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Computerized and Subjective Assessments of Post-thaw Motility of Semen from Finnish Ayrshire AI Bulls in Relation to Non-return Rates

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Andersson, M., T. Hellman, B-G. Holmström and L. Jokinen: Computerized and subjective assessments of post-thaw motility of semen from Finnish Ayrshire AI bulls in relation to non-return rates. Acta vet. scand. 1992, 33, 89-93. – A semen analyser (Lazymot), was used to evaluate post-thaw motilities in 296 batches of semen from 74 Ayrshire bulls used for artificial insemination (AI). Motility was also assessed subjectively. A significant correlation was observed between assessments of motility using the Lazymot analyser and the subjective assessments. There was no correlation between post-thaw motility assessments and non-return rates in relation to the batches examined, which met Finnish criteria for use in AI. This suggests that criteria for post-thaw semen motility should not be increased beyond the present requirement for 40% motile spermatozoa.

sperm motility; non-return rate; dairy bull.

Introduction

Sperm motility assessment is widely used to evaluate bovine ejaculates. Subjective assessment of post-thaw motility is more difficult than initial motility studies of diluted semen because the thawing process and post-thaw incubation time affect the motility.

Few computerised post-thaw motility studies related to fertility have been conducted using semen samples accepted for use in artificial insemination (AI). The conception rate of unfrozen bovine semen exhibiting a low progressive motility has been studied by *Swanson & Herman* (1944). In the study reported below, motilities of semen meeting current Finnish criteria for use in AI were investigated subjectively and by means of a motility analyser (Lazymot) and the results were correlated to fertility. Recently, several such analysers have been marketed including the Autosperm, CellSoft, HTM-S and Lazymot analysers. Interest in automated methods for analysing motility in artificial insemination stations has been increasing. Through use of such methods motility assessment could be more uniform than if it was based on examinations by 2 or 3 technicians, which inevitably involve differences in acceptance criteria. If a positive correlation between a motility parameter and non-return rates could be demonstrated, frozen semen could be categorized by value of this parameter. Only semen of high quality could be used. Low quality semen could be rejected. Any improvement in non-return rate would allow the cost of the semen analyser to be recouped.

Materials and methods

Ejaculate densities were measured at 6 AI centres, using a photometer. The freezing extender consisted of lactose solution, egg yolk, glycerol and neomycin sulfate. The final glycerol concentration in the diluted semen was 3.5 vol %. The semen was frozen after an equilibration time of about 4 h. Frozen semen pellets from ejaculate batches selected for use in AI were stored in liquid nitrogen and sent from the 6 AI centres to a semen laboratory. In all, 296 batches from 74 bulls were investigated. Each semen pellet (0.1 ml) was thawed in 1 ml of 3.2% sodium citrate (PH 7, 37°C), and incubated for 10 min in a water bath at 37°C before semen analysis. Progressive motility was evaluated subjectively and using a Lazymot sperm motility analyser (Lazymot, BTG, Biotechnik GmbH, Düsseldorf, FRG). The Lazymot analyser consists of a unit with computer software and a printer. The Doppler shift of light from a helium-neon laser scattered by the moving spermatozoa is measured to allow their motility to be quantified. The analyser allows determination of the concentrations, motilities, progressive motilities, velocities and progressive velocities of spermatozoa under temperature-controlled conditions. Ten chambers can be filled with semen samples. According to the manufacturer, concentrations of the semen samples should lie within the range 3-250 x 10^6/ml. We used concentrations varying from 20 to 45 x 10^6/ml (haemocytometer count). Cows and heifers were inseminated with semen from bulls so that inbreeding was avoided. Percentage non-return rates (NR%) within 60 days of 9953 first inseminations using pellets from the same batches studied here were calculated. The insemination dose contained 10-15 x 10^6 motile spermatozoa. Only accepted semen was used for AI. The Finnish criteria for use in AI are based on initial progressive motility and post-thaw motility. The initial progressive motility must be at least 60% and the post-thaw motility must be at least 40%. Semen of low density (density of less than 0.4 x 10^9/ml as evaluated with a photometer) is not frozen.

