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Conference paper

ACROSINE AND ASPARTATE AMINOTRANSFERASE ACTIVITIES IN BOAR SEMEN DURING THE FREEZING PROCESS AS INDICATIVE OF THE CELL STATUS

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Abstract

Acrosine and Aspartate aminotransferase (AAT) activities after a cold shock as well as a number ot tradicional morphological parameters of semen quality were measured after collection in 36 ejaculates from four boars. Activities average of both enzymes in fresh semen were 2.5 and 45 mIU per 10^9 spermatozoa respectively. Activities of Acrosine and AAT tested at different stages during the freezing process showed a parallel pattern of changes. Both enzymes slightly increased their activities after addition of egg yolk and rose markedly following addition of glycerol, indicating a damage of cells after the addition of this compound. There was a substantial fall in enzyme activity in the frozen-theawed semen. The activities of the enzymes in the fresh semen did not provide useful predictions of capacity of the semen to be successfully frozen and thawed but are a good indicative of the cell status during freezing process. Percentage cells with normal acrosomes afterr thawing is significatively correlated with ORT index ($P<0.025$) and correlated (No significative) with the percentage cells with normal acrosomes in the fresh semen.

Introduction

Several enzymes are involved in the metabolism of mammalian spermatozoa but previously published studies have focussed mainly on two: Acrosine and aspartate aminotransferase (AAT). Activities of these enzymes in semen are considered by some researchers to provide an indication of semen quality (Bower et al., 1973; Brown, and Harrison, 1978; Ciereszko and Strzezek, 1989; Ciereszko et al., 1990; Ciereszko et al., 1992; Strzezek, 1981; Strzezek et al., 1984; Strzezek and Swidowicz, 1986; Strzezek and Ciereszko, 1987; Strzezek 1990).

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Acrosine is a trypsin-like enzyme that is found exclusively in the head of the mammalian spermatozoa, including that of the boar (Polakoski and Zaneveld, 1984). It is located on the internal acrosomal membrane (Bacetti, 1979) and has an important role i penetration of the ovum by the sperm (Bedford and Cross, 1978; Brown and Harrison, 1978).

Aspartate aminotransferase (AAT) is located in the mitochondria (mAAT) and in the cytoplasm (cAAT) of the spermatozoa (Ciereszko and Strzezek, 1989) and its synthesis is controlled by androgens (Dubiel et al., 1987). AAT is involved in synthesis of glutamate from aspartate. Under the influence of various chemical and physical factors (eg. cold shock) both enzymes tend to be released from the spermatozoa and the levels released are throught to be indicative of reduction in cell membrane integrity.

The purpose of this paper was the evaluation of the spermatozoa damage in each one of the freezing steps by measuring both enzymes activites throughout the wole freezing process.

Materials and Methods

Semen was collected once a week over a period of nine weeks from four Large White boars aged two to four years (Total = 36 ejaculates). Only the rich fraction of each ejaculate was used.

