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Evaluation of bovine sperm telomere length and association with semen quality

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ABSTRACT

The study aimed to evaluate if the sperm telomere length can be considered as a new biomarker for sperm quality in bulls. Sperm Telomere Length was evaluated by Monochrome Multiplex Quantitative PCR in group A (n = 8) and group B (n = 8) bulls, classified according to standard semen analysis. Also, this parameter was measured before and after Percoll gradient separation within bulls that produced semen of satisfactory quality. Sperm telomere length, measured as T/S ratio (average ratio of telomere repeats copy number to a single copy gene), was higher in group A than in group B bulls (0.77 ± 0.03 vs 0.43 ± 0.06; P < 0.01). Sperm telomere length was positively correlated with motility, viability and membrane integrity, and it was negatively correlated with sperm anomalies. Furthermore, Percoll gradient selected sperms with higher T/S ratio than unselected sperms (1.19 ± 0.02 vs 0.67 ± 0.03). These results suggest that sperm telomere length can be used as a new marker of bovine semen quality.

1. Introduction

The efficiency of Artificial Insemination (AI) programs in cattle is strictly correlated with variations in sperm qualitative characteristics. Indeed, semen quality is an important factor affecting both AI and in vitro fertilization efficiency [1], as a high individual variability in fertility is recorded among bulls [1,2]. Although several in vitro fertility tests have been developed to predict the potential fertility of a bull, it is still necessary to assess many parameters to accurately estimate bull fertility. It follows the need to identify new reliable markers of semen quality in cattle.

Recently, sperm telomere length (STL) has been suggested to be associated with sperm quality in men [3]. Telomeres are heterochromatic structures with the noncoding highly conserved hexanucleotide repeat sequence 5′-TTAGGG-3′. They are specific chromatin structures located at the end of the eukaryotic chromosomes and contribute to the chromosome stability and genome integrity. These guanine-rich repeats are highly conserved during evolution, and their role is related to their length and structure [4]. They play a main role in meiosis by facilitating chromosomal alignment, pairing, synapsis and crossing over. Kozik et al. [5] demonstrated that the length of telomeres in human, bovine and porcine sperm is higher than in somatic cells and that the lengthening of telomeric DNA during mammalian spermatogenesis is important for both meiotic and postfertilization functions. It is also known that STL is maintained throughout aging in cattle, as well as in humans [6]. In addition, it was reported that sperm telomeres (ST) have an active role in the sperm-specific chromosome architecture formation [7]. Spermatozoa present a high telomerase activity which compensate for telomere erosion occurring during cellular division [8], preventing the age-associated shortening of the chromosomes, and their instability, ensuring the transmission of intact chromosomes over generations [4,8]. Moreover, ST are carried on the zygote in fertilization, acting like a zero-time length mark for the next cell divisions [9].

During early spermatogenesis, the telomeres migrate and link, individually, with the nuclear membrane forming clusters. In this way, the DNA is reorganized in nucleoprotamines and the telomeric DNA is packaged in nucleosomes associating with a part of sperm histones. This migration is believed to have an essential role in

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fertilization, pronucleus formation and early development [10] The importance of STL in reproduction is also highlighted by its correlation with telomere length in embryos and offspring, observed in rodents [11] and humans [12].

Furthermore, sperm telomere length is higher in human spermatozoa selected by both Percoll density gradient [13] and swim-up [14] method, commonly used for discriminating high-quality sperms in assisted reproduction, also reporting less DNA damage [15–17].

Therefore, STL has recently been proposed as an additional new diagnostic and prognostic biomarker of sperm quality in men [3].

We hypothesized that STL can be a potential biomarker of semen quality also in cattle, in which, to the best of our knowledge, STL analysis has not yet been investigated.

Therefore, the aim of this study was to evaluate whether STL could be an additional new parameter for the evaluation of sperm quality in bulls (*Bos taurus*). In particular, a first objective of the study was to assess whether STL varies between bulls suitable and unsuitable for reproductive use, and if STL is correlated to other sperm qualitative traits. Further objectives of the work were to evaluate the effect of Percoll gradient separation of frozen-thawed sperm on STL and to investigate whether STL differs among bulls with different in vitro fertilizing ability.