Pearson correlation coefficients were calculated using the Statistical Analysis System (1985).

Results

The average progressive motility for the 296 semen batches determined using the Lazymot analyser was 39.8%, (range 22 to 56%). Average total motility was 58.8%. The average motility determined subjectively was 51.6%, (range 40 to 65%).

Post-thaw motility determined subjectively was found to correlate statistically significantly with motilities determined using the Lazymot analyser ($p < 0.001$). Values for post-thaw motility measured subjectively also correlated significantly ($p < 0.001$) with values for mean velocities of motile spermatozoa and of progressive spermatozoa measured using the Lazymot analyser.

Motility measured subjectively and by analyser, and density values obtained photometrically were not significantly correlated with NR% values. Results are summarized in Table 1.

The average non-return rate of the 9 semen batches with the lowest Lazymot progressive motility values (< 30%) was 55.8% compared to 61.2% of all inseminations in this study.

Discussion

Use of bovine unfrozen semen exhibiting a low progressive motility (< 45% motile spermatozoa) in AI has been shown to be associated with a low conception rate *(Swanson & Herman,* 1944). In the study reported here,

Table 1. Pearson correlation coefficients of Lazymot values, subjective motility estimations and non-return rate of post-thaw semen (9953 inseminations).

	Pdens.	Smot.	Conc.	Mot.	Vmot.	Progr.	Vprogr.	NR%
Pdens.	1.00000[1)]	0.05290	–0.06241	0.17907	0.11369	0.16931	0.10547	0.05180
	0.0000[2)]	0.3644	0.2954	0.0020	0.0507	0.0035	0.0700	0.3745
	296[3)]	296	296	296	296	296	296	296
Smot.	0.05290	1.00000	–0.17517	0.24721	0.38926	0.33451	0.37632	–0.00188
	0.3644	0.0000	0.0031	0.0001	0.0001	0.0001	0.0001	0.9743
	296	296	296	296	296	296	296	296
Conc.	–0.06241	–0.17517	1.00000	0.06995	–0.14440	–0.02997	0.20595	–0.08570
	0.2954	0.0031	0.0000	0.2408	0.0150	0.6157	0.0005	0.1504
	296	296	296	296	296	296	296	296
Mot.	0.17907	0.24721	0.06995	1.00000	0.82411	0.94054	0.73605	0.07006
	0.0020	0.0001	0.2408	0.0000	0.0001	0.0001	0.0001	0.2295
	296	296	296	296	296	296	296	296
Vmot.	0.11369	0.38926	–0.14440	0.82411	1.00000	0.94954	0.92335	0.02302
	0.0507	0.0001	0.0150	0.0001	0.0000	0.0001	0.0001	0.6933
	296	296	296	296	296	296	296	296
Progr.	0.16931	0.33451	–0.02997	0.94054	0.94954	1.00000	0.84189	0.06070
	0.0035	0.0001	0.6157	0.0001	0.0001	0.0000	0.0001	0.2979
	296	296	296	296	296	296	296	296
Vprogr.	0.10547	0.37632	–0.20595	0.73605	0.92335	0.84189	1.00000	0.00973
	0.0700	0.0001	0.0005	0.0001	0.0001	0.0001	0.0000	0.8676
	296	296	296	296	296	296	296	296
NR%	0.05180	–0.00188	–0.08570	0.07006	0.02302	0.06070	0.00973	1.00000
	0.3745	0.9743	0.1504	0.2295	0.6933	0.2979	0.8676	0.0000
	296	296	296	296	296	296	296	296

[1)]Correlation coefficient
[2)]Signifiance level
[3)]No. batches

Pdens. = Photometer measured density of ejaculated semen.
Smot. = Subjective post-thaw motility
Conc. = Concentration of post-thaw semen (Lazymot).
Mot. = Motility (%) of post-thaw semen (Lazymot).
Vmot. = Mean velocity (μm/s) of post-thaw semen (Lazymot.)
Progr = progressive motility (%) of post-thaw semen (Lazymot).
Vprogr. = Mean velocity (μm/s) of progressively motile post-thaw semen (Lazymot).
NR% = Non-return rates within 60 days of first inseminations.

