for the equine species. These results can be explained by a maximum activity of the hypothalamus-pituitary-gonadal axis during late spring and summer for the equine species, which decreases consecutively with age [14].

In the present study, frozen-thawing evaluation of semen samples showed that age and seasonality significantly affect sperm kinetics traits. In young stallions, the sperm progressive motility and sperm mean velocity showed the highest values during summer, which decreased during winter ($P = .01$). This seasonal difference was minor in aged stallions, denoting lower seminal quality for cryopreservation compared with young and middle stallions but greater stability of sperm kinetics throughout the year.

The sperm progressive motility is one of the main potential fertility parameters in the postthawing seminal evaluation. In European countries, 35% rapid motile spermatozoa after thawing is considered as a minimum standard requirement for appropriate fertility [15,16]. In the present study, most CPS maintained over 35% post-thawing sperm progressive motility. Only the aged/winter group showed slightly lower values with 30.12 ± 4.95%. Even though the evaluation of additional parameters might be necessary for a full understanding, the post-thawing sperm motility observed for CPS seems to be sufficiently high for successful artificial insemination. However, the post-thawing sperm progressive motility only was weakly correlated with the fresh sperm progressive motility ($r = 0.33$), showing a higher correlation to the age of the stallions ($r = -0.68$) and the fresh normal sperm morphology ($r = 0.52$).

The sperm membrane fatty acid composition undergoes seasonal changes, and the long chain polyunsaturated fatty acids content increases from the nonbreeding into the breeding season [17]. This structural change might be related to the stability of membrane, kinetic properties, and freezability along the year. The worsening of sperm kinetics observed in our study during winter, especially in young stallions, could be related to structural changes in the sperm cell membrane [12].

On the other hand, frozen-thawing in equine semen is associated with increased production of reactive oxygen species [18], leading the sperm to suffer oxidative stress and a potential organelle membrane damage and DNA fragmentation [19]. Fragmentation of sperm DNA in a semen sample begins immediately with thawing and increases with the thawing time [20]. Moreover, the observed seasonal changes in postthawing sperm DNA fragmentation could be associated with reactive oxygen species generated during sperm cryopreservation, which increased significantly when cryopreservation was performed during winter [21].

In the present study, both age and seasonality affected postthawing sperm DNA fragmentation. Thus, the young/summer group had the lowest value and the aged/winter group had the highest value. However, the higher value observed in our study was slightly below (2.7% less) the average reported for Thoroughbred stallions, which is the breed with the highest postthawing DNA fragmentation reported in the literature [1]. In contrast to our results, an investigation carried out in 121 stallions of seven different breeds reported that postthawing sperm DNA fragmentation was the most variable trait, fundamentally attributable to breed and seasonality, but not to the age as was observed in our study ($r = 0.54$). However, it is important to highlight that sperm DNA fragmentation observed in the CPS was in the range reported for other breeds ranging from 8.7 ± 2.3% in the Oldenburg warmblood to 19.3 ± 3.0% in the Thoroughbred breed [1]. These authors also reported a subtle difference in postthawing DNA fragmentation between summer and winter (11.9 ± 1.1% vs. 12.9 ± 1.0%).

Post-thawing sperm DNA fragmentation is a very important property because it may lead to dysfunctional or defective sperm, affecting the sperm functional competence. In animals and humans, there is a documented relationship between fragmented DNA in sperms and decreased fertility or embryo-fetal losses [22–25]. In conventional laboratory analyses, sperms with

Table 3
Correlation (r) among age, fresh seminal traits, and postthawing sperm traits from purebred Chilean stallions.

	AGE	FGSV	SC	FSPM	FNSM	PTSPM	PTSMV	PTDNAF
AGE	1	0.34	−0.64	−0.27	−0.63	−0.68	−0.56	0.54
FGSV	—	1	−0.46	−0.08	−0.25	−0.07	0.03	−0.01
SC	—	—	1	0.12	0.35	0.33	0.30	−0.19
FSPM	—	—	—	1	0.18	0.33	0.18	−0.28
FNSM	—	—	—	—	1	0.52	0.41	−0.57
PTSPM	—	—	—	—	—	1	0.59	−0.72
PTSMV	—	—	—	—	—	—	1	−0.55

FGSV, free gel seminal volume; SC, sperm concentration; FSPM, fresh sperm progressive motility; FNSM, fresh normal sperm morphology; PTSPM, postthawing sperm progressive motility; PTSMV, postthawing sperm mean velocity; PTDNAF, postthawing DNA fragmentation.

fragmented chromatin cannot be differentiated from normal sperms. That is why it is very important to make a more exhaustive laboratory evaluation regarding the sperm DNA integrity incorporating new techniques to avoid unwanted fertility losses [24,26,27]. In addition, it is necessary to keep in mind that the chromatin susceptibility to denaturation during the frozen-thawing process is correlated to the level of DNA damage shown before cryopreservation, emphasizing the relevance to select seminal samples with high DNA integrity [28].

The present study did not consider fertility tests of the evaluated semen but should be included in future studies about the freezing-thawing effects on sperm quality in semen of CPS.

5. Conclusion

This first study about the post-thawing sperm quality in accordance with age and seasonality, in semen of CPS, unveils that in this preliminary experimental condition, the age and the seasonality affect the post-thawing kinetics and DNA fragmentation. Therefore, it is highly recommended to consider these variables during selection of this breed for reproductive programs when semen cryopreservation is used.

Acknowledgments

The authors would like to thank the Universidad Santo Tomás for funding this study through the “Fondos internos de investigación, proyecto regular TAS 0000028290”.