2. Material and methods

Unless otherwise stated, reagents were purchased from Merck Life Science S. r.l. (Milano, Italy). The IVM medium was TCM 199 supplemented with 15% bovine serum (BS), 0.5 μg/mL FSH, 5 μg/mL LH, 0.8 mM L-glutamine and 50 μg/mL gentamycin. The IVF medium was Tyrode's modified medium [18] without glucose and bovine serum albumin (BSA), supplemented with 5.3 SI/mL heparin, 30 μM penicillamine, 15 μM hypotaurine, 1 μM epinephrine and 1% of BS. The IVC medium consisted of synthetic oviduct fluid (SOF) medium with 30 μL/mL essential amino acids, 10 μL/mL non-essential amino acids and 5% BS [19].

2.1. Experimental design

Sixteen Holstein Friesian (*Bos tTaurus*) bulls (4–6 y age) maintained at an authorized National Semen Collection Center (Centro Tori Chiacchierini, Civitella D'Arna, Italy) under uniform management conditions, routinely used for semen collection, were selected for the trial. Semen collection was carried out weekly by artificial vagina (IMV, L'Aigle, France) in spring (March–April).

In Experiment 1 bulls were divided in two quality groups according to standard criteria currently applied at the Semen Collection Center: Group A, i.e. suitable (3 ejaculates per 8 bulls) and Group B, i.e. unsuitable (3 ejaculates per 8 bulls) for reproductive use. More specifically, the discriminating criteria employed were post-thawing sperm motility (>and <60%, respectively) and sperm anomalies (<and >20%, respectively). Subsequently, in our laboratory sperm viability, morphology, membrane integrity, as well as DNA fragmentation and STL were evaluated after thawing in both groups.

In Experiment 2, the same assessments were carried out on Group A bulls (7 ejaculates per bull) before and after Percoll selection of frozen-thawed samples. In addition, the in vitro fertilizing ability of Group A bulls was evaluated on abattoir-derived cumulus–oocyte complexes (N = 1298, over 7 replicates, i.e. on average 162 per bull) to assess whether STL could predict cleavage and blastocysts yields.

2.2. Freezing procedure

Fresh ejaculates were diluted at 37 °C with an animal free protein extender BioXcell (IMV-technologies, L'Aigle, France) to a final concentration of 30×10^6 spermatozoa/ml. The diluted semen was mixed up at room temperature (25 °C) for 10 min and automatically loaded into 0.5 mL French straws using computer-controlled filler (IMV Technologies, L'Aigle, France). After filling, straws were placed into a cooling box and subjected to a combined cooling with equilibration period of 3 h at 5 °C. Freezing was performed using a programmable controlled rate freezer (IMV technology, L'Aigle, France) until temperature of straws reached −145 °C. Then straws were plunged into liquid nitrogen (−196 °C) for storage.

2.3. Percoll separation

Frozen bovine sperm were thawed in a water bath at 37 °C for 40 s and selected by centrifugation (25 min at 300×*g*) on a Percoll discontinuous gradient (45 and 80%). The pellet was reconstituted into 2 mL of Sperm-TALP medium [18] and centrifuged twice, at 160 and then at 108×*g* for 10 min each. The pellets were reconstituted in Sperm-TALP medium and used for different analyses.

2.4. Assessment of sperm qualitative characteristics

Sperm motility was examined by phase contrast microscopy (magnification: ×40) on a clean slide maintained on thermo-regulated stage at 37 °C. The percentage of motile spermatozoa was subjectively determined by analyzing five different microscopic fields for each semen sample [20]. Sperm viability and morphology were assessed by Trypan Blue/Giemsa technique [21]. Briefly, 5 μL of semen and 5 μL 0.27% Trypan blue were spread, fixed in 37% formaldehyde and stained with 7.5% Giemsa overnight. A total of 100 spermatozoa were microscopically (magnification: ×40) analyzed per slide and differentiated for viability as: acrosome intact live (AIL), acrosome intact dead (AID), acrosome lost live (ALL) and acrosome lost dead (ALD), and for morphology as normal and abnormal. Anomalies includes proximal droplets, head, mid-piece and tail defects [22] The percentage of AIL sperm was considered as sperm viability. The percentage of the total morphological anomalies was estimated by deducting the spermatozoa with normal morphology.

