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Relationship between ATP Content and Motility in Bovine Spermatozoa with Reference to the Effects of the Bull and the A.I.Centre

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Söderquist, L., and E-M. Stålhammar: Relationship between ATP content and motility in bovine spermatozoa with reference to the effects of the bull and the A.I.centre. Acta vet. scand. 1991, 32, 353–359. – Deep-frozen semen from 28 bulls belonging to 6 different A.I.centres was studied after thawing and the ATP content in the spermatozoa was assayed using a bioluminescence technique. The sperm motility was subjectively estimated under a phase contrast microscope and the sperm concentration of each ejaculate was calculated in a haemocytometer. The overall mean ATP content was 16.6 nmoles ATP/spermatozoa $\times 10^8$. There was a significant variation in ATP content between A.I.centres. Significant differences between bulls in ATP content were found as well as a significant correlation between ATP concentration and the number of motile spermatozoa. This may indicate that ATP assessment may be useful as an additional, objective laboratory test.

sperm motility; artificial insemination; adenosine triphosphate; fertility.

Introduction

The worldwide use of deep-frozen bull semen for artificial insemination (A.I.) has increased the need for reliable indicators of sperm quality. It is very common in A.I. centres to use post-thaw sperm motility as the only quality indicator of frozen and thawed semen, and ejaculates are discarded if freezing survival is judged to be too low. The effectiveness of a variety of laboratory methods for evaluation of frozen bovine semen for prediction of fertility was examined in a field trial by *Linford et al.* (1976). It was found that the best prediction of frozen semen fertility was achieved by estimating the motility before processing or the percentage of dead and morphologically abnormal cells. In Swedish A.I.centres, sperm motility is used as the only criterion of sperm quality after freezing. A minimum sperm motility of 50 % post-thaw is required. However, this subjective method is of limited exactness and there is variation between semen evaluations carried out by different persons.

The movement of the spermatozoa is the major energy-demanding process in live spermatozoa. It was therefore suggested at an early stage (*Mann* 1945) that adenosine triphosphate (ATP) and motility are related, and that the frequency of the flagellar motion and thus forward movements depends upon the concentration of ATP. Measurement of ATP content in spermatozoa may therefore provide an objective and additional method to conventional estimation. Several investigations have been made concerning the relationship between ATP content and sperm motility in both fresh and

frozen-thawed bovine semen using a bioluminescent technique (*Brackett & Williams* 1967, *Brooks* 1970, *Prinzen* 1977, *Pfetsch* 1979, *Kähn* 1980, *Vélez Cuevas* 1982).

A pilot study was earlier performed in Sweden concerning the relationship between ATP content and post-thaw sperm motility in bovine semen (*Söderquist & Larsson* 1985). Our knowledge of the variation within and between bulls is insufficient. No information is available concerning the effect of A.I.centre.

The aim of the present study was to evaluate the relationship between ATP content and motility in bovine spermatozoa with reference to the effects of the bull and the A.I. centre.

Materials and methods

Material

Deep-frozen semen from a total of 28 bulls (13 Swedish Friesian and 15 Swedish Red and White) used for commercial A.I. was used. The bulls had documented good fertility and were taken from 6 different A.I. centres (Table 1). The semen was collected at the A.I.centres using an artificial vagina. It was processed and frozen in French mini straws according to conventional methods, using the extender Laiciphos 478 (I.M.V., B.P.76.10, Bd Clémenceau – 61300 L'Aigle, France) and stored at –196°C in liquid nitrogen. Straws from 3 separate freezing operations per bull, i.e. a total of 82 freezing operations (Table 1), were sent from the A.I. centres to the semen laboratory where the analyses were performed.

Table 1. Number of bulls and their breed in the 6 A.I.centres.

A.I.centre	Swedish Friesian		Swedish Red and White	
	Bulls	Ejaculates	Bulls	Ejaculates
I	5	15	–	–
II	2	6	3	9
III	–	–	5	13*
IV	–	–	5	15
V	1	3	2	6
VI	5	15	–	–
Total	13	39	15	43

*) In 2 bulls only 2 freezing operations were analysed.

Determination of ATP

Five straws from each freezing operation were thawed at + 30–35°C for 12 s and the contents were thereafter pooled. From the pool of each freezing operation duplicate samples were immediately taken for analysis of ATP (Adenosine-Tri-Phosphate) concentration. The ATP content was determined by a method based on the ATP-Luciferase-Reaction which produces measurable bioluminescence. The bioluminescence was measured with a LKB Luminometer (LKB Luminometer 125 001; LKB-Produkter AB, Bromma, Sweden). Trichloroacetic acid (12 %; v/v) was used for the extraction and the ATP content was expressed both as nmoles ATP/ml and nmoles ATP/spermatozoa × 10^8. This method has previously been used by *Söderquist & Larsson* (1985).

The ATP determinations were performed in 2 equal aliquots of each semen sample, and the coefficient of variation (CV%) was found to be 8.6 %. The coefficient of variation was calculated using the formula $(\sqrt{D} \times 100)/\bar{x}$ $D = \sum d^2/2n$, where d = difference between duplicate measurements and n = number of duplicate determinations.

