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Age-dependent and seasonal changes in the activity of platelet-activating factor acetylhydrolase (PAF-AH) of boar seminal plasma in relation to the content of calcium ions

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The objective of this study was to determine the activity of platelet-activating factor acetylhydrolase (PAF-AH) and the content of calcium ions (Ca^{2+}) of boar seminal plasma in relation to season and animal age. Wide fluctuations in PAF-AH activity and Ca^{2+} content of plasma were observed. PAF-AH activity ranged from 334.2 to 524.3 nmol PAF hydrolyzed/min/mg protein, whereas the Ca^{2+} content from 2.4 to 3.4 mg/100 ml. In some cases PAF-AH activity was positively correlated with Ca^{2+} content. Both the PAF-AH activity and Ca^{2+} content of plasma depended on the season and age of the boars. The age-related and seasonal changes observed in PAF-AH activity suggest that the enzyme may play a role in boar reproductive functions.

KEY WORDS: boar / calcium / PAF-AH / reproduction / seminal plasma

The platelet activating factor – PAF – 1-O-alkyl-2-acetyl-*sn*-glycero 3-phosphoryl-choline, a naturally occurring membrane phospholipid compound belonging to the acetylated glycerophospholipid family, is implicated in various physiological and pathological processes in animals [Harper 1989, Venable *et al*. 1993]. PAF was first discovered as a chemical mediator released by sensitized basophils that caused blood platelet aggregation [Benveniste *et al*. 1972]. Synthesis and degradation of PAF are controlled by the activity of membrane-bound cytosolic and extracellular enzymes, particularly platelet-activating factor acetylhydrolase (PAF-AH) – EC 3.1.1.47 [Snyder 1995, Stafforini *et al*. 1997 and Soubeyrand and Manjunath 1998].

Recently, a great interest has been given to the action of PAF and its metabolizing enzymes in mammalian reproductive processes. As regards the male reproductive tract, PAF effects on motility, capacitation and acrosome reaction of spermatozoa have been well documented [Luconi *et al*. 1995, Muguruma and Johnston 1997, Huo and Yang 2000, Wu *et al*. 2001, Kordan and Strzeżek 2002].

Interestingly, the activity of PAF-metabolizing enzymes, particularly PAF-AH, facilitates the action of PAF in the processes accompanying spermatozoon-egg interactions [Wu *et al*. 2001]. It was shown that PAF-AH may help to stabilize the plasmalemma of spermatozoa and prevent the premature acrosome reaction that may be caused by PAF [Wu *et al*. 2001]. PAF-AH has been identified in the male and female reproductive tracts. PAF-AH occurs in the seminal plasma of many laboratory and livestock species, *i.e*. bull, human, boar, rooster rabbit, stallion and rat [Hough and Parks 1994, Kordan 2001]. However, the reproductive significance of PAF-AH in the seminal plasma has yet to be defined. Moreover, PAF-AH takes part in the neutralization of spontaneously damaged phospholipids, including the plasmalemma of spermatozoa, induced by lipid peroxidation.

This study aimed at determining the activity of platelet-activating factor acetylhydrolase (PAF-AH) and the content of calcium ions (Ca^{2+}) in boar seminal plasma, in relation to season and animal age.

Material and methods

Animals and semen collection

Ejaculates were collected once a week from three Polish Landrace boars at the age of 8 months, from February 1999 through December 2001. All the boars were housed under the same conditions in a climate-controlled environment, in individual pens, and were given a commercial porcine diet. Water was available *ad libitum*.

The study was approved by the local Ethics Committee. The official regulations (Directive on Animal Protection, Government Gazette of the Republic of Poland, No. 111, item 724, of August 21, 1997) on animal protection were followed.

A total of 236 ejaculates were collected using the "gloved-hand" technique. The gel portion was removed using double gauze and the semen samples were centrifuged at 10, 000 × g for 15 min at room temperature. The seminal plasma obtained was used immediately or stored at -20°C (253. 2 K) until required.