The functional integrity of sperm was assessed by the Hypo osmotic swelling (HOS) test [23]. The test was performed by mixing 50 μL of sperm sample with 0.50 mL of hypo-osmotic medium (0.73 g sodium citrate and 1.35 g fructose in 100 mL of distilled water, 150 mOsm/L) and incubating for 45 min at 37 °C.A sample drop (20 μL) was placed on a slide and covered with coverslip. A total of 200 spermatozoa were counted in different fields (magnification: ×40) under phase contrast microscope and spermatozoa with swollen tail were classified as HOS positive.

DNA fragmentation index was assessed by Tunel assay [24] using a commercially available kit (In Situ Cell Death Detection Kit, Roche, Indianapolis, USA). Semen samples were fixed with 4% (w/v) paraformaldehyde in phosphate buffered saline (PBS) for 30 min at room temperature. After fixation, the samples were centrifuged at 300×*g* for 10 min, resuspended in a permeability enhancing solution containing 0.1% Triton X-100 in 0.1% sodium citrate for 10 min. Then the cells were washed twice in PBS-PVP and incubated in Tunel reaction mixture for 1 h at 37 °C in a dark and humidified atmosphere. At the end of the incubation, slides were washed in PBS-PVP labeled with Hoechst 33,342 1 mg/mL for 30 min at room temperature, washed again in PBS-PVP and dropped (20 μL) on a glass slide in glycerol and overlaid with a coverslip. At least 200 spermatozoa were analyzed in each sample by using a fluorescent microscope (Eclipse E–600; Nikon, Japan) under ultraviolet light

with excitation DAPI (460 nm for blue fluorescence), and FITC (520 nm for green fluorescence) filters. Digital images of each spermatozoa were acquired using NIS-Elements-F software and a high-resolution color digital camera (Digital Sight DS-Fi 1C; Nikon, Japan) and the numbers of total (blue) and Tunel -positive (green) nuclei were recorded.

2.5. In vitro embryo production

Abattoir-derived oocytes were matured, fertilized and cultured in vitro according to our standard procedure [19]. Briefly, bovine ovaries were recovered from a local abattoir and transported to the laboratory, in physiological saline at 30–35 °C. Cumulus–oocyte complexes (COCs) were aspirated from follicles of 2–8 mm in diameter and only those with uniform cytoplasm and multilayered cumulus cells were selected in Groups of 20–25 COCs and matured in 400 μL of IVM medium, covered with mineral oil, in four well plates (NuncTM, Roskilde, Denmark), for 22 h at 39 °C, and 5% CO_2 in air. In vitro matured COCs were washed and transferred into 300 μL of IVF medium covered with mineral oil. Percoll separated sperm were diluted with IVF medium and added in the fertilization wells at the concentration of 1×10^6 sperm/mL. Gametes were co-incubated for 20 h at 39 °C, in 5% CO_2 in air, after which presumptive zygotes were vortexed for 2 min to remove cumulus cells in Hepes-TCM with 5% BS, washed twice in the same medium and randomly distributed into 400 μL of IVC medium and incubated in a humidified mixture of 5% CO_2, 5% O_2 and 88% N_2 in air at a temperature of 39 °C. On Day 7 cleavage and blastocyst rates were recorded.