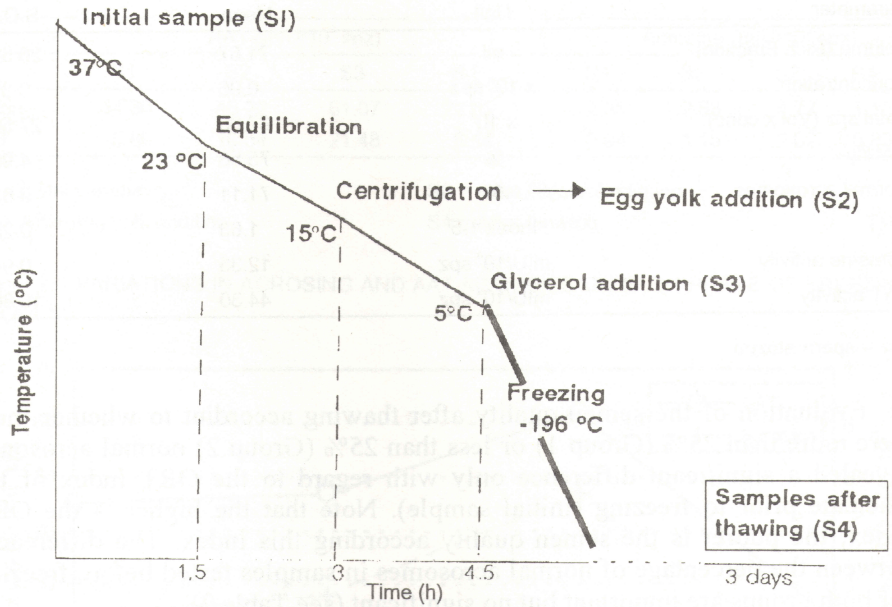
Morphological examination of the ejaculates was carried out immediately after collection and ejaculates were then processed and frozen according to the Pursel and Johnson (1971) method.

In order to determine the initial semen quality, before freezing, the following parameters were evaluated:

- Volume of the ejaculate (rich fraction) and sperm cell concentration, using Bürker camera and spectrophotometric method as modified by Sáiz Cidoncha et al. (1992).
- Percentage of cells motility, by microscopic examination
- Percentage of spermatozoa with normal acrosomes (Pursel and Johnson 1972).
- Osmotic resistance test (ORT) index (1-5), according to Shilling et al., (1984, 1986).
- Acrosine activity, after a cold-shock, according to the Huang-Yang and Meizel (1975) and Schleuning (1984) methods.
- AAT activity, after a cold-shock, by the Ciereszko and Strzezek (1989) metod as modified by Strzezek (1990).

Semen samples were taken for both enzymes activity measurement at the following steps (Fig 1): After addition of egg yolk, after addition of glycerol and after thawing.

Figure 1. - DIAGRAM SHOWING SAMPLING TIMES IN RELATION TO THE TIME-TEMPERATURE CURVE DURING BOAR SEMEN FREEZING (Pursel and Johnson, 1971)



Aliquots of semen were thawed three days after freezing and the activities of Acrosine and AAT as well as the percentage of spermatozoa with normal acrosomes were measured. Each semen sample was classified into one of the two groups as follows:

*Group 1: More than 25% normal acrosomes after thawing
*Group 2: Less than 25% normal acrosomes after thawing

in order to study the possible relationship between the semen quality after thawing and some of the initial parameters of the ejaculates.

Results

The mean values and standard deviations for all the parameters of semen quality measured in the 36 ejaculates (initial samples) after collection are shown in Table 1.

No significant relationship has been found between AAT and/or Acrosine activities with the other quality parameters in the fresh semen.

Table 1. - MEANS AND STANDARD DEVIATIONS (SD) OF SEMEN QUALITY PARAMETERS MEASURED IN THE INITIAL SAMPLES OF THE 36 EJACULATES (BEFORE FREEZING)

Parameter	Unit	Mean	S.D.
Volume (Rich Fraction)	ml	71.67	20.52
Concentration	x 10^9 spz	0.96	0.37
Total spz (Vol x conc)	x 10^9	67.06	27.55
Motility	%	75.56	4.98
Normal acrosomes	%	71.11	9.63
ORT	Index 1-5	1.63	0.25
Acrosine activity	mIU/10^9 spz	12.35	0.94
AAT activity	mIU/10^9 spz	44.30	16.86

spz = spermatozoa

Evaluation of the semen quality after thawing accordint to whether there were more than 25% (Group 1) or less than 25% (Group 2) normal acrosome, revealed a significant difference only with regard to the ORT index of the ejaculate prior to freezing (initial sample). Note that the higher is the ORT index, the poorer is the semen quality according this index. The differences between the percentage of normal acrosomes in samples tested before freezing for both groups are important but no significant (see Table 2).