Sperm motility and sperm concentration

The pooled semen samples were incubated at + 35°C and aliquots were taken for estimation of post-thaw sperm motility and for calculation of sperm concentration. The progressive sperm motility was subjectively esti-

mated at 30 min post-thaw under a phase contrast microscope at + 37°C (× 450).

The sperm concentration of each freezing operation was calculated twice in a haemocytometer (Bürker chamber), according to the method of *Bane* (1952), and the mean value was used to calculate the ATP concentration expressed as nmoles/spermatozoa $\times 10^8$.

The number of spermatozoa with forward motility was calculated by multiplying the estimated percentage of motile spermatozoa (30 min post-thaw) by the estimated number of spermatozoa per ml.

Statistical methods

Data were analysed according to the method of least-squares as applied in the general linear model procedure (GLM) of the Statistical Analysis System (SAS Institute Inc. 1982). As both breeds were represented in only 2 out of the 6 A.I.centres a model including the complex "A.I.centre-breed" was designed.

The model chosen as the most appropriate to estimate variance components and correlations for semen characteristics was:

$Y_{ijk} = \mu + AIbr_i + b_{ij} + e_{ijk}$

where:

Y_{ijk} = the observation of the kth freezing operation

μ = general mean

$AIbr_i$ = effect of ith A.I.centre-breed

b_{ij} = effect of jth Bull within ith A.I.centre-breed

e_{ijk} = random error

Results

Overall means, standard deviations and the ranges for the post-thaw sperm characteristics for the 6 A.I.centres are presented in Table 2. Correlations for semen characteristics between A.I.centres and bulls are presented in Table 3.

The overall mean sperm motility was 54.5 % (range 30.0–65.0 %). No significant difference in sperm motility between A.I. centres was found. The overall mean ATP

Table 2. Overall means, standard deviations and range of semen characteristics in 6 A.I.centres.

A.I.centre		Sperm motility (%) 30 min post-thaw	ATP content nmoles/ml	ATP content nmoles/spz×10^8	Sperm conc. ×10^6/ml
I	(n= 5)	54.3±5.0	11.5±3.2	16.2±4.5	73.7±13.2
		(45.0–60.0)	(6.2–18.2)	(8.6–23.8)	(54.2–96.9)
II	(n=15)	54.3±7.8	11.1±2.9	16.8±4.4	66.3±11.8
		(40.0–65.0)	(6.6–16.0)	(8.9–25.8)	(45.0–82.5)
III	(n=13)	54.2±8.4	7.5±1.4	8.5±1.8	89.5±15.0
		(30.0–60.0)	(5.6–10.1)	(6.0–11.7)	(68.4–124.2)
IV	(n=15)	55.3±6.7	22.0±3.0	21.1±3.4	106.7±25.9
		(45.0–65.0)	(17.1–26.5)	(12.6–27.4)	(80.7–191.7)
V	(n= 9)	53.9±5.5	17.7±4.3	18.4±5.4	100.7±28.9
		(45.0–60.0)	(9.5–23.7)	(9.6–24.0)	(73.4–151.1)
VI	(n=15)	54.7±6.7	14.1±2.5	18.4±3.6	78.1±13.3
		(40.0–60.0)	(9.5–1.2)	(10.9–25.8)	(63.2–110.0)
Total	(n=82)	54.5±6.6	13.9±5.6	16.6±5.5	84.7±23.1
		(30.0–65.0)	(5.6–26.5)	(6.0–27.4)	(45.0–191.7)

spz = spermatozoa

Table 3. Least square analysis for semen characteristics between A.I. centres (1) and bulls (2), respectively (n=82).

Source of variation	Sperm motility% 30 min post-thaw	ATP content nmoles ATP per ml	ATP content nmoles ATP per spz x 10^8	Sperm conc. per ml x 10^6	Number of motile spz x 10^6
(1) A.I.centre-breed	ns	***	***	***	***
(2) Bull (A.I.centre-breed)	ns	ns	*	ns	ns

spz = spermatozoa
Levels of significance:
ns = not significant
* = $p \leq 0.05$
** = $p \leq 0.01$
*** = $p \leq 0.001$

content was 13.9 nmoles ATP per ml and 16.6 nmoles ATP/spermatozoa x 10^8. There was a significant variation in ATP content (both expressed as nmoles ATP/ml and nmoles ATP/spermatozoa x 10^8) between A.I.centres. Straws from A.I.centre III had lower values than the others. The mean sperm concentration was 84.7 x 10^6/ml with a variation from 45.0 to 191.7 x 10^6/ml between the 6 different A.I.centres. As is shown in Table 3, no significant differences with regard to the investigated characteristics were found between bulls, except for ATP content (nmoles ATP/spermatozoa x 10^8; $p \leq 0.05$).

The overall correlation between the semen characteristics are presented in Table 4 and within bull correlations in Table 5.