2.6. Sperm telomere length by Monochrome Multiplex Quantitative PCR (MMQPCR)

The average telomere length in sperm was performed by Monochrome Multiplex Quantitative PCR (MMQPCR) according to the method described by Cawthon [25]. Genomic DNA was extracted directly from sperm samples by the phenol-chloroform DNA method. The quantity and quality of DNA samples were measured using a NanoDrop spectrophotometer where the ratio of the absorbance was used to assess the purity of DNA ($1.7 < A260/280 < 1.9$ was considered acceptable). MMQPCR was performed into a 96-well CFX RT-PCR System in a 10 μL reaction total mix containing 20–30 ng of genomic DNA, iTaq Universal SYBR Green Supermix and forward and reverse primer sequences for telomere and beta-globin (defined as single copy gene-scg), as reported by Brown et al. [26]. The thermal cycling profile followed Iannuzzi et al. indications [27]. The dissociation curve confirmed the specificity of both primers as shown in Fig. 1. Their specificity was also tested by agarose gel electrophoresis (Supplementary Fig. 1) showing specific bands at 79 and 144 bp. Each sample was assayed in triplicates in three different runs, with negative control (NTC) included. Only samples that presented a coefficient of variation <5% were considered for the analysis (4 samples presented a CV > 5% and they were repeated by re-extracting their DNA). The STL was measured as a T/S ratio (average ratio of telomere repeat copy number to a single copy gene) for each sample and calculated using the ΔΔCt method [28].

2.7. Statistical analysis

In Experiment 1, in order to evaluate differences in sperm qualitative traits at thawing (before Percoll separation) between A and B groups, one-way univariate ANOVA for repeated measures (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp) was carried out after logit transformation of all variables expressed as percentages. A preliminary analysis ruled out an ejaculate effect for all variables but motility ($p < 0.05$). Therefore, only for this parameter, the ejaculate factor was also included in the analysis. Furthermore, the relationships between the TS ratio and the remaining variables were analyzed by Kendall's tau correlation after logit transformation of percentages.

In Experiment 2, to evaluate differences in group A bulls in sperm qualitative traits between total sperm population (pre-Percoll) and selected population (post-Percoll), ANOVA for repeated measures was carried out with Percoll treatment as fixed and bull as random factors. Also, in this case variables expressed as percentages were transformed into their corresponding logit value.

Finally, differences among bulls in STL, sperm qualitative characteristics and cleavage and blastocysts rates, were assessed by one-way univariate ANOVA, with bull as fixed factor. A value of $P < 0.05$ was considered statistically significant.

3. Results

In Experiment 1, bulls of group A had both higher ($P < 0.05$) volume and motility of fresh semen ($P < 0.05$), but similar sperm concentration (Table 1). In addition, Group A showed increased percentages ($P < 0.01$) of motility, viability and HOS + sperm, with a lower incidence of total anomalies ($P < 0.01$) and Tunel + sperm after thawing, as shown in Table 1. Besides, T/S ratio was higher ($P < 0.01$) in group A compared to group B, while no differences were recorded in sperm DNA fragmentation. Furthermore, T/S ratio was positively correlated ($P < 0.01$) to motility, viability, HOS + sperm and negatively correlated to total sperm anomalies, as shown in Table 2.

In Experiment 2, Percoll separation selected a sperm population with higher ($P < 0.01$) STL that also showed higher ($P < 0.01$) motility and lower ($P < 0.05$) DNA fragmentation (Table 3).

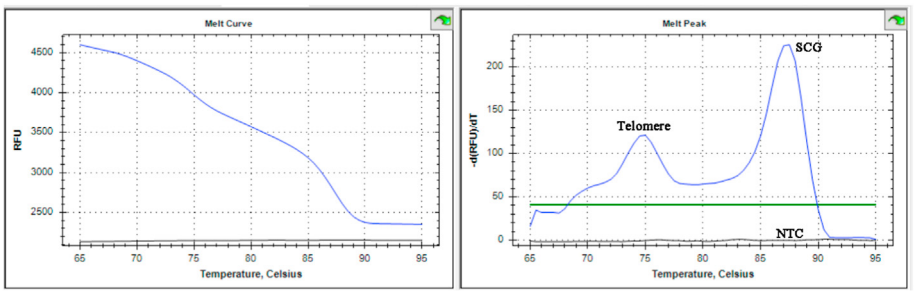

Fig. 1. Representative dissociation curve (blue) for telomere and scg using CFX RT-PCR System (Bio-Rad). The 74 °C read correspond to the Ct (cycle threshold) value for the telomere amplification while the 87 °C read correspond to the Ct value for the scg amplification. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Sperm qualitative traits and T/S ratio of bulls judged suitable (Group A; 3 ejaculates per 8 bulls) or unsuitable (Group B; 3 ejaculates per 8 bulls) for reproductive use. All parameters were recorded at thawing, with the exception of volume, concentration and fresh motility. Data are reported as mean ± SE.