Table 2 - RELATIONSHIP BETWEEN THE PRE-FREEZING PERCENTAGE OF NORMAL ACROSOMES AND ORT INDEX WITH THE PERCENTAGE OF NORMAL ACROSOMES IN THE SEMEN AFTER FREEZING-THAWING PROCESS

	Group 1 (NA> 25%)			Group 2 (NA< 25%)			
Parameter	N	Mean	S.D.	N	Mean	S.D.	P<
ORT index	12	1.1	0.12	24	1.9	0.31	0.025
% NA (before freezing)	12	75.3	5.6	24	69.0	10.5	N.S.

NA = % normal acrosomes

As shown in Table 3 and Fig. 2 the activities of Acrosine and AAT in the semen after cold shock tratment of samples taken before freezing and at various steps in the freezing process follow similar patterns.

A very high correlation (r= 0.682) between values for Acrosine and for AAT activities is observed in every measurements.

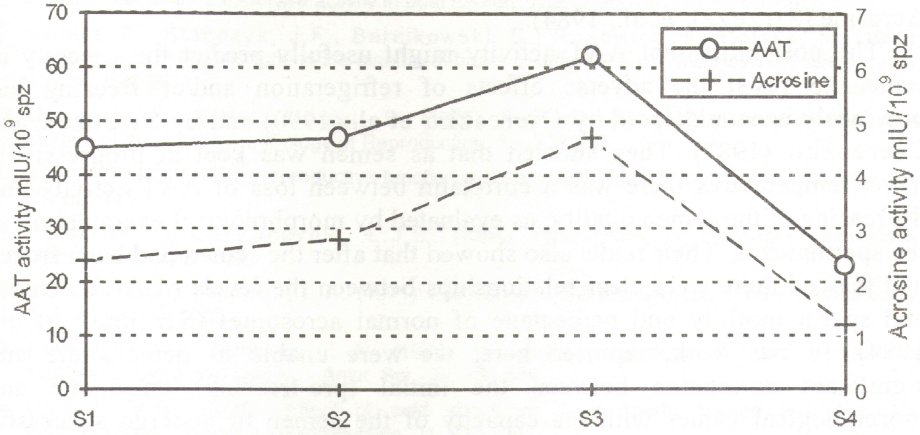
Activities of both enzymes rose significantly (P<0.001) after the egg yolk addition and after glycerol addition (P<0.001)

Table 3. - ACTIVITIES OF ACROSINE AND AAT IN SEMEN SAMPLES TESTED AFTER COLLECTON AND DURING THE FREEZE-THAWING PROCESS (TIMES OF SAMPLING AS SHOWN IN FIG. 1)

	AAT (mIU/10^9 spz)				Acrosine (mIU/10^9 spz)			
	S1	S2	S3	S4	S1	S2	S3	S4
Mean	44.30	46.22	61.07	22.82	2.35	2.88	4.77	1.13
S.D.	15.86	16.54	21.48	6.14	0.94	1.15	2.02	0.86

S1 = Initial sample
S2 = After egg yolk addition
S3 = After Glycerol addition
S4 = After thawing

Figure 2. - VARIATIONS IN ACROSING AND AAT ACTIVITIES ON EACH PHASE OF FREEZING PROCESS



Discussion and conclusions

The search for simple, accurate indicators of quality (i.e. fertilizing ability) in fresh and frozen boar semen has led a number of researchers to study the enzymes activites, asuch as Acrosine, AAT and hyaluronidase, in the semen. Brown and Harrison (1978) studied Acrosine activity in fresh samples but they were unable to demonstrate any close relationship between this and the most traditional morphological parameters of semen quality. Our results with Acrosine and AAT, reported here, are essencially similar to those of these authors.

In our study we have demonstrated a close relationship between the activities of Acrosine and AAT in semen. changes in activity of the two

enzymes during the successive stages of the freezing process were also found to follow a similar pattern.