The overall correlation and the correlation within bull between sperm motility and the content of ATP (nmoles ATP/spermatozoa x 10^8) were significant ($r = 0.28$; $p \leq 0.05$ and $r = 0.33$; $p \leq 0.05$, respectively). Significant correlations were also found between ATP content (nmoles ATP/ml) and the number of motile spermatozoa ($r = 0.55$; $p \leq 0.001$ overall and $r = 0.50$; $p \leq 0.001$ [within-bull]).

Discussion

In the present study the mean level of ATP (± S.D.) determined in frozen and thawed bovine semen was higher (16.6 ± 5.5 nmoles ATP/spermatozoa x 10^8) than previously reported (10.0 ± 4.7 nmoles ATP/spermatozoa x 10^8; *Foulkes & MacDonald* 1979). Differences in the extracting methods as well as in semen quality may have contributed to the somewhat higher values recorded in this study.

In addition, considerable variation was also seen in the ATP levels of frozen and thawed semen, both within A.I.centres and totally. This may indicate that the samples with low levels of ATP had reduced viability. However, the overall correlation and the correlation within bull between ATP concentration and the calculated number of motile spermatozoa was good ($r = 0.55$; $p \leq 0.001$ and $r = 0.50$; $p \leq 0.001$, respectively). No significant difference between A.I.centres was found with respect to sperm motility,

Table 4. Overall correlations between semen characteristics (82 freezing operations).

	A	B	C	D	E
A. Sperm motility (%) 30 min. post-thaw	–	0.16 ns	0.28 *	–0.15 ns	0.32 **
B. Mean ATP content (nmoles) per ml		–	0.74 ***	0.50 ***	0.55 ***
C. Mean ATP content (nmoles)/spz × 10^8			–	–0.17 ns	–0.04 ns
D. Mean sperm conc. × 10^6 per ml				–	0.88 ***
E. Number of motile spz × 10^6					–

spz = spermatozoa
Levels of significance:
ns = not significant
* = $p \leq 0.05$
** = $p \leq 0.01$
*** = $p \leq 0.001$

Table 5. Correlations between semen characteristics within bull (28 bulls and 82 freezing operations).

	A	B	C	D	E
A. Sperm motility (%) 30 min. post-thaw	–	0.22 ns	0.33 *	–0.17 ns	0.43 ***
B. Mean ATP content (nmoles) per ml		–	0.58 ***	0.40 **	0.50 ***
C. Mean ATP content (nmoles)/spz × 10^8			–	–0.43 ***	–0.22 ns
D. Mean sperm conc. × 10^6 per ml				–	0.80 ***
E. Number of motile spz × 10^6					–

spz = spermatozoa
Levels of significance:
ns = not significant
* = $p \leq 0.05$
** = $p \leq 0.01$
*** = $p \leq 0.001$

since only semen from bulls with a minimum sperm motility of approximately 50 % post-thaw was available. The reason for the wide range in mean ATP content between A.I.centres is difficult to explain fully, but differences in laboratory routines in the A.I.centres are probably the major cause.

Based upon the limited number of freezing operations examined in this study, it was found that the mean ATP content (nmoles ATP/spermatozoa $\times 10^8$) within each A.I. centre ranked almost identically with the average 56-day non-return rate of the individual A.I.centre. However, the value of the ATP test for predicting fertility remains to be further evaluated.

The variation seen in the sperm concentration/straw between freezing operations within A.I.centre and totally (45.0–191.7 spermatozoa/ml $\times 10^6$) agrees with earlier observations in our semen laboratory (*Larsson*, unpublished). The low sperm concentration (66.3 ± 11.8 spermatozoa/ml $\times 10^6$) seen in A.I.centre II is due to the A.I.centre policy of producing straws with 15×10^6 spermatozoa per mini straw or 60×10^6/ml.

The significant correlation between ATP concentration and the number of motile spermatozoa as well as the significant difference in ATP content between different bulls would suggest that ATP assessment may be useful as an additional, objective laboratory test.

However, other possible sources of variation regarding sperm ATP content and its relation to fertility remain to be evaluated.

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Sammanfattning

Sambandet mellan ATP innehåll och motilitet i tjursperma med särskild hänsyn till effekten av tjur och tjurstation.

ATP innehållet i djupfryst sperma från 28 tjurar tillhörande 6 olika tjurstationer undersöktes efter upptining med hjälp av en bioluminescence metod. Spermiemotiliteten bedömdes subjektivt i faskontrastmikroskop. Spermiekoncentrationen framräknades med hjälp av Bürkerkammare.

Medel ATP innehållet (± S.D.) i upptinad sperma var 16.6 ± 5.5 nmol ATP/spermie × 10^8. Medel ATP innehållet varierade påtagligt mellan olika tjurstationer. Den observerade signifikanta skillnaden mellan tjurar i spermiernas ATP innehåll, liksom den signifikanta korrelationen mellan antalet rörliga spermier och ATP innehållet indikerar att mätning av ATP innehållet i sperma skulle kunna användas som en objektiv laboratorietest.

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