

EFFECT OF EXTENDER ON ASPARTATE AMINOTRANSFERASE ACTIVITY IN BOAR SEMEN DURING PRESERVATION

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Abstract

The objective of this study was carried out to determine the influence of extenders on AST activity during preservation of boar semen. A total of 37 ejaculates were used for this study. The semen was diluted in dilution ratio 1+8 in extenders Androhep (A), Safecell Plus (SCP) and SUS and was stored at a temperature of 17°C. AST activity was evaluated at 24h, 96h, 168h and 240h after semen dilution and 24h, 48h, 72h and 96h after thawing. The total mean value of the AST activity was 72.69, 89.52, 84.40 mU/10⁹ spermatozoa in A, SCP and SUS, respectively. There were found significant differences between A and SCP extender (P<0.05). Significant differences in the AST activity were noted at 96h vs. 168h and 240h storage time in SCP (P<0.05). The gradual increase in the AST activity was observed at all extenders during storage time where total the highest difference was after 96h vs. 168h (P<0.05). The mean values of the AST activity after thawing were 128.27 mU/10⁹ in A, 141.99 mU/10⁹ in SCP and 118.44 mU/10⁹ in SUS. Significant differences in the AST activity were noted SCP and SUS extender (P<0.05). In conclusion, the results of this study showed gradual increase in the AST activity indicating the membrane damage of spermatozoa during storage time. The effect of extenders on the activity of AST was significant during storage time after dilution and after thawing.

Key Words: Boar semen, aspartate aminotransferase, extenders, storage time

Aspartate aminotransferase (AST) is an intracellular enzyme of spermatozoa and an increase in the level of this enzyme in seminal plasma is considered as a determinant of cellular damage (Larson et al. 1996). This enzyme is important for the metabolism and function of spermatozoa (Strzeżek et al. 1981). Pandey and Singh (1996) reported the significant effect of diluents on the extracellular activity of transaminases during storage that reflect the importance of protective properties of the ingredients of diluents.

The aim of the experiment was carried out to determine the influence of extenders on AST activity during preservation of boar semen.

Material and Methods

Semen samples from 37 fertile hybrid AI boars (1 to 3 years) were used for studying the effect of 3 extenders on quality of AST activity. In this study were used extenders Androhep (Minitüb, Germany), Safecell Plus (IMV, France) and SUS (Hema Malšice, Czech Republic). The semen was diluted (dilution ratio 1+8) in extenders and was stored at a temperature of 17°C up to 240h. AST activity was evaluated at 24h, 96h, 168h and 240h after semen dilution. The rest of the ejaculate was cryopreserved using the straw freezing procedure describe by Westendorf et al. (1975) and modified by Minitüb (Tiefenbach, Germany). Straws were thawed in a water bath at 38°C for 40s and extended in a dilution ratio of 1+8 in Androhep (A), Safecell Plus (SCP) and SUS (38°C). AST activity was evaluated 24h, 48h, 72h and 96h after thawing. The AST activity was measured with a BIOLATEST kit (Lachema, Brno, Czech Republic) and with an ENCORE spectrophotometer and calculated per 10⁹ spermatozoa. A 2ml sample diluted semen was centrifuged at 1 700 rpm for 10min and the supernatant was used to assess the AST activity.

Basic statistical characteristics of the results arithmetic means, standard deviations and significance (P) were calculated by the QC Expert program. Statistical significance

was checked by the analysis of variance ANOVA – Fisher's test at significance levels of P<0.05, P<0.01 and P<0.001.

Results and Discussion

The mean values of AST activity with A, SCP and SUS extender during storage time are showed in Table 1. The total mean value of the AST activity was 72.69, 89.52, 84.40 mU/10⁹ spermatozoa in A, SCP and SUS, respectively. There were found significant differences between A and SCP extender (P<0.05). Azawi et al. (1990) and Pandey et al. (2001) reported also significant differences in AST activity during preservation in different extenders. On the contrary, Frydrychová et al. (2010) noted that between tested extenders were not found significant differences in the AST activity. This might be attributed to the protective properties of ingredients in extenders. Significant differences in the AST activity were noted at 96h vs. 168h and 240h storage time in SCP extender (P<0.05). The gradual increase in the AST activity was observed at all extenders during storage time where total the highest difference was after 96h vs. 168h (P<0.05).

