

The effect of seminal plasma alkaline phosphatase, fructose and aspartate-amino-transferase on non-return rate in bulls

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ABSTRACT

In this study some of biochemical constituens of Simmental bull (n=31) seminal plasma were deterimined using spectophotometric analysis for asparte-amino-transferase (AST), alanine-amino-transferase (ALT), alkaline phosphatase (ALP), adenosine-triphosphate (ATP), fructose (FRU) and Citrate (CIT). The fertility of bulls (n= 18) was measured by non-retun rate 90 after insemination (NR) with deep frozen semen in strows with 6×106 of spermatozoa . There was significant positive correlation between NR and ALP (0.725, P=0.00147) and significant negative correlation between NR and AST (-0.659, P= 0.00554) and FRU (-0.562, P= 0.0153), respectively. It is quite evident that inseminations using ejaculates with high activity of ALP (> 3530 U/L) results in better NR (69% vs. 65%, P=0.028). However, biochemical complexity od seminal plasma attemps to perform such simple correlations among seminal plasma components and NR are likely to produce inconsistent results.

Key words: bovine, bull, seminal plasma, alkaline phosphatase, non-return rate

INTRODUCTION

The bull semen is is combined secretion of testis, epididymis and the male accessory glands (prostate, seminal vesicles) wich form the bulk of the fluid portion of semen known as seminal plasma. The composition and reproductive functions of seminal plasma are well described in generaly (Henault et al. 1995, Maxwell et al. 1997, Vishwanath and Shannon 1997) and in detail by many authors (Killian et al. 1993, Ahmad et al. 1996, Henault and Killian 1996) especially concernig the role of plasma in artificial insemination proces (Töpfer-Petersen et al. 1998). Summarising all these research we could extract from present knowledge that seminal plasma provides a medium for motility of sperm after ejaculation and enhance fertilisation of the egg (Holt 1996).

In consonance with literature mentioned above, it would be likely that selected seminal plasma biochemical constituens - particularly enzymes - and some sugar that provide ATP through glycolysis play important role in sperm motility and fertility of the bull.

This study was undertaken to investigate the effect od particullarly biochemical constituens of seminal plasma on fertility in bull. We will show, in contrast to to widely accepted point of view, that impact on bull fertility is transmited only by very few inducers investigated here.

MATERIAL AND METHODS

The study was performed on 31 Simmental bulls aged 12 to 14 months individualy penned on AI station. The bulls were offered basal diet consisted of the mixture including 4 kg of hay, 20 kg of corn silage, 3 kg of concentrate feed and vitamin-mineral mix. The total daily nutriens was therefore 11.5 kg od dry matter, 112.6 MJ of metabolic energy and 698 g of crude protein.

Semen samples were obtained from bulls using an artificial vagina; from each bull two consecutive ejaculates were collected and pooled together in order to drain as possible the epidydimis and accessory glands. After this, pooled sample was splited in two portions: a portion to used immidiately for chemical analysis of ejaculate and an aliquot for artificial insemination.

Ejaculate specimens were centrifuged on 3000 g to separate seminal plasma from the spermatozoa.

Supernatant of the seminal plasma was analysed for asparte-amino-transferase (AST), alanine-amino-transferase (ALT), alkaline phosphatase (ALP), adenosine-triphosphate (ATP), fructose (FRU) and Citrate (CIT) using Boehringer commercial kits[3] on automatic photospectometric analyser.[4]

Because of low volume of seminal fluids from some

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ejaculates, certain investigations were unable to perform. So, the number of tests varies among the chemical analyses as shown in section of Results.

The semen freezing extender used in this experiment was egg yolk-citrate-glycerol with ethylen-di-amino-tetra-acetic acid. After cooling the semen was stored in 0.5 mL French strows with 6×106 of spermatozoa and immersed into the liquid nitrogen till insemination.

For artificial insemination we used randomly selected cows and heifers on the dairy farms in north-east Slovenian region. Non-return rate (NR%) of 18 bulls was recorded 90 days after insemination computing the field insemination records and expressed the the results as percentage of pregnant animals.

All collected data were analysed using descriptive statistic methods, correlation including partial correlation methods, and t-test for independent samples with algorithms from SPSS software[5]

RESULTS AND DISCUSSION

In Table 1 the results of descriptive statistics are summarized to present physiological concentration of biochemical constituens investigated here (except of NR90%). The reference values for yearling simmental bulls are not widely available in present literature. For example, Luthra et al. (1994) indicates the average concentration of 23.5 mmol/L of fructose for Simmental bulls 12-15 months of age wich is slightly higher than our results. In the same study they found significantly higher sperm concentration in the semen of bulls with lower concentration of fructose. Also, bulls with higher concentration of fructose had higher sprem motility. Fructose is syntesized in accessory gland of reproductive tract and spermatozoa convert fructose anaerobically to lactate wich leads, at least in bull, to consecutive high motility (Storey 2008).

[5] SPSS Statistics17.0 Command Syntax Reference 2008, SPSS Inc., Chicago, USA.

In Table 2, correlation analysis between fructose concentration and non-return data indicate significant negative relationship. This is consistent with idea of Lutra at. al. (1994) although we do not examined the sperm concentration. However, this relationship is partially influenced also by presence od AST in the seminal plasma. If we exclude AST from this calculaton correlation coefficient between FRU and NR intends to be lower t.i. -0.379 (P=0.163). Increase level of seminal fructose was allways accompanied with increase activity of AST (Table 2; r=0.44, P=0.167). The correlation coefficient between AST and NR was negative and significant (-0.659, P=0.00554) This suggest that failure in sperm cellular membrane after freezing and leakage of transaminases had detrimental effect of NR results, wich agree with findings of Dhami and Sahni (1993) for ALT (r=-0.736) but not for AST. Ejaculates analysed here with activity of AST above the median value (>126 U/L) have not significant lower NR (65% vs. 67% in below median value, P=0.222). In contrast, study of Herak et al. (1993) indicate some higher level of AST activity in bulls with lower NR (256 U/L vs. 218 U/L).