PAF-AH assay

PAF-AH activity was determined using [^{3}H]-acetyl-PAF (hexadecyl-acetyl-*sn*-glycero3-phosphorylcholine (NEN Research Products, Boston MA) as a substrate. Acetylhydrolase activity was determined by measuring the release of [^{3}H]-acetate from PAF according to Safforini *et al*. [1987] and Safforini *et al*. [1990] with some

modifications [Kordan and Strzeżek [2000]. Approximately 15 mg/ ml of protein were employed in each PAF-AH assay. Briefly, 40 µl of substrate consisting of 100 µM PAF supplemented with [^{3}H]-acetyl-PAF (11.25 µCi/µM) suspended in 0.1 M HEPES buffer, pH 7.2 (SIGMA) were incubated with 10 µl of seminal plasma in an atmosphere of 5% CO_2 (Incubator SANYO, MCO 175-T) for 2 h at 37°C (310.2 K). The reaction was stopped by adding 50 µl of 12.5% trichloroacetic acid (TCA) (SIGMA) followed by neutralizing with 1.5 ml of 100 mM sodium acetate (pH 7.4). Labelled acetate was recovered by reverse-phase chromatography (RH-FPLC) on octadecyl gel columns (octadecyl C_{18}-silica gel, JT BAKER) pre-equilibrated with absolute ethanol and 100 mM sodium acetate. Elution of [^{3}H]-acetate was conducted with 100 mM sodium acetate (pH 7.4). [^{3}H]-acetate released from [^{3}H]-acetyl-PAF was measured with a liquid scintillation meter (BECKMAN LS 5000 TD) with 70% efficiency for tritium, and expressed as the nmol PAF hydrolyzed/min/mg protein (PAF-AH activity).

Calcium ions (Ca^{2+}) determination

Determination of Ca^{2+} in the seminal plasma was performed according to Tisdall and Kramer [1925]. Briefly, 1 ml of saturated ammonium oxalate ($C_2H_8N_2O_4$) and 2 ml of distilled H_2O (MILLIPORE) were added to 2 ml of plasma. The samples were thoroughly mixed and centrifuged at 600 × g for 10 min at room temperature. The supernatant was decanted and calcium oxalate precipitate was washed twice with 4 ml of 2% ammonia solution (600 × g for 10 min). The resulting supernatant was decanted and the precipitate was re-dissolved in 2 ml of 1N H_2SO_4. Blank sample solution consisted of 0.1 ml of 2% NH_4, and 2 ml H_2SO_4. All samples were kept in a water bath at 100°C for 1 min and titrated with 0.01 N potassium permanganate ($KMnO_4$) to give a colour change (light pink), persisting for at least one minute. The Ca^{2+} content of plasma was calculated according to the formula:

$$Ca^{2+} \text{ content (mg\%)} = 10(a - b)$$

where:

a – volume of $KMnO_4$ used for titrating the test sample;

b – volume of $KMnO_4$ used for titrating the blank samples.

Statistical

Values are expressed as means and their standard deviations (SD). Significance of differences between the means was estimated at $P \leq 0.5$ with Duncan's multiple range test. The relationship between the variables was evaluated using Pearson correlation coefficients. Analysis was conducted using the STATISTICA computer programme (STATISTICA for Windows, Inc., Tulsa, USA).

Results and discussion

The level of many seminal plasma components is dependent on the age of the animal and season. This view is supported by evidence showing that photoperiod, an environmental factor, influences the yearly secretion of the seminal plasma components [Borkowski and Strzeżek 1994]. As regards boar seminal plasma, proteins with antiperoxidative properties facilitate the natural defense mechanism, which relies on increased secretion during the periods when spermatozoa are highly susceptible to environmental conditions [Strzeżek 1999]. PAF-AH is implicated in the neutralization of spontaneously damaged phospholipids of the spermatozoa plasmalemma caused by lipid peroxidation [Wu *et al*. 2001].

Recently, PAF-AH of boar seminal plasma has been purified and characterized [Kordan 2001]. Sample of the purified enzyme (44-fold) is highly dependent on the concentrations of calcium ions in the incubation mixture. Hence, in this study, the effect of Ca^{2+} content of seminal plasma on PAF-AH activity was analysed in relation to the age of the boars and season.

PAF-AH activity of the seminal plasma is species-dependent. The enzyme activites in the seminal plasma of bull, stallion, rat, rabbit, human and rooster were found 105, 99, 20, 0.99, 0.80 and 0.42 nmol/min/mg protein, respectively [Hough and Parks 1994]. The secretory sites of PAF-AH have been identified in the seminal vesicle glands and prostate [Kordan 2001].