Sperm parameters	Group A	Group B
Ejaculate volume (mL)	8.5 ± 0.4[a]	7.1 ± 0.5[b]
Concentration (x10^6/ml)	1031.8 ± 42.4	1005.6 ± 70.8
Fresh motility (%)	83.8 ± 1.1[A]	74.4 ± 1.2[B]
Total anomalies (%)	16.6 ± 3.7[A]	65.2 ± 6.2[B]
Post-thawing motility (%)	67.50 ± 3.13[A]	40.91 ± 3.92[B]
AIL (%)[a]	89.04 ± 3.31[A]	53.55 ± 2.39[B]
HOS+ (%)[b]	67.00 ± 3.09[A]	36.00 ± 1.38[B]
Tunel + (%)[c]	6.50 ± 1.01[a]	8.36 ± 1.83[b]
T/S ratio[d]	0.77 ± 0.03[A]	0.43 ± 0.06[B]

[A, B] Values within columns with different superscripts are different; P < 0.01.
[a, b] Values within columns with different superscripts are different; P < 0.05.
[a] AIL = acrosome intact live.
[b] HOS+ = membrane integrity.
[c] Tunel + = DNA fragmentation index.
[d] T/S = average ratio of telomere repeats copy number to a single copy gene.

However, a bull effect (P < 0.01), as well as an interaction bull x treatment (P < 0.05) was found for all traits but DNA fragmentation.

No differences in T/S ratio were recorded in the selected sperm population among the eight suitable bulls, despite differences in motility, viability, membrane integrity, anomalies, cleavage and blastocyst rates (Table 4).

4. Discussion

In this work, the hypothesis that STL can be a prognostic biomarker for semen quality in cattle was tested. To the best of our knowledge, this is the first report on STL measurement in bovine sperm and its correlation with sperm qualitative characteristics. The results of this preliminary work demonstrated that STL is higher in bulls judged suitable for reproductive use and it is correlated with most sperm qualitative characteristics (Table 1). It was also proven that spermatozoa selected with Percoll density gradient have longer telomeres. However, within suitable bulls, STL did not vary among bulls with different in vitro fertilizing ability.

A first objective of the study (Experiment 1) was to evaluate whether STL differ between suitable (Group A) and unsuitable (Group B) bulls for reproductive use, and if STL is correlated to other sperm qualitative traits. The discriminating criteria employed at the Semen Collection Center were post-thawing sperm motility (>and <60%, respectively) and sperm anomalies (<and >20%, respectively).

Table 3
Sperm qualitative traits and T/S ratio at thawing (Pre-Percoll separation) and post-Percoll separation in bulls judged suitable (Group A; 3 ejaculates per 8 bulls) for reproductive use.

	Pre-Percoll separation	Post-Percoll separation
	mean ± SE	mean ± SE
Motility (%)	69.55 ± 0.99[A]	75.60 ± 1.31[B]
AIL[a](%)	76.82 ± 2.53	73.59 ± 2.19
Total anomalies (%)	12.13 ± 1.24	8.70 ± 0.86
HOS+ (%)[b]	62.75 ± 1.22	62.41 ± 1.07
Tunel + (%)[c]	11.68 ± 0.83[a]	10.80 ± 0.82[b]
T/S ratio[d]	0.67 ± 0.03[A]	1.19 ± 0.02[B]

[A, B] Values within columns with different superscripts are different; P < 0.01.
[a, b] Values within columns with different superscripts are different; P < 0.05.
[a] AIL = acrosome intact live.
[b] HOS+ = membrane integrity.
[c] Tunel + = DNA fragmentation index.
[d] T/S = average ratio of telomere repeats copy number to a single copy gene.

