Acta vet. scand. 1985, *26*, 308—312.

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# RELATIONSHIP BETWEEN ATP CONTENT AND POST THAW MOTILITY IN BULL SEMEN

By

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SÖDERQUIST, L. and K. LARSSON: *Relationship between ATP content and post thaw motility in bull semen.* Acta vet. scand. 1985, *26*, 308—312. — The ATP content in frozen and thawed semen from 17 bulls was determined by a bioluminescence method.

The post thaw motility was assessed by phase contrast microscopy by subjective estimation of the percentage of sperm cells with forward motility. The concentration of spermatozoa was counted in a Bürker chamber. The correlation between ATP content and the number of sperm cells with forward motility was high.

Bovine; spermatozoa.

The widespread use of artificial insemination for cattle breeding has led to a requirement of techniques for semen quality assessment to avoid the use of low quality semen. Laboratory methods for evaluation of bovine semen are currently used to select bulls for artificial insemination and to select ejaculates of satisfactory standard for bulls at A.I. centres.

It is well established that there is a close relationship between sperm metabolism and sperm motility. At an early stage *Mann* (1945) showed that ATP (Adenosine-Tri-Phosphate) content and motility are related. Thus measurement of ATP content could provide an objective method for estimation of semen viability. Later *von Prinzen* (1977) and *Foulkes & MacDonald* (1979) using a bioluminescence method, also showed that the ATP content was highly correlated to the forward motility of sperm cells in samples of fresh, frozen and thawed semen. According to *Foulkes & MacDonald* (1979) no ATP is detected

in seminal plasma or diluent. The purpose of the present experiment was to compare the ATP content and the number of sperm cells with progressive motility in frozen and thawed bull semen.

## MATERIAL AND METHODS

Deep frozen semen from 17 young (14—24 months old) bulls (15 Swedish Red and White and 2 Ayrshire) used for commercial A.I. was investigated. Five French ministraws originating from a single frozen ejaculate from each of the 17 bulls were thawed at 30—35°C for 12 s before being pooled. The ATP content was determined by a method based upon the ATP-Luciferase Reaction (*Olsson et al.* 1983) which produces a measurable bioluminescence. A LKB Luminometer 1250 001 was used. Two samples (100 µl each) were immediately pipetted from each semen pool into test tubes and placed on ice, 100 µl ice cold TCA (Tri Chloroacetic Acid, 12 %) was added and the ingredients were thoroughly mixed. All the samples were placed on ice for at least half an hour. Two hundred µl Tris/EDTA/Triton (100 nM/4 mM/0.2 %) was then added and admixed. From the mixture 25 µl was taken and pipetted into a test tube containing 0.8 ml Tris/EDTA/Triton and mixed before 200 µl of ATP monitoring reagent (LKB-SVERIGE AB, Box 305, 161 26 Bromma) was added and the amount of ATP was read on the printer. For internal calibration 10 µl of ATP standard was added and read. Double samples from each semen pool were assayed for ATP content.

The coefficient of variation (CV%) was calculated by using the formula $\frac{(\sqrt{D} \times 100)}{\bar{x}}$, $D = \Sigma d^2/2n$ where d = difference between duplicate measurements and n = number of duplicate determinations. The pooled semen was incubated at + 35°C and the sperm motility was assessed by phase contrast microscopy at 30 min post thawing. The concentration of spermatozoa in each pool was counted twice in a Bürker chamber and the mean values were used. The number of sperm cells with forward motility was calculated by multiplying the estimated percentage of motile spermatozoa by the total number of sperm cells in the thawed and pooled semen from each bull/ejaculate.

## RESULTS AND DISCUSSION

The mean sperm concentration in the thawed samples was $91.9 \pm 21.3 \times 10^6$ spermatozoa per ml. Post thawed motility varied between 30 and 65 %.

The mean concentration of ATP (± s) in frozen and thawed semen was 12.27 ± 3.84 nmol ATP per ml. The variation of the ATP levels in the investigated bulls/ejaculates ranged between 5.1 and 21.4 nmol ATP per ml (Table 1). The coefficient of variation for the ATP samples was low (CV % = 4.32) and the

Table 1. ATP content, motility and concentration in frozen thawed semen from 17 A.I. bulls.