The level of PAF-AH activity of plasma was from 334.2 to 524.3 nmol PAF hydrolyzed/min/mg protein. It was observed that PAF-AH activity was dependent on the age of the boars (Fig. 1) and season (Tab. 1). It should be noted that boar spermatozoa

Table 1. Means and their standard deviations (SD) for PAF-AH activity of boar seminal plasma as affected by season over three years

Season		PAF-AH activity (nmol PAF hydrolyzed/min/mg protein)		
		1999 yr. (n=80)	2000 yr. (n=80)	2001 yr. (n=80)
Autumn	mean	342.0[a]	360.8[a]	400.6[a]
	SD	28.9	33.5	44.0
Winter	mean	332.2[a]	400.9[b]	430.2[a]
	SD	48.3	44.0	38.7
Spring	mean	334.2[a]	445.5[b]	524.3[b]
	SD	33.2	40.1	47.8
Summer	mean	394.5[b]	485.5[c]	488.7[b]
	SD	40.2	49.2	39.9

[a,b,c]Within columns means bearing different superscripts differ significantly at P≤0.05.

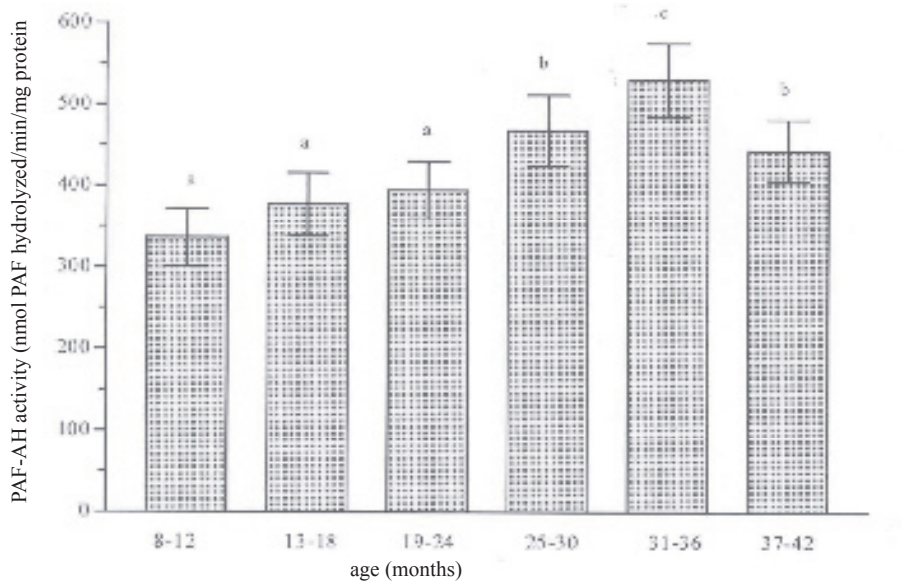

Fig. 1. Effect of boars' age on PAF-AH activity of seminal plasma (total n = 236). Values are means ± SD. Means marked with different letters are significantly different at P≤0.05.

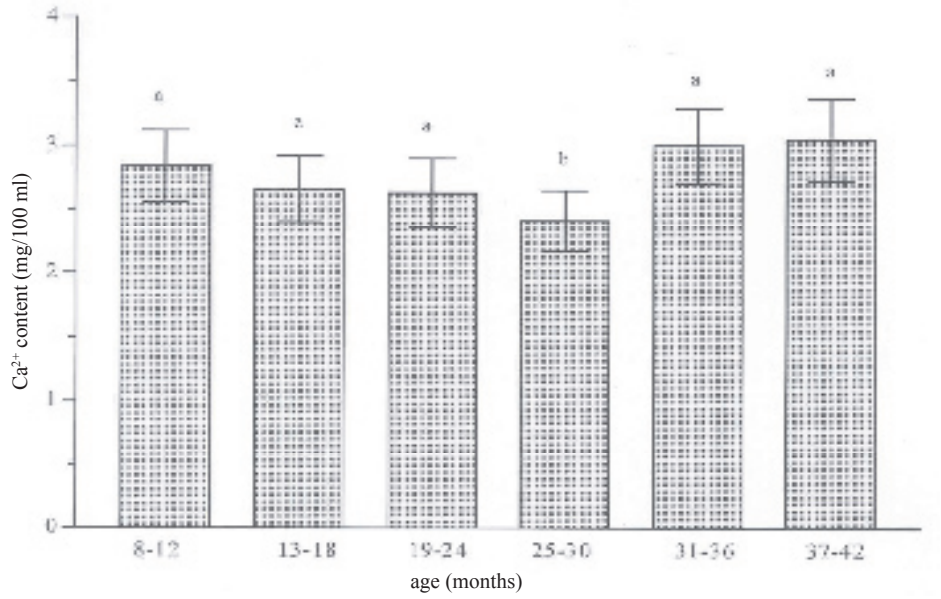

Fig. 2. Effect of boars' age on Ca^{2+} content of seminal plasma (total n = 236). Values are means ± SD. Means marked with different letters are significantly different at P≤0.05.

