Interbreed differences in Bovine semen quality characteristics before and after cryopreservation

Sara Ataei-Nazari[1], Filip Benko[1], Michal Ďuračka[2], Peter Ivanič[3], Eva Tvrdá[1,4]

[1]Institute of Biotechnology, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2 – 94976 - Nitra, Slovakia
[2]AgroBioTech Research Centre, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2 – 94976 - Nitra, Slovakia
[3]Slovak Biological Services a.s., Breeding station in Lužianky - 951 41 - Lužianky, Slovakia
[4]Chair of Animal Breeding and Biotechnology, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Sciences, Kreutzwaldi 62 – 51006 - Tartu, Estonia

Abstract
The current experiment was conducted to evaluate the semen parameters in Holstein, Simmental and Limousin breeds before and after cryopreservation. Semen was collected from 5 bulls from each breed. The computer-assisted sperm analysis was used to evaluate the motility parameters including total and progressive motilities and secondary kinematic parameters including path velocity, linear velocity, curvilinear velocity, lateral amplitude of head displacement, beat cross frequency, straightness and linearity in fresh and post-thawing semen samples. Fresh semen parameters were not affected by the type of species, whereas there were some post-thawing differences in semen quality characteristics. Post-thawing total and progressive motilities were highest in Holstein semen followed by Simmental and Limousin breeds. Moreover, progressive velocity was highest, medium and lowest respectively in Holstein, Simmental and Limousin breeds. The results from the current demonstrate that the semen from Holstein as a dairy breed has more satisfactory quality characteristics comparing to other two breeds which can be critical in fertility and conception rate and the sperm cells of this breed are more resistant to cryopreservation comparing to Simmental and Limousin breeds. Further research to explore the underlying mechanisms controlling these superior quality parameters would be beneficial in acquiring strategies to reinforcement the strengths and mitigate the weaknesses.

Keywords: Bovine semen, Cryo-resistance, Interbreeds differences, Quality characteristics.

1. Introduction

The profitability of dairy and beef cattle herds depends on optimum reproductive rates to a large extent. The quality of the bulls semen has an undeniable importance on reproductive success of Bovine breeds in either natural or artificial inseminations [1, 2]. Although many studies have been done to reduce the deleterious effects of cryopreservation on semen characteristics necessary for an acceptable reproductive rate, still much work is remained to be done to further mitigate cryopreservation-induced damage to sperm cells. The concentration, morphology and motility of the sperm cells are the key important factors involved in selection of a semen sample for cryopreservation which are used as a predictor of fertility in *in vitro* and *in vivo* conditions [3]. Progressive but not total motility has been reported to have association with pregnancy rates [4]. In addition to concentration and motility, the computer-assisted sperm analysis (CASA) can give various valuable kinematic parameters including path velocity (VAP), linear velocity (VSL), curvilinear velocity (VCL), lateral amplitude of head displacement (ALH), beat cross frequency (BCF), straightness (STR) and linearity (LIN). The kinematic parameters of VSL, VCL and VAP has been reported to have correlation

* Corresponding author: Eva Tvrdá, +421-37-641-4918, eva.tvrda@uniag.sk

with pregnancy rate. Comparing to progressive motility, VAP showed to be better predictor of fertilizing potential of fresh or post-thawed bull semen and comparing to VCL and VSL, VAP showed the highest correlation with fertility making it as the most reliable kinematic parameter for estimation of sperm fertilizing capacity [5, 6]. Many research reported the difference in semen quality characteristics of various Bovine breeds which can affect the fertility and conception rates [7-9]. Moreover, sperm cells antioxidant properties has been showed to be inter-breeds and inter-individual in some specific breed [10, 11]. Determining these differences in sperm parameters among various breeds and exploring the underlying mechanisms responsible for these differences would help in acquiring strategies to improve the strengths and mitigate the weaknesses in various breeds which can finally lead to optimum sperm cryo-resistance.

Hence, the objective of the current study was to investigate if there are any breed-specific differences in sperm cells characteristics including motility and secondary kinematic parameters in fresh semen samples of Holstein dairy breed, Simmental dual-purposed and Limousin beef breeds and also to evaluate how these quality characteristics response to cryopreservation.