| Bull no. | Mean ATP content (nmol/ml) | % motile spermatozoa 30 min post thaw | Mean no. of spermatozoa/ ml $\times 10^6$ | Estimated no. of motile spermatozoa/ ml $\times 10^6$ |
|---|---|---|---|---|
| 1 | 5.1 | 30 | 88.6 | 26.6 |
| 2 | 8.0 | 45 | 101.0 | 45.5 |
| 3 | 8.8 | 35 | 67.3 | 36.4 |
| 4 | 9.8 | 50 | 72.9 | 36.5 |
| 5 | 9.8 | 40 | 83.8 | 33.5 |
| 6 | 10.4 | 50 | 78.4 | 39.2 |
| 7 | 10.8 | 50 | 92.0 | 46.0 |
| 8 | 12.1 | 40 | 104.5 | 41.9 |
| 9 | 12.5 | 60 | 92.5 | 55.5 |
| 10 | 12.7 | 55 | 92.0 | 50.6 |
| 11 | 12.8 | 65 | 84.0 | 54.6 |
| 12 | 13.0 | 60 | 62.6 | 37.6 |
| 13 | 13.0 | 60 | 93.2 | 55.9 |
| 14 | 14.6 | 65 | 83.1 | 54.0 |
| 15 | 15.6 | 55 | 94.6 | 52.0 |
| 16 | 18.2 | 50 | 157.5 | 78.8 |
| 17 | 21.4 | 60 | 114.0 | 68.4 |

correlation between ATP content and the calculated number of sperm cells with forward motility was high ($r = 0.855$; $P < 0.001$) (Fig. 1).

No ATP was detected in seminal plasma or diluent. Thus the ATP content in spermatozoa seems to indicate the actual number of viable sperm cells in a sample.

Assessment of the ATP content might be useful as an additional test to motility estimation and other laboratory methods.

However, possible sources of variation regarding sperm cell ATP content remain to be evaluated.

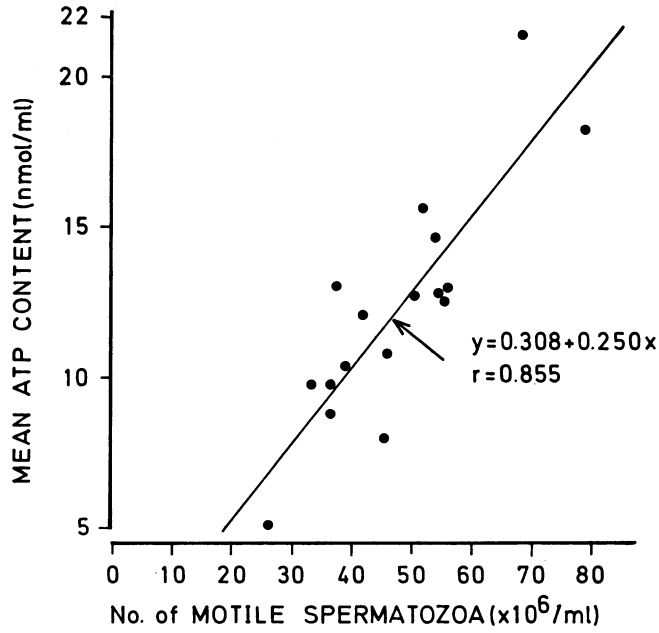


Figure 1. Mean ATP content and the number of motile spermatozoa in frozen thawed semen from 17 A.I. bulls.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Lars Drejare, Nordavel, for providing the bovine semen, Karin Ullman for technical assistance and Dr. Thomas Olsson for his kind help with the ATP methodology.

The authors are indebted to the National Veterinary Institute for the loan of the Luminometer used in this study.

This study has been supported by grants from the Swedish Agricultural Market Board.

REFERENCES

*Foulkes, J. A. & B. J. MacDonald:* The relationship between ATP content and motility of bovine spermatozoa. Theriogenology 1979, *11*, 313—319.

*Mann, T.:* Studies on the metabolism of semen. 1. General aspects. Occurrence and distribution of cytocrome, certain enzymes and co-enzymes. J. Biochem. 1945a, *39*, 451—458.

*Mann, T.:* Studies on the metabolism of semen. 2. Glycolysis in spermatozoa. J. Biochem. 1945b, *39*, 458—465.

*Olsson, T., H. Gulliksson, M. Palmeborn, K. Bergström & A. Thore:* Methodological aspects on the firefly luciferase assay of adenine nucleotides in whole blood and red blood cells. Scand. J. clin. Lab. Invest. 1983, *43*, 657—664.

*von Prinzen, R.:* ATP-bestimmungen (Adenosin-Tri-Phosphat) mit einer Schnellmethode and frischen und tiefgefrorenen Bullenspermien (A method for the rapid determination of ATP (Adenosine-Tri-Phosphate) in fresh and frozen bull sperm). Zuchthyg. 1977, *12*, 105—108.

SAMMANFATTNING

*Sambandet mellan ATP innehåll och upptiningsmotilitet i tjursperma.*

Djupfryst sperma från 17 tjurar undersöktes efter upptining med hjälp av en bioluminescence metod på sitt ATP innehåll.

Spermiemotiliteten fastställdes i faskontrastmikroskop genom subjektiv bedömning av den procentuella andelen spermier med progressiv framåtgående rörelse. Spermiekoncentrationen framräknades med hjälp av Bürkerkammare.

Korrelationen mellan ATP innehåll och andelen spermier med progressiv framåtgående rörelse var hög.

*(Received April 26, 1985).*

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