## THE INFLUENCE OF THE GENOTYPE ON THE QUANTITATIVE TRAITS OF BOVINE SEMEN

Vidmantas Pileckas, Jonas Kutra, Algirdas Urbšys Institute of Animal Science, Lithuanian Veterinary Academy, R. Žebenkos 12, LT-82317 Baisogala, Radviliškio r. Lithuania, e-mail: vidmantas@lgi.lt

**Summary.** Neither the genotype no the season have any influence on the main qualitative traits and the amount of defective fresh and cryopreserved semen of beef and dairy bulls provided the animal have good feeding and housing conditions. Charolais bulls had the highest ejaculate volume (7.97 cm³) but the lowest sperm concentration in fresh semen (1.24×10 9/cm³). The highest sperm concentration (1.39×10 9/cm³) was determined in the fresh semen of Simmental bulls. The best postthaw sperm motility (40.9%) was found in the Limousine semen, yet the best survival 5 h after thawing was determined for the Salers x Aberdeen Angus semen. Though the postthaw motility of the latter spermatozoa was the lowest (38.5%). Thus, sperm viability in the frozen semen does not depend on the postthaw sperm motility. However, these traits are interdependent with regard to individual genotype (in case of our studies – Sales x Aberdeen Angus and Simmental breeds).

Key words: bulls, genotype, semen, quality.

## GENOTIPO ĮTAKA BULIŲ SPERMOS KOKYBINIAMS RODIKLIAMS

Vidmantas Pileckas, Jonas Kutra, Algirdas Urbšys Lietuvos veterinarijos akademijos Gyvulininkystės institutas R. Žebenkos g. 12, LT-82317 Baisogala, Radviliškio r.; el. paštas: vidmantas@lgi.lt

**Santrauka.** Stabiliai šeriant ir laikant, nei genotipas, nei sezoniškumas šviežios ir kriokonservuotos mėsinių ir pieninių veislių bulių spermos pagrindiniams kokybiniams rodikliams ir brokuojamam kiekiui įtakos nedaro. Šarolė veislės bulių vidutinis ejakuliato tūris buvo didžiausias (7,97 cm³), o spermatozoidų koncentracija šviežioje spermoje – mažiausia (1,24×10 9/cm³). Didžiausia spermatozoidų koncentracija (1,39×10 9/cm³) buvo šviežioje Simentalio bulių spermoje. Judriausi atšildyti spermatozoidai (40,9 proc.) buvo Limuzino veislės bulių spermoje, tačiau iki 5 val. atšildyti geriausiai išgyveno Salers x Aberdino Anguso, nors pastarieji spermatozoidai buvo nejudriausi (38,5 proc.). Taigi spermatozoidų gyvybingumas atšildytoje kriokonservuotoje bulių spermoje nepriklauso nuo jų judrumo. Tačiau atskiro genotipo (mūsų tyrimų atveju – Salers × Aberdino Anguso ir Simentalio veislių) bulių spermoje šie rodikliai susiję.

Raktažodžiai: buliai, genotipas, spermos kokybė.

**Introduction.** In Europe beef cattle were mostly raised in the countries having short winters and comparatively warm climate. After introduction of quotas for milk and milk products and with the increasing demand for beef, the development of beef cattle herds has also spread in the countries of dairy cattle production. Crossing of dairy cows with beef bulls is also spreading (Galvijų ūkis, 2001).

Beef cattle husbandry has not been developed in Lithuania. However, at present specialized beef cattle farms are being established with the aim to raise beef cattle or beef and dairy cattle hybrids. Dairy cows are mostly inseminated with Limousin, Simmental, Charolais and Sales x Aberdeen Angus semen for production of better quality beef. Aberdeen Angus cattle are noted for being polled and thus suitable for group housing. The birth weight of calves is low and therefore the semen of the breed is suitable for heifer insemination. It is thought that reproduction traits of beef cattle are lower due to their physiological peculiarity when all the body resources are mobilized towards weight increase.

Bovine semen quality traits are often influenced by various ambient factors (Chenoweth *et al.*, 1994, Fabbrocini *et al.*, 2000), and the conception rate of beef cattle depends on semen quality (Mickelsen and Memon, 1993).

The number of live spermatozoa (Shannon and Vishwanath, 1995, Мордань, 2002) and the breed (Holt, 2000) are also considered to be the factors of great importance for the conception rate of cows and heifers. It is often stated that semen quality traits of beef bulls are worse than those of dairy bulls (Ahmad *et al.*, 2003, Amann *et al.*, 2000, Correa *et al.*, 1997, Den Daas *et al.*, 1998). However, sometimes part of the semen that does not meet the requirements for bovine semen is rejected. It is expedient to investigate and evaluate the quality of bovine semen of the main beef breeds bred in Lithuania (Charolais, Limousin, Simmental, etc.) and to determine the dependency of quality parameters of fresh and frozen semen on the genotype of bulls.