2. Materials and methods

Samples collection

Samples of semen were collected from bulls (5 bulls in each breed) at the Slovak Biological Services (Nitra, Slovakia) with the same age condition kept in similar environmental, housing and nutritional conditions. Three semen samples were collected regularly from each animal through using a sterilized artificial vagina. The collected samples were immediately transferred into sterile pre-heated vials and transported into the laboratory within 20 minutes.

Freezing procedure

The ejaculates related to each bull in each breed were extended to 11×10^6 spermatozoa/ml with Triladyl (Minitüb GmbH, Germany), comprising 20% (w/v) egg yolk, Tris, citric acid, sucrose, buffering agents, glycerol, antibiotics, and distilled water. Then the semen samples were transferred into 0.25 ml French straws, cooled at 4°C for 2 h, and subsequently frozen according to the following schedule: –3°C/min from +4 to –10°C; –40°C/min from –10 to –100°C; –20°C/min from –100 to –140°C using a digital freezer (Digitcool 5300 ZB 250; IMV, France). Lastly, the straws were transferred into liquid nitrogen (–196°C) and stored for 30 days. The straws were thawed at 37°C in a water bath for 20 s immediately before the analysis.

The CASA system (version 14.0 TOX IVOS II, Hamilton-Thorne Biosciences, Beverly, CA, USA) was used to evaluate the sperm motility in fresh and post-thawing semen samples as previously described [12]. Total and progressive motilities were defined as the proportion of spermatozoa moving at a velocity greater than 5 μm/s and proportion of spermatozoa moving at speeds higher than 20 μm/s respectively. The secondary kinematic parameters including VAP (μm/s), VSL (μm/s), VCL (μm/s), ALH (μm), BCF (Hz), STR (%) and LIN (%) were also evaluated. The samples were diluted in a ratio of 1:40 with physiological saline solution (sodium chloride 0.9% w/v, Bieffe Medical, Grosotto, Italy). Then, 10 microliters of the samples were put into the pre-warmed (37°C) Makler counting chamber (depth 10 μm; Sefi Medical Instruments, Haifa, Israel). Sperm motility, count, and concentration were measured in 10 fields, counting a minimum of 30 cells/fields at a frame rate of 60 Hz and a magnification of 175x.

Data were analysed by GLM procedure of SAS software in a complete randomized design. The model included the effect of breed. Least-square means were computed and tested for differences by the Tukey's test. Difference between least-squared means was significant at $P < 0.05$.

3. Results and discussion

The motility and secondary kinematic parameters of the fresh semen samples of the bulls are presented in Table 1. Total and progressive motilities were not statistically different in various breeds; however, the values were higher in Holstein comparing to other two breeds. There was no difference in VAP, VSL, VCL, ALH, BCF, STR and LIN affected by type of breed for fresh semen samples. Regarding post-thawing semen samples, total and progressive motilities were significantly higher in Holstein comparing to

Limousin breed, whereas Simmental was in the middle. This pattern of difference was also seen for VAP, VSL and VCL, however the differences did not reach statistical significance for VAP and VCL. These kinematic parameters represent various forms of motilities. The VAP shows the sperm head average velocity along its path which is computed by smoothing the actual path according to algorithms in the CASA. VCL represent the velocity of sperm head on time-average basis along its actual curvilinear path (measured in in two dimensions). The VSL indicate sperm head mean velocity along the straight line between its first and last detected position.

The ALH measures the lateral displacement of sperm head about its average path expressed as maximum or average of such displacement. LIN estimates a curvilinear path reflecting the straightness of the sperm path (VSL/VCL×100; %). The STR reflects the righteousness of motion (VSL/VAP×100, %). These breed specific difference in post-thawed semen samples, despite the lack of difference in fresh samples demonstrate the influence of cryopreservation.

Table 1. The difference between sperm characteristics in different breeds before cryopreservation.

parameters	Holstein	Simmental	Limousin	SEM[1]	P-value
MOT (%)	88.14	84.85	85.00	3.03	0.69
PROG (%)	61.71	57.28	56.85	2.55	0.35
VAP (μm/s)	39.18	43.2	40.98	2.27	0.48
VSL (μm/s)	41.66	45.87	42.95	2.19	0.40
VCL (μm/s)	60.7	63.08	60.15	2.94	0.75
ALH (μm)	3.6	3.64	3.58	0.12	0.94
BCF (Hz)	17.6	18.4	17.38	0.91	0.70
STR (%)	21.00	20.88	20.85	0.71	0.98
LIN (%)	20.14	21.00	19.71	0.64	0.37

[1]Standard Error of the Mean.
MOT – motility, PROG – progressive motility, VAP – path velocity, VSL – progressive velocity, VCL – track speed, ALH – lateral amplitude, BCF – beat frequency, STR – straightness, LIN – linearity.