Authors’ contributions: Rodrigo Castro contributed to conceptualization, methodology, validation, formal analysis, data curation, and writing—original draft. Pamela Morales contributed to project administration, funding acquisition, data curation, and writing—review and editing. Victor H. Parraguez contributed to investigation and writing—original draft.

References

[1] Greiser T, Sieme H, Martinsson G, et al. Breed and stallion effects on frozen-thawed semen in warmblood, light and quarter horses. Theriogenology 2020;142:8–14.
[2] Wrench N, Pinto C, Klinefelter G, et al. Effect of season on fresh and cryopreserved stallion semen. Anim Reprod Sci 2010;119:219–27.
[3] Aurich C. Seasonal influences on cooled-shipped and frozen-thawed stallion semen. J Equine Vet Sci 2016;43:1–5.
[4] Darr C, Morales L, Scanlan T, et al. Sperm mitocondrial function is affected by stallion age and predicts post-thaw motility. J Equine Vet Sci 2017;50:52–61.
[5] Barrier I, Kempfer A, Lemasson N, et al. Prediction of the fertility of stallion frozen-thawed semen using a combination of computer-assisted motility analysis, microscopical observation and flow cytometry. Theriogenology 2017;97:186–200.
[6] Ministerio de Agricultura. Declara monument natural al caballo de pura raza chilena. Biblioteca del congreso nacional de Chile [internet]; 2011. https://www.leychile.cl/N?i=1024797&f=2011-04-26&p=. [Accessed 24 January 2020].
[7] Perez V, Huber A. El gran libro del caballo chileno: Historia y tradición de un Pais. Santiago: Creación y desarrollo; 2004.
[8] Mora M, Briones R, Galecio J, et al. Main musculoskeletal injuries associated with lameness in Chilean Rodeo horses. Arch Med Vet 2014;46:419–24.
[9] Brito L, Greene L, Kelleman A, et al. Effect of method and clinician on stallion sperm morphology evaluation. Theriogenology 2011;76:745–50.
[10] Giaretta E, Munerato M, Yeste M, et al. Implementing an open-access CASA software for the assessment of stallion sperm motility: relationship with other sperm quality parameters. Anim Reprod Sci 2017;176:11–9.
[11] Sellem E, Broekhuijse M, Chevrier L, et al. Use of combinations of in vitro quality assessments to predict fertility of bovine semen. Theriogenology 2015;84:1447–54.
[12] Janett F, Thun R, Niederer K, et al. Seasonal changes in semen quality and freezability in the Warm- blood stallion. Theriogenology 2003;60:453–61.
[13] Pereira D, Ozanam F, Roser J. Reproductive characteristics of stallions during the breeding and non-breeding season in a tropical region. Trop Anim Health Prod 2012;44:1703–7.
[14] Turner R. Declining testicular function in the aging stallion: management options and future therapies. Anim Reprod Sci 2019;207:171–9.
[15] Kirk E, Squires E, Graham J. Comparison of in vitro laboratory analyses with the fertility of cryopreserved stallion spermatozoa. Theriogenology 2005;64:1422–39.
[16] Vidament M. French field results (1985-2005) on factors affecting fertility of frozen stallion semen. Anim Reprod Sci 2005;89:115–36.
[17] Aurich C, Ortega C, Peña F, et al. Seasonal changes in the sperm fatty acid composition of Shetland pony stallions. Theriogenology 2018;107:149–53.
[18] Baumber J, Ball B, Linfor J, et al. Reactive oxygen species and criopreservation promote DNA fragmentation in equine spermatozoa. J Androl 2003;24:62–628.
[19] Linfor J, Meyers S. Detection of DNA damage in response to cooling injury in equine spermatozoa using single-cell gel electrophoresis. J Androl 2002;23:107–13.
[20] López-Fernández C, Crespo F, Arroyo F, et al. Dynamics of sperm DNA fragmentation in domestic animals. II the Stallion. Theriogenology 2007;68:1240–50.
[21] Mislei B, Bucci D, Malama E, et al. Seasonal changes in ROS concentrations and sperm quality in unfrozen and frozen-thawed stallion semen. Theriogenology 2020;144:89–97.
[22] Karoui S, Diaz C, Gonzalez-Marin C, et al. Is sperm DNAfragmentation a good marker for field AI bull fertility? J Anim Sci 2012;90:2437–49.
[23] Leach M, Aitken R, Sacks G. Sperm DNA fragmentation abnormalities in men from couples with a history of recurrent miscarriage. Aust N Z J Obstet Gynaecol 2015;55:379–83.
[24] Santi D, Spaggiari G, Simoni M. Sperm DNA fragmentation index as a promising predictive tool for male infertility diagnosis and treatment management – meta-analyses. Reprod Biomed Online 2018;37:315–26.
[25] De la Torre J, Crespo F, Arroyo F, et al. Effect of sperm dosage transportation in stallions: Effect on sperm T DNA fragmentation. Anim Reprod Sci 2019;206:38–45.
[26] Fatehi A, Bevers M, Schoevers E, et al. DNA damage in bovine sperm does not block fertilization and early embryonic development but induces apoptosis after the first cleavages. J Androl 2006;27:176–88.
[27] Ahmadi A. Fertilizing ability of DNA-damaged spermatozoa. J Exp Zool 1999;284:696–704.
[28] Evenson D, Sailer B, Jost L. Relationship between stallion sperm deoxyribonucleic acid (DNA) susceptibility to denaturation in situ and presence of DNA strand breaks: implications for fertility and embryo viability. Biol Reprod Mono 1995;1:655–9.