The purpose of the study was to investigate the dependency of semen qualitative characteristics (fresh semen ejaculate volume, concentration, sperm motility, percentage of frozen semen doses, amount of defective semen, frozen sperm motility and survival after thawing) of beef bulls on the genotype of bulls.

Material and Methods. The study varied out at the Animal Reproduction Department of the Institute of Animal Science of the Lithuanian Veterinary Academy and the joint-stock company "Marijampolės regiono veislininkystė". The semen from beef and dairy bulls with the

sperm motility of no less than 7 points (70%) and concentration not lower than  $0.8 \times 10^9$  /cm<sup>3</sup> were used in the study. Sperm motility was evaluated visually with the hot stage microscope by examining 300x smear. Mixed ejaculates were used in the study. Spermatozoa concentration was determined with a photoelectrocalorimeter. Sperm motility was evaluated after semen thawing water bath in 10 seconds at 40°C and also after 5 h storage at 38°C (Pakėnas, 1993). The extender of the following composition was prepared for semen dilution: lactose - 11.5 g, egg yolk - 20 cm<sup>3</sup>, glycerol - 5 cm<sup>3</sup>, redistilled water -100 cm<sup>3</sup>, penicillin 50000 a.u./100 cm<sup>3</sup>. After evaluation, semen was diluted at a rate of 1:1 with 27±1°C extender, exposed to 19±1°C temperature for 15 minutes and diluted finally with 19±1°C extender. Diluted semen was packaged into straws and placed into one-layer cassettes. The cassettes were transferred into the fridge and cooled at 4±2°C for 4 hours. The semen was deep-frozen using Lithuanian technique at -150±5°C in liquid nitrogen. Freezing cycle lasted 8 minutes. The semen was thawed after 3 days. Spermatozoa motility was evaluated right after thawing and after 5 h storage at 38±0.5°C.

Statistical analysis of the data was performed using Microsoft® Excel 2000 ANOVA analysis of variance and Correlation tools. Tests of significance were accepted at P < 0.05.

**Results and Discussion.** A total of 998 ejaculates, including 531 of Limousin, 271 of Simmental, 133 of Charolais and 63 of Salers × Aberdeen Angus bulls, have been analyzed. Ejaculates from Holstein bulls have been analysed for comparison. 3.2% Limousin, 6.2% Salers × Aberdeen Angus, 2.4% Simmental and 1.0% Charolais bull semen have been rejected as not corresponding to the fresh semen requirements.

*Ejaculate volume.* The average volumes of the first and second ejaculates from Salers × Aberdeen Angus and Charolais bulls were similar (4.51-4.53 cm³ and 4.25-4.38 cm³, respectively) and higher than the average ejaculate volume of the two other breeds. The Charolais breed bulls had the highest total average volume of both ejaculates – 7.96 cm³ (P<0.05; Fig. 1). Thus, this indicator, Charolais bulls are equal to dairy bulls and stand out significantly among the other beef breeds which had the total average volume of both ejaculates ranging from 5.17 to 5.65 cm³.

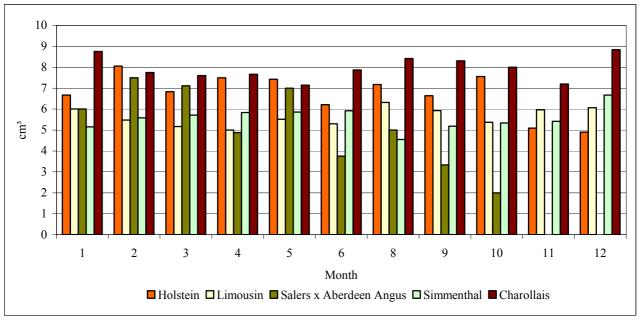


Fig. 1. Average ejaculate volumes of different breeds

Sperm motility in fresh semen. The average sperm motility in the fresh semen was similar all the year round for all beef breeds and accounted for 69.0 and 70.7% in the first and second ejaculate, respectively. However, estimations of the sperm motility in the mixed ejaculates indicated that the fresh semen of Salers × Aberdeen Angus bulls had 6.0% lower sperm motility (P<0.01) than the average sperm motility of bulls representing the population (Fig. 2).