Table 2. The difference between sperm characteristics in different breeds after cryopreservation.

parameters	Holstein	Simmental	Limousin	SEM[1]	P-value
MOT (%)	58.42[a]	44.42[ab]	32.28[b]	6.60	0.03
PROG (%)	36.00[a]	24.28[ab]	18.71[b]	4.44	0.03
VAP (μm/s)	36.21	29.51	24.55	3.41	0.07
VSL (μm/s)	38.4[a]	30.01[ab]	25.18[b]	3.46	0.04
VCL (μm/s)	54.68	47.84	39.10	4.79	0.09
ALH (μm)	3.00	2.92	2.37	0.24	0.17
BCF (Hz)	16.02	15.00	12.24	1.39	0.16
STR (%)	19.57	19.28	15.85	1.67	0.24
LIN (%)	18.14	17.42	15.42	1.63	0.49

[1]Standard Error of the Mean.
a, b – values in columns with different letters differ significantly (P≤0.05).
MOT – motility, PROG – progressive motility, VAP – path velocity, VSL – progressive velocity, VCL – track speed, ALH – lateral amplitude, BCF – beat frequency, STR – straightness, LIN – linearity.

The proportional reduction in semen evaluated parameters in response to cryopreservation are presented in Table 3. The detrimental effects of cryopreservation on sperm motility characteristics were lowest in Holstein bulls and highest in Limousin bulls, whereas Simmental bulls were in the middle, however the differences were statistically significant for motility. In addition to intrinsic fertility, the post–thawed bovine sperm ability to fertilise an oocyte depends on its cryopreservation resilience. The breed specific differences observed in current work was also reported in other works in various bovine breeds [7-9, 13-

16]. In the studies of Hoflack, Opsomer [15] and Morrell, Valeanu [9], the quality characteristics of semen from dairy bulls of Holstein and Swedish red was superior comparing to semen samples from beef bulls of Belgian Blue, Limousin, Charolais, Simmental, Hereford and Angus which is in agreement with our current work. Many factors may contribute to sperm cell cryo-resistance including semen and sperm cell proteins profile, membrane polyunsaturated fatty acids content, intracellular calcium content and antioxidant system [17, 18]. Although breed specific differences in adaptability to environmental conditions scrotal circumference and also intensive selection in dairy sires for production traits in offspring and sperm quality was proposed as underlying mechanisms controlling the observed breed-induced differences [9], further research is needed to explore the exact contributing mechanisms in various breeds.

Table 3. Cryopreservation-induced reduction in sperm characteristics in different breeds.

parameters	Holstein	Simmental	Limousin	SEM[1]	P-value
MOT (%)	32.78[a]	46.70[ab]	61.82[b]	7.90	0.05
PROG (%)	40.14	56.81	66.45	8.18	0.09
VAP (μm/s)	13.35	30.64	37.66	8.40	0.13
VSL (μm/s)	14.45	33.67	38.94	8.34	0.12
VCL (μm/s)	13.37	22.98	35.62	7.88	0.16
ALH (μm)	15.10	19.40	32.68	7.01	0.20
BCF (Hz)	10.56	17.75	26.50	8.57	0.43
STR (%)	6.59	7.83	24.68	7.84	0.21
LIN (%)	9.82	16.71	21.72	8.35	0.60

[1]Standard Error of the Mean.
a, b – values in columns with different letters differ significantly (P≤0.05).
MOT – motility, PROG – progressive motility, VAP – path velocity, VSL – progressive velocity, VCL – track speed, ALH – lateral amplitude, BCF – beat frequency, STR – straightness, LIN – linearity.

4. Conclusions

The results demonstrate that comparing to Simmental and Limousin breeds, Holstein bulls have semen with higher motilities and secondary kinematic parameters. There might be some underlying mechanisms which not only cause higher sperm quality characteristics in fresh semen samples of Holstein bulls but also can exert more cryopreservation and cryo-resistance comparing to Simmental and Limousin bulls. Further research to explore these underlying mechanisms are warranted.

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