This agrees with the results reported by other authors who have found that Acrosine and AAT activities in semen after cold shock tended to be closely correlated (Brown and Harrison, 1978; Ciereszko and Strzezek, 1989; Strzezek, 1981). This encourages the idea that just one of these enzymes, rather than necessarily both, may be suitable as an indicator of sperm cell status in boar semen. AAT activity would appear to be the better choice to evaluate because the methodology is comparatively straightforward (Mann, 1981). Quantification of Acrosine levels is more difficult due to the dialysis step that is necessary to eliminate inhibitors of proacrosine-acrosine transformation (Polakoski and Zaneveld, 1984). An added incentive to measure AAT rather than Acrosine is the fact that AAT seems to be slightly more correlated with traditional parameters of boar semen quality than Acrosine (Strzezek et al., 1984).

The possibility that AAT activity might usefully predict the capacity of semen to resist the adverse effects of refrigeration and/or freezing has previously been addressed by Ciereszko et al. (1990) and by Strzezek and Ciereszko (1987). They showed that as semen was kept at progressively lower temperatures there was a correlatin between loss of AAT activity and decreasing of the semen quality, as evaluated by morphological examination of the spermatozoa. Their reults also showed that after the semen had been frozen and thawed there were clear relationships between the levels of AAT activity and sperm motility and percentage of normal acrosomes (Strzezek et al., 1984). In our work, reported here, we were unable to demonstrate any significant correlation between the initial (pre-freezing) enzymatic and morphological values with the capacity of the semen to undergo successful freezing and thawing. Only with respect to the pre-freezing ORT index did we observe a significant relationship with another post-thawing semen quality parameter, namely the percentage of normal acrosomes (Table 2).

The sharp and highly significant increasing in activity of both enzymes after a cold-shock observed in our samples tested after the glycerol addition is indicative of a marked loss of membrane integrity in the spermatozoa and associated liberation of enzymes. This has also reported by Pursel (1979) and by Cierezszko et al. (1990 and 1992). If, as seems likely, the loss of membrane integrity caused by the action of glycerol, and by the frezzing-thawing process is a primary cause of the low fertility of frozen boa semen, more research is needed to help the spermatozoa protection during freezign. In particular the possible damaging effects of glycerol as well as its protective effects on sperm cells remain to be fully elucidated.

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AKTIVNOSTI AMINOTRASFERAZE AKROSINA I ASPARTATA U SJEMENU NERASTA ZA VRIJEME PROCESA ZAMRZAVANJA, INDIKATIVNI ZA STANJE STANICE

Sažetak

Aktivnosit aminotransferaze akrosina i aspartata (AAT) nakn hladnog šoka kao i brojni uobičajeni morfološki pokazatelji kakvoće sjemena mjereni su nakon skupljanja u 36 ejakulata četiriju nerasta. Aktivnosti su u prosjeku oba enzima u svježem sjemenu prosječno iznosile 2.5 odnosno 45 mIU na 10^9 spermatozoa. Aktivnosti akrosina i AAT testirane u raznim fazama procesa zamrzavanja dale su usporedni uzrok promjena. Oba su enzima neznatno povećala aktivnosti nakon dodavanja žumanjka a znatno su porasle nakon dodavanja glicerola, pokazujući oštećenje stanica nakon dodavanja ovog spoja. Znatan pad aktivnosti enzima zapažen je u zamrznutom-otopljenom sjemenu. Aktivnosti tih enzima u svježem sjemenu nisu pružile korisna predviđanja o sposobnosti sjemena za uspješno zamrzavanje i odmrzavanje ali su dobar pokazatelj stanja stanica u procesu zamrzavanja. Postotak stanica u normalnim akrosomima nakon odmrzavanja u značajnoj je korelaciji s ORT indeksom ($P<0.025$) i ne značajno s postotkom stanica s normalnim akrosomima u svježem sjemenu.

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