Spermatozoa concentration in fresh semen and semen dilution. Charolais bulls were noted for the lowest sperm concentration in the first and second fresh semen ejaculates (Fig. 3). The concentration was, respectively, 7.5 and 8.4% lower (P<0.001) than the average of bulls

representing the population of beef breeds. The bovine sperm concentration of other beef breeds was similar to that of Holstein breed bulls, i.e. 1.33 and  $1.37 \times 10^9$  /cm<sup>3</sup>.

Increased amounts of fresh second ejaculate semen for rejection was observed for Limousin and Simmental bulls in separate months, but no correlations between this indicator and season or breed have been found.

The semen of Charolais was found to be most suitable for dilution because not even one ejaculate has been rejected. However, the collection of the semen was not distributed evenly, i.e. 61.5% of the yearly amount of semen suitable for dilution was collected in summer and autumn. On the contrary, Salers × Aberdeen Angus bulls produced 83.6% of semen suitable for dilution in winter and spring

months. Regarding other beef breeds, semen was collected evenly all the year round like with dairy breeds. Thus, the above mentioned excesses could be explained not by some specific traits of individual breeds but as a

consequence of divergence from optimum semen collection regimes (semen collection frequency, lack of exercise) that should be adapted not only to the separate breed but to the individual bull, too.

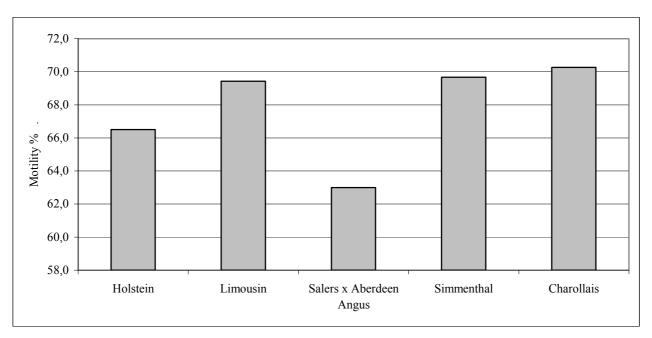


Fig. 2. Sperm motility in fresh semen

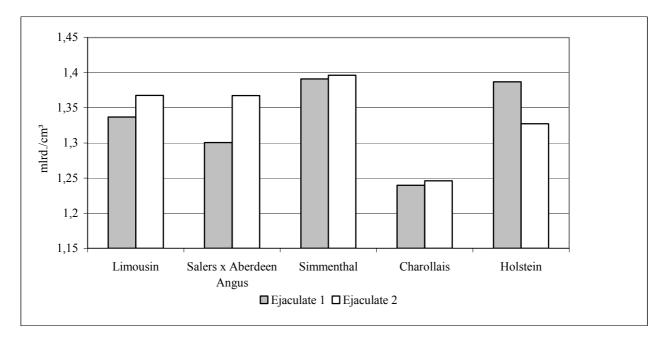


Fig. 3. Average concentration of spermatozoa in fresh semen from different bovine breeds

Dilution rate and volume of diluted semen. Charolais bulls were distinguished by the dilution rate of fresh semen because its dilution rate was the lowest, on the average 1:6.5 (Fig. 4). Obviously, sperm concentration in fresh semen was the decisive factor as the correlation coefficients with the dilution rate were, respectively, r=-0.93 and r=-0.96 for different ejaculate concentration. Nevertheless, the average volume of the diluted Charolais se-

men (60.67 cm<sup>3</sup>) was even 20.4% (P<0.005) higher than that of other beef breeds due to high general volume of fresh semen (Fig. 5).

Dilution rates (1:7.21-1:7.52) and the average volume of diluted semen (48.4-55.0 cm<sup>3</sup>) of other bulls were similar to those of beef and dairy breeds, i.e. 1:7.25 and 51.8 cm<sup>3</sup>, respectively.