As expected, in frozen-thawed sperm TL was higher in Group A that also showed improved motility, viability, membrane integrity (HOS+), a lower incidence of sperm anomalies and DNA fragmentation. Besides, STL was positively correlated to sperm motility, viability and membrane integrity and negatively correlated with sperm anomalies, whereas no correlation was found with DNA fragmentation. An association between STL and both sperm motility and viability was also previously reported in men [3]. In contrast to our study, a negative correlation was also observed between STL and sperm DNA fragmentation in humans [3–29]. Although unsuitable bulls in our study had indeed higher incidence of Tunel + sperm and shorter telomeres, we failed to show an association between these parameters, likely due to the low cases. It is known that the oxidative radicals are normally produced during the sperm maturation, leading to sperm DNA breaks that affect mainly telomeres (G-rich sequences) then non-telomeric DNA. In this way, telomeres act like very sensitive marker of DNA integrity, contributing to cause and also increase sperm DNA fragmentation [3–30]. In contrast, another hypothesis suggests that STL is a consequence of DNA fragmentation, related to several stressors impairing male fertility. For this reason, it is not possible to rule out that DNA fragmentation could occur in the mature sperm nucleus, as a late consequence of the telomere dysfunction.

The lower STL in Group B is in accordance with Ferlin et al. [31] who observed shorter STL in infertile men. In our study, a notable difference in STL, as well as in other sperm qualitative characteristics, was detected between the two groups. This was due to the

Table 2
Kendall's tau_b correlation among sperm qualitative traits at thawing. *Cc* indicates the correlation coefficient.

		T/S[a]	AIL[b] %	Total anomalies %	HOS+[c] %	Tunel+[d] %
Cc	T/S[a]	1.000	0.513[b]	−0.439[b]	0.435[b]	−0.129
P value			0.000	0.000	0.000	0.211
Cc	AIL[b] %	0.513[b]	1.000	−0.393[b]	0.598[b]	−0.052
P value		0.000		0.000	0.000	0.616
Cc	Total anomalies %	−0.439[b]	−0.393[b]	1.000	−0.694[b]	0.149
P value		0.000	0.000		0.000	0.150
Cc	HOS+[c] %	0.435[b]	0.598[b]	−0.694[b]	1.000	−0.115
P value		0.000	0.000	0.000		0.267
Cc	Tunel+[d] %	−0.129	−0.052	0.149	−0.115	1.000
P value		0.211	0.616	0.150	0.267	

[a] T/S = average ratio of telomere repeats copy number to a single copy gene.
[b] AIL = acrosome intact live.
[c] HOS+ = membrane integrity.
[d] Tunel + = DNA fragmentation index.

Table 4
Sperm qualitative traits, cleavage and embryo yields after Percoll separation. Data are reported as mean ± SE.