results obtained using the Lazymot analyser were compared with subjective assessments of semen motility. Motility measured subjectively correlated significantly with motility measured using the Lazymot analyser. Motility measured using the Lazymot analyser was higher and progressive motility was lower than motility assessed subjectively. No correlation between motility assessment and the fertility of post-thaw semen was observed, whether using the Lazymot analyser or assessing motility subjectively in semen batches meeting Finnish criteria for use in AI. However, the average non-return rate of the 9 batches with the lowest Lazymot progressive values was shown to be lower than the average non-return rate in this study. The Cellsoft semen analyser has also been used to study the correlations between sperm motility and the fertility of frozen bovine semen. No significant correlation was found *(Budworth et al.* 1988).

In earlier preliminary work with the Lazymot analyser we noted that if 3 pellets were thawed rather than 1 in the same volume of diluent (1 ml), results for motility were about 10% higher. Spermatozoa concentration should therefore be kept as constant as possible when the Lazymot analyser is used. This might explain why some investigators have obtained unsatisfactory results using the Lazymot anlyser. Another reason for such results might be the inability of the instrument to distinguish between spermatozoa and debris (*Agrawal & Vanha-Perttula* 1989, *Brotherton* 1988, *Schirren et al.* 1988). In one study (*Heiskanen et al.* 1991), the progressive motility of diluted stallion semen (milk extender) determined using the Lazymot analyser was significantly different ($p < 0.001$) from that determined subjectively. The Lazymot analyser was unable to distinguish between particles of the size of spermatozoa in milk extender and spermatozoa themselves. Values for motility were therefore too low (*Heiskanen et al.* 1991). In the same study, mean values for progressive motility for fresh, undiluted stallion semen measured using the Lazymot analyser and subjectively did not differ significantly. Other automatized semen analysers have also been reported to give rise to problems with debris. In 1 study on monkey semen, the CellSoft was unable to discriminate between intact sperm heads and debris (*Yeung et al.* 1988).

In conclusion, we suggest that semen should be diluted with clear fluids, and that the sperm concentrations should be standardized to allow comparable results to be obtained using the Lazymot system. Neither the motility results obtained using the Lazymot system nor subjective motility assessments of semen pellets meeting Finnish criteria for use in AI correlated with NR% values. Motility assessments of thawed bovine semen using the Lazymot analyser were no worse than subjective motility assessments for selecting semen for use in AI. Because motility assessments did not correlate with NR%, setting a motility requirement above 40% for post-thaw semen would not be likely to increase NR%. However, setting a lower subjective post-thaw motility requirement than 40% would probably decrease fertility. An additional study using batches with a subjective motility of less than 40% and less than 30% would elucidate the role of post-thaw motility on fertility. This could not be undertaken in this study while cows inseminated were owned by private farms.

Because automatized systems are expensive and errors can arise because of debris, subjective assessment will remain the fundamental method for motility estimations in AI stations.

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Sammanfattning

Automatiserad och subjektiv motilitetsbedömning av upptinad sperma från finska Ayrshire semintjurar i relation till icke omlöparprocenten.

En sperma-analysator (Lazymot), användes till att undersöka 296 upptinade frysoperationer av sperma från 74 semintjurar av Ayrshire ras. De upptinade spermadosernas motilitet bedömdes också subjektivt. Sperma-analysatorns motilitetsvärden var signifikant korrelerade till den subjektiva motilitetsbedömningens värden.

Varken sperma-analysatorns eller den subjektiva bedömningens motilitetsvärden var korrelerade till icke omlöparprocenten i denna studie bestående av selekterade frysoperationer.

Detta resultat visar att krav på högre spermamotilitet än 40% på djupfryst tjursperma inte är motiverade.

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