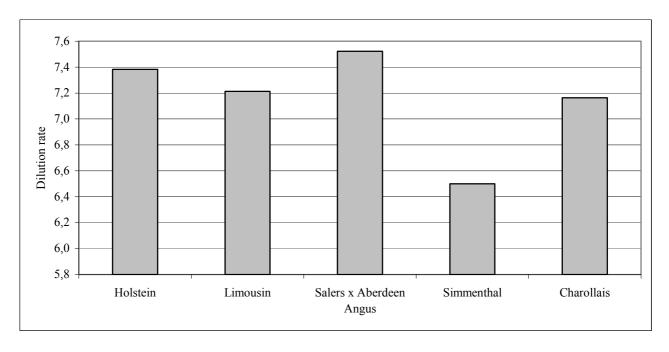


Fig. 4. Average semen dilution rates for different breeds

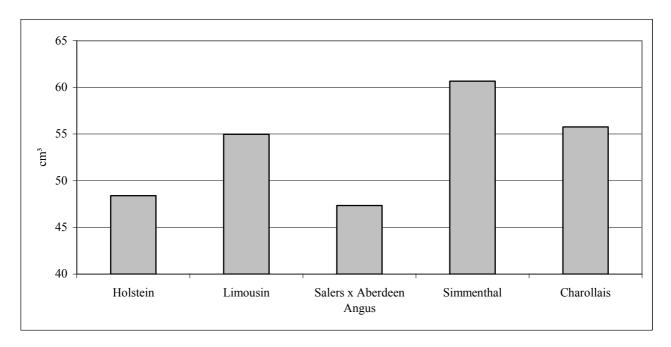


Fig. 5. Average volumes of diluted semen

**Post-thaw sperm motility and survival.** No significant differences were found between different beef breeds for the both post-thaw sperm motility and motility after 5 h semen incubation at 38±0.5 °C. The effect of season on sperm motility is still lower.

The examining of the samples of frozen semen in which thawed spermatozoa survived for 5 h or more at  $38\pm0.5$  °C indicated no dependency of sperm survival on sperm motility after thawing (r = 0.21). However, sperm survival of Salers x Aberdeen Angus and Simmental bulls was highly dependent on post-thaw sperm motility, respectively, r = 0.68 and r = 0.73. Sperm survival of

Charolais and Limousin bulls was almost not influenced by post-thaw sperm motility or even had inverse dependency, respectively, r = 0.25 and r = -0.26.

## Conclusions

- 1. Neither the genotype non season have any influence on the main quality indicators of fresh and frozen semen from beef and dairy bulls provided the conditions of feeding and housing are adequate.
- 2. There are no significant differences of spermatozoa motility after semen thawing among bulls of different beef cattle breeds.
  - 3. Sperm survival in the frozen semen does not de-

pend on post-thaw sperm motility. However regarding the individual genotype (Salers x Aberdeen Angus and Simmental in our study), these traits were interdependent.

## References

- Ahmad Z., Anzar M., Shahab M., Ahmad N., Andrabi S. M. H. Sephadex and sephadex ion exchange filtration improves the quality and freezability of low-grade buffalo semen ejaculates. Theriogenology. 2003. Vol. 59. N 6. P. 1189-1202.
- Amann R., Seidel G., Mortimer R. Fertilizing potential in vitro of semen from young beef bulls containing a high or low percentage of sperm with a proximal droplet. Theriogenology. 2000. Vol. 54. P. 1499-1515.
- 3. Chenoweth P.J., Risco C.A., Larsen R.E., Velez J., Tran T., Chase C.C. Effects of dietary gossypol on aspects of semen quality, sperm morphology and sperm production in young Brahman bulls. Theriogenology. 1994. Vol. 42. P. 1-13.
- Correa J., Pace M., Zavos P. Relationships among frozenthawed sperm characteristics assessed via the routine semen analysis, sperm functional tests and fertility of bulls in an artificial insemination program. Theriogenology. 1997. Vol. 48. P. 721-731.
- Den Daas J.H., De Jong G., Lansbergen L.M., Van Wagtendonk-De Leeuw A.M. The relationship between the number of spermatozoa inseminated and the reproductive efficiency of individual dairy bulls. J. Dairy Sci. 1998. Vol. 81. P. 1714-1723.
- Fabbrocini A., Del Sorbo C., Fasano G., and Sansone G. Effect of differential addition of glycerol and pyruvate to extender on cryopreservation of mediterranean buffalo (B. bubalis) spermatozoa. Theriogenology. 2000. Vol. 54. P. 193-207.
- Galvijų ūkis. Sudaryt. P. Bendikas ir A. Kersnauskas. Kaunas: "Aušra". 2001. P. 8-9.
- Holt W. Fundamental aspects of sperm cryobiology. The importance of species and individual differences. Theriogenology. 2000. Vol. 53. P. 47-58.
- Mickelsen W.D., Memon M.A. Relationship of post-thaw semen evaluation to pregnancy rates in beef cows. Agri-Practice. 1993. Vol. 14. P. 24-28.
- Pakėnas P. Veislinių bulių laikymo ir naudojimo Lietuvos technologija. Vilnius: Academia. 1993. P. 3, 6, 46.
- Shannon P., Vishwanath R. The effect of optimal and suboptimal concentrations of sperm on the fertility of fresh and frozen bovine semen and a theoretical model to explain the fertility differences. Anim. Reprod. Sci. 1995. Vol. 39. P. 1-10.
- 12. Мордань Г.Г. Методы оценки биологической полноценности спермы быков-производителей и оптимальное количество спермиев в дозе для осеменения. Автореф. дис. канд. сельск. наук. Жодино. 2002. С. 9, 11, 15