Table 2 presents comparison the AST activity in boar semen after thawing in extenders during storage time. The mean values of the AST activity after thawing were 128.27 mU/10⁹ in A, 141.99 mU/10⁹ in SCP and 118.44 mU/10⁹ in SUS. Significant differences in the AST activity were noted SCP and SUS extender (P<0.05). There was noted increased AST activity at all extenders and the largest after 72h storage time. Increasing in AST activity in supernatant after thawing indicated increase in the membrane damage of spermatozoa during cryopreservation and thawing procedure. Bower et al. (1971) and Strzeżek et al. (1984) observed most intensive leaking AST when boar semen was frozen. According to some authors this may be a direct cause of lower biological value of boar semen after thawing, most probably due to the effect of dead cells on motile live spermatozoa.

Table 1. Comparison of mean values AST- supernatant (mU/10⁹ spermatozoa) in boar semen in different extenders during storage periods (mean ± SD)

Extender	AST				
	Storage time				
	24h	96h	168h	240h	Total
A	70.49±64.14	77.95±90.23	67.19±37.53	75.13±50.69	72.69±63.19 ^A
SCP	91.15±78.55	112.68±80.17 ^a	76.57±40.27 ^b	77.66±41.25 ^b	89.52±64.13 ^B
SUS	75.55±59.75	86.45±69.59	70.71±46.49	104.88±111.56	84.40±76.23
Total	79.06±67.91	92.36±81.08 ^a	71.49±41.40 ^b	85.89±75.19	

^{a,b} means within the row ^{a,b} P<0.05 ^{A,B} means within the column ^{A,B} P<0.05

Table 2. Comparison of mean values AST- supernatant (mU/10⁹ spermatozoa) in boar semen in different extenders during storage periods after thawing (mean ± SD)

Extender	AST				
	Storage time				
	24h	48h	72h	96h	Total
A	122.96±86.17	121.45±86.89	134.68±95.33	133.97±95.44	128.27±90.34
SCP	137.67±95.36	137.95±102.00	150.22±103.45	142.14±86.06	141.99±96.10 ^A
SUS	106.40±75.75	114.55±100.65	128.98±101.64	123.83±77.82	118.44±89.30 ^B
Total	122.34±86.31	124.65±96.38	137.96±99.70	133.31±86.28	

^{A,B} means within the column ^{A,B} P<0.05

Conclusion

The results of this study showed gradual increase in the AST activity indicating the membrane damage of spermatozoa during storage time. The effect of extenders on the activity of AST was significant during storage time after dilution and after thawing.

References

- AZAWI OI, ZNAD MM, AL-JARRAH LH. The correlation between the viability of bovine spermatozoa preserved at ambient temperature and the activity of transferases. *Anim Reprod Sci* 22, 1990, 319-323.
- BOWER RE, CRABO BG, PACE MM AND GRAHAM EF. Effects of dilution and glycerol on the release of glutamic-oxaloacetic transaminase (GOT) from boar spermatozoa. *J Anim Sci* 36, 1973, 319-323.
- FRYDRYCHOVÁ S, ČEŘOVSKÝ J, LUSTYKOVÁ A, ROZKOT M. Effects of long-term liquid commercial semen extender and storage time on the membrane quality of boar semen. *Czech J Anim Sci* 55, 2010, 160-166.
- LARSON K., EINARSSON S., NICANDER L. Influence of thawing diluents on vitality, acrosome morphology ultra structure and enzyme release on deep frozen boar spermatozoa. *Livestock Production Science* 5, 1996, 293.
- PANDEY RP AND SINGH BK. Studies on extracellular Transaminase activity of preserved boar semen with BTS, Kiev and BL-1 dilutors. *Indian J Anim Reprod* 17, 1996, 127-129.
- PANDEY RP, SINGH BK. Effect of dilutors on extracellular enzyme activity of preserved boar semen. *Indian J Anim Sci* 71, 2001, 826-828.
- Strzezek J, Glogowski J, Magierska E, Lubarda Z and Jablonowska Cz. Some aspect of cryobiochemistry of boar semen. *Proc. Xth Int. Congr. Anim Reprod AI* 2, 1984, 224-226.
- STRZEŽEK J, TORSKA J, AL-TAHA TJ. The use of enzymatic tests to get better frozen semen yields. *Practical Biotechnology* 10, 1981, 16-19.
- WESTENDORF P, RICHTER L & TREU H. ZUR TIEFGEFRIERUNG VON EBERSPERMA. LABOR-UND BESAMUNGsergebnisse mit dem Hulsenberger Paillettenverfahren. *Deutsche Tierarztliche Wochenschrift* 82, 1975, 261-267.

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