

EVALUATION OF POST THAWED SEMEN MOTILITY OF HOLSTEIN BULLS BY COMPUTER ASSISTED SPERM ANALYSIS (CASA)

ABSTRACT

The objective of this study was to evaluate the quality of thawed semen of Holstein bull by computer assisted sperm analysis (CASA). A total sample of 40 straws imported from USA (total bulls = 4; 5 ejaculates per bull; 2 straws per ejaculate) was analysed by CASA using an IVOS. Semen motility was evaluated for kinetics parameters after thawing. The percentage of total mobile spermatozoa (%TMOB) and the percentage of progressive spermatozoa (%PROG) were determined. Several velocities parameters were also determined: average path velocity (VAP, $\mu\text{m/s}$), curvilinear velocity (VCL, $\mu\text{m/s}$) and straight-line velocity (VSL, $\mu\text{m/s}$). Statistical analysis was conducted using SAS software (version 1997). Analysis of variance was carried out using the GLM procedure. DUNCAN test was used to compare motile variable between bulls. Our results showed that the percentages of TMOB and PROG spermatozoa were higher in the semen of 3 bulls compared to the fourth (% TMOB = 50, 56 and 60 % vs 34 %, $p < 0.01$; % PROG = 19, 31 and 28 % vs 11 %, $p < 0.01$). The VAP, VCL and VSL were also higher in the semen of the same 3 bulls compared to the fourth one (VAP = 83, 86 and 109 $\mu\text{m/s}$ vs 70 $\mu\text{m/s}$, $p < 0.01$; VCL = 132, 138 and 187 $\mu\text{m/s}$ vs 116 $\mu\text{m/s}$, $p < 0.01$; VSL = 70, 75 and 89 $\mu\text{m/s}$ vs 59 $\mu\text{m/s}$, $p < 0.01$). Our study suggested that semen from 3 bulls had a good quality compared to the fourth one. It was concluded that their semen can be used in an artificial insemination (AI) program. However, the semen of the fourth bull was not allowed to be utilized in an AI program because it had low kinetics parameters. So, we concluded that imported Holstein bull semen should be assessed by CASA before its use in AI.

Keywords semen motility, Holstein bull, CASA, quality, AI.

1. INTRODUCTION

Artificial insemination (AI) is the most valuable breeding management tool available to dairy cattle breeders to improve the genetic potential and performance of their herds. In Tunisia, the holstein dairy cattle are inseminated by imported frozen semen of high indexed bulls in order to improve fertility and performance of milk production of our herds.

The aim of this study was to determine the sperm motile parameters of imported frozen semen after thawing using a computer assisted sperm analysis (CASA).

2. MATERIAL AND METHODS

2.1. Samples:

The study took place in the laboratory of the control of semen quality in the Ministry of Agriculture of Tunisia. A total of 40 straws imported from USA (total bulls = 4; 5 ejaculates per bull; 2 straws per ejaculate) was used in this study.

2.2. Semen motility:

The straws were thawed in the water bath at 37°C during 30 sec (1). After that, the semen was diluted in the Bioxell® extender (IMV France) at the dilution rate of 1/4. This later was previously placed in a water bath at 37° C and incubated for 10 minutes at the same temperature. Sperm motility was assessed using Hamilton Thorn Motility Analyzer (HTM, version 12.1 M) as described by Gerard et al. (2). For this purpose, 2 drops per straw were placed in the microcell chamber and evaluated for percentage of total motile sperm (%TMOB), percentage of progressive sperm (%PROG) and the kinetic parameters: average path velocity (VAP, $\mu\text{m/s}$), straight line velocity (VSL, $\mu\text{m/s}$) and curvilinear velocity (VCL, $\mu\text{m/s}$).

2.3. Statistical analysis:

Statistical analysis was conducted with SAS software (version 1997). Analysis of variance was carried out using the GLM procedure. DUNCAN test was performed to compare motile variable between bulls. Statistical significance was considered at $p < 0.05$.

3. RESULTS AND DISCUSSION

The results showed that the percentage of total motile (%TMOB) and progressive sperm (%PROG) in post-thawed semen of Holstein bulls (Table 1) were higher in bulls 1, 2 and 3 compared to those of bull 4 ($p < 0.01$). Alapati et al. (3) reported that the percentage of motile sperm must be above 33 %. For the percentage of progressive sperm, Galli et al. (4) said that this later should be above 22%. These motile parameters are important because they were highly correlated with sperm viability and fertility (5).

The kinetic sperm parameters (VAP, VSL and VCL) were higher in the semen of bulls 1, 2 and 3 compared to those of the semen of bull 4 ($p < 0.01$). These parameters obtained in bull 4 were considered lower according to what has been reported by Hallap et al. (6). Consequently, we can say that semen of bull 4 had the lowest quality and could decrease the fertility in our herds.

	Bull 1	Bull 2	Bull 3	Bull 4
%TMOB	50 ^a	56 ^a	60 ^a	34 ^b
%PROG	19 ^a	31 ^a	28 ^a	11 ^b

a, b: $p < 0.01$

Table 1: The percentage of total motile (%TMOB) and progressive sperm (%PROG) in post-thawed semen of Holstein bulls.

	Bull 1	Bull 2	Bull 3	Bull 4
VAP ($\mu\text{m/s}$)	83 ^a	86 ^a	109 ^b	70 ^c
VSL ($\mu\text{m/s}$)	132 ^a	138 ^a	187 ^b	116 ^c
VCL ($\mu\text{m/s}$)	70 ^a	75 ^a	89 ^a	59 ^b

a, b, c: $p < 0.01$

a, b: $p < 0.01$

Table 2: The kinetic sperm parameters in post-thawed semen of Holstein bulls.

3. CONCLUSION

We concluded that semen of bulls 1, 2 and 3 can be used in an artificial insemination (AI) program. However, semen of the bull 4 cannot be used in an AI program because of its low motile and kinetics parameters, because it could decrease the fertility of our herds. It can be concluded that imported Holstein bull semen should be assessed in the laboratory before its use in AI program.

5. REFERENCES

1. Mazur P., 1984. Freezing of living cells: mechanisms and implications. *Am. J. Physiol.*, 247: 125-142.
2. Gerard O., Ponsart C., Petit M., Humblot P., 2008. Technical practices and evolution of semen processing and artificial insemination in cattle. *Renc. Rech. Ruminant*, 15: 351-354.
3. Alapati R., Stout M., Saenz J., Gentry G.T., Godke R.A., Devireddy R.V., 2009. Comparison of the permeability properties and post-thaw motility of ejaculated and epididymal bovine spermatozoa. *Cryobiology*, 59(2): 164-170.

4. Galli A., Basetti M., Bulduzzi D., Martnonna M., Bornaghi V, Maffii M., 1991. Frozen bovine semen quality and bovine cervical mucuspenetration test. *Theriogenolgy*, 34(4): 837-844.
5. Kommisrud E., Graffer T., Steine T., 1996. Comparison of two processing systems for bull semen with regardto post-thaw motility and non return rates. *Theriogenology*, 45: 1515-1521.
6. Hallap T., Haard M., Jaakma U., Larsson B., Rodriguez-Martinez H., 2004. Does cleansing of frosen-thawed bull semen befote assessment provide simples that relate better to potential fertility? *Theriogenology*, 62(3-4): 702-7013.

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