	Bulls							
	1	2	3	4	5	6	7	8
Motility (%)	70.00 ± 2.58^{AB}	72.50 ± 3.59^{AB}	71.67 ± 3.07^{AB}	70.00 ± 2.58^{AB}	63.33 ± 3.33^{B}	65.00 ± 2.24^{B}	73.33 ± 1.67^{AB}	78.33 ± 1.67^{A}
AIL[a] (%)	80.90 ± 3.75^{A}	80.67 ± 1.81^{A}	81.42 ± 1.44^{A}	81.11 ± 1.33^{A}	81.67 ± 3.26^{A}	56.33 ± 5.75^{B}	78.60 ± 3.51^{A}	48.00 ± 8.16^{B}
Tot. anomalies (%)	7.33 ± 2.51^{ab}	5.83 ± 0.91^{a}	9.17 ± 1.14^{b}	8.33 ± 1.26^{ab}	4.50 ± 0.99^{a}	6.83 ± 1.01^{ab}	6.67 ± 1.84 ^{ab}	12.50 ± 4.81^{b}
HOS+[b] (%)	71.50 ± 1.84^{A}	59.83 ± 2.33^{B}	65.67 ± 2.93^{A}	64.17 ± 2.33^{A}	60.33 ± 2.63^{B}	51.67 ± 3.46^{B}	61.67 ± 1.59^{B}	58.33 ± 2.93^{B}
Tunel +[c] (%)	8.67 ± 1.52	10.00 ± 2.21	8.67 ± 2.12	12.33 ± 4.09	13.00 ± 1.79	15.33 ± 3.26	11.50 ± 1.86	13.83 ± 2.10
T/S ratio[d]	1.22 ± 0.05	1.19 ± 0.06	1.19 ± 0.10	1.05 ± 0.06	1.24 ± 0.06	1.29 ± 0.06	1.01 ± 0.04	1.19 ± 0.05
Cleavage (%)	74.49 ± 1.07^{A}	71.97 ± 2.84^{A}	73.47 ± 3.30^{A}	76.14 ± 3.48^{A}	77.34 ± 4.98^{A}	56.49 ± 5.05^{B}	70.3 ± 4.64^{A}	53.40 ± 5.10^{B}
TMBL/TOT (%)	32.16 ± 2.11^{A}	27.59 ± 3.15^{A}	26.79 ± 2.74^{A}	30.06 ± 3.06^{A}	28.89 ± 4.78^{A}	14.70 ± 4.09^{B}	29.84 ± 5.12^{A}	12.07 ± 4.03^{B}

[A, B] Values within columns with different superscripts are different; P < 0.01.
[a,b] Values within columns with different superscripts are different; P < 0.05.
[a] AIL = acrosome intact live.
[b] HOS+ = membrane integrity.
[c] Tunel + = DNA fragmentation index.
[d] T/S = average ratio of telomere repeats copy number to a single copy gene.

strict criteria used for selecting suitable and unsuitable bulls: indeed, the latter group had motility and anomalies scores that normally discourage their use for reproduction. These preliminary findings suggest that STL can be considered a new marker to distinguish between normal and abnormal spermatogenesis, as it is correlated to most sperm qualitative characteristics.

To further confirm this, in Experiment 2 STL was evaluated within Group A bulls before and after Percoll gradient separation, a common method used to process semen in assisted reproduction. Interestingly, the Percoll selected sperm population exhibited higher STL than total sperm fraction, similar to what previously described in men [16], along with improved motility and decreased incidence of DNA fragmentation [17]. Unexpectedly, the other sperm traits did not improve after separation, mainly due to the bull effect. Undoubtedly, the separation method was efficient to select a sperm population, with longer telomeres.

It is known that a high individual variability in fertilizing ability exist among bulls considered suitable for reproductive use [1,2]. For this reason, a further objective was to assess if STL varies in bulls with different in vitro fertility. Unfortunately, our findings did not confirm the hypothesis that STL could be a new predictive fertility marker. Indeed, no differences were found among bulls after Percoll separation in STL, as well as DNA fragmentation, despite variations in the other parameters, including cleavage and blastocyst rates after IVF. Therefore, STL can be used as a semen quality marker for a heterogeneous population of bulls, but fails to predict the in vitro fertilizing ability within a more homogeneous population of bulls, already selected as suitable based on post-thawing motility and anomalies scores. It is worth noting that Percoll method selected a sperm population with higher STL, eliminating previous individual differences. For this reason, it will be very interesting to evaluate in the future whether STL in fresh semen could be used to preliminarily screen bulls for fertility, for potential applications.

In conclusion, it was demonstrated that the STL can be consider a new biomarker of sperm quality in cattle. Indeed, STL was higher in bulls suitable vs unsuitable for reproduction, as well as after Percoll separation. Further studies are, however, needed to better understand the role of STL in sperm function, spermatogenic activity and fertility. Finally, in future perspective it will be worth evaluating whether STL could predict in vitro or in vivo fertilizing ability of bulls in a larger number of non-selected samples.

CRediT author statement

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Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.theriogenology.2020.09.019.

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