The role of ATP in sperm metabolism is well known (Minelli et al. 1995). The spermatozoa metabolize different substrate to obtain energy in the form of ATP. This is mainly achieved by exploiting the catabolic routes of the glycolytic Embden-Mayerhof pathway, wich is anaerobic and produces 2 moles of ATP per mole of glucose and the aerobical Krebs citric-acid cycle, wich produce 36 moles of ATP per mole of glucose (Jones and Murdoch 1996). Dephosphorylation of ATP is fundamental for activity of spermatozoa, so this relationship suppose to be correlated with their molitilty. ATP content in seminal plasma (Table 1) is much lower as found in the sperm cell (Söderquist 1991). This may be the reason that we did not find the correlation between ATP and NR% and agree with results of McLaughlin et al. (1994) for human spermatozoa.

The alkaline phosphatase in seminal lasma has two sources. The major part is prostatic and very smaller part comes from sprem cell itself. We found (Table 1) slightly less activity of ALP in seminal plasma as Pangawkar et al. (1988) 3653 U/L vs. 5100 U/L and 7100 U/L after freezing, respectively.

Table 1: Descriptie statistics of biochemical constituens in seminal plasma before freezing: asparte-amino-transferase (AST), alanine-amino-transferase (ALT), alkaline phosphatase (ALP), adenosine-triphosphate (ATP), fructose (FRU), citrate (CIT) and non-return rate 90 day after insemination (NR) of the same ejaculate

	AST U/L	ALP U/L	CIT mmol/L	ATP mmol/L	FRU mmol/l	ALT U/L	NR 90 day %
N	29	28	30	31	31	28	18
Mean	142	3653	4,33	7,48	19,65	5,0	66,13
Std. Error of Mean	18,6	392	,125	4,15	1,135	,76719	,740
Median	126	3530	4,4	76	19,800	4,5	65,25
Std. Deviation	100,43	2076,46543	0,686	23,10	6,319	4,06	3,142
Minimum	3,00	866,00	2,30	9,00	6,60	,00	60,30
Maximum	332,00	8055,00	5,220	133,00	29,00	20,00	71,20

Table 2: Correlation matrix among biochemical constituens in seminal plasma before freezing (asparte-amino-transferase AST, alanine-amino-transferase ALT, alkaline phosphatase ALP, adenosine-triphosphate ATP, fructose FRU and citrate CIT) of Simmental bulls and non-return rate 90 day after insemination (NR) of the same ejaculate. Values in cell are indicated as Pearson correlation coefficient (first row), two tailed probability (second row) and number of observations (third row)

	ALT U/L	ALP U/L	ATP mmol/L	FRU mmol/L	CIT mmol/L	NR 90 day %
AST	-0.153 0.436 28	-0.448 0.017 28	-0.104 0.592 29	0.441 0.017 29	0.179 0.352 29	-0.659 0.006 16
ALT	1	0.162 0.410 28	-0.0875 0.658 28	0.0365 0.854 28	0.0104 0.958 28	-0.358 0.899 15
ALP		1	0.129 0.506 29	-0.488 0.007 29	-0.180 0.350 29	0.725 0.001 15
ATP			1	0.174 0.350 31	-0.204 0.280 30	0.257 0.303 18
FRU				1	0.046 0.808 30	-0.562 0.015 18
CIT					1	-0.273 0.290 17
NR						1

Observed difference between fresh and post-thawed semen might reflect initial damage of the spermatozoan membrane during freezing, with subsequent increase in its permeability, resulting in leakage of ALP in the seminal plasma. The positive significant correlation of ALP vs. NR (0.562, P= 0.0153) shows the potential of prostate gland through their secretion on the biochemical enviroment necessary for oocyte fertilisation. There is no reliable information availible in the literature regarding the fertility of post-thawed semen and ALP activity in seminal plasma in the bull. For the buffalo (*Bubalus bubalis* v. Surti) found Dhami and Kodagali (1990) sperm post-thaw motility had significantly (P<0.01) negative correlations with the release of ALP (−0.673) from the sperm cell.

Using linear correlation coefficient, it was detected strong negative impact of AST activity and FRU concentration in seminal plasma on 90 day non-return rate; beside of this the positve impact of ALP activity was also measured.

However, ejaculates with activity of AST above the median value (>126 U/L) have not significant lower NR (65% vs. 67%, P=0,222) but ejaculates with above median concentration of fructose (>19,8 mmol/L) show slight tendency to lower NR (65% vs. 68%, P=0.065). It is quite evident that inseminations using ejaculates with high activity of ALP (> 3530 U/L) results in better NR (69% vs. 65%, P=0.028).

CONCLUSIONS

The presented results do not support the view that the sperm fertility, measured by Non-return method, could be seriously affected during the release of sperm cell enzymes into the fresh seminal plasma. Contrary to results for the cell enzymes, the plasmal concentration of mainly prostatic ALP indicated some value as an indicator of ejaculate fertility. Hence, only ALP before-freezing activity above 3653 U/L can be attributed to better fertility in the bull. To summerise from this study, biochemical complexity od seminal plasma attemps to perform such simple correlations among seminal plasma components and NR, are likely to produce inconsistent results and their role in the assessment of sperm function must therefore be called into question.

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