are a rich source of phospholipids with *sn*-2 position of glycerol containing long-chain polyunsaturated fatty acids which can be fragmented into short-chain fatty acids during lipid peroxidation [Parks and Lynh 1992, Strzeżek *et al*. 1999]. Moreover, the modified phospholipids may enhance substrate availability for PAF-AH. This may be the reason for the very high PAF-AH activity of boar seminal plasma compared with those of other animal species.

In boars younger than 37 months an increase in PAF-AH activity was parallel to their age (Fig. 1). Maximum enzyme activity was found in boars aged 31-36 months, followed by a drop at 37-42 months. This phenomenon might be associated with the boar attainment of reproductive maturity. Observed was seasonal variation in PAF-AH activity within the respective year (Tab. 1), the maximum being found in the summer. This might be attributed to intensive secretion of the enzyme in the male reproductive tract as a result of accumulation of toxic products in the sperm structure, or intensive PAF synthesis. Moreover, it has been shown [Strzeżek *et al*. 1992] that boar spermatozoa are susceptible to induced membrane lipid peroxidation and ejaculates have been associated with poor spermatozoa quality when collected during the summer.

Table 2. Means and their standard deviations (SD) for Ca^{2+} content of boar seminal plasma as affected by season over three years

Season		Ca^{2+} content (mg/100 ml)		
		1999 yr. (n=80)	2000 yr. (n=80)	2001 yr. (n=80)
Autumn	mean	2.4[a]	2.6[a]	3.2[a]
	SD	0.3	0.5	0.5
Winter	mean	2.8[a]	2.9[a]	2.9[b]
	SD	0.4	0.5	0.3
Spring	mean	2.9[a]	2.5[a]	3.4[a]
	SD	0.3	0.2	0.3
Summer	mean	3.0[b]	3.3[b]	3.1[a]
	SD	0.3	0.4	0.4

[a,b] Within columns means bearing different superscripts differ significantly at $P \leq 0.05$.

The Ca^{2+}content of boar seminal plasma ranged from 2.4 to 3.4 mg/100ml and was associated with both the age of the boars and season. The lowest Ca^{2+} content was found in boars aged 25-30 months (Fig. 2). Furthermore, higher Ca^{2+} content was found during the summer (1999 and 2000) than in other seasons (Tab. 2).

The correlation coefficients between PAF-AH activity and Ca^{2+} content of the seminal plasma in relation to the age of the boars and seasons are presented in Tables 3 and 4. PAF-AH activity was positively correlated with Ca^{2+} content of plasma of

Table 3. Correlation coefficients between PAF-AH activity and Ca^{2+} content of seminal plasma as related to the age of the boars (n = 236)

Boar age (months)	No of ejaculates	Correlation coefficient
8-12	40	-0.11
13-18	40	+0.17
19-24	40	+0.47*
25-30	40	-0.25
31-36	38	+0.61*
37-42	38	+0.30

*P≤0.05.

Table 4. Correlation coefficients between PAF-AH activity and Ca^{2+} content of boar seminal plasma as related to season (n = 236)

Season	No of ejaculates	Correlation coefficient
Autumn	60	+0.29
Winter	60	-0.07
Spring	58	+0.47*
Summer	58	+0.63*

*P≤0.05.

boars at the age of 19-24 and 31-36 months. Significant correlations were also found between PAF-AH activity and Ca^{2+} content during the spring and summer. The earlier study [Kordan 2001] showed that PAF-AH activity was stimulated by calcium ions. Similar calcium stimulatory effects were shown for the unpurified form of PAF-AH from stallion seminal plasma [Hough and Parks 1994]. In contrast, PAF-AH isolated from bovine seminal plasma was found calcium-independent [Hough and Parks 1997, Soubeyrand *et al*. 1998].

Summarizing the results presented here it may be stated, that an increase in the PAF-AH activity of the seminal plasma of boars older than 2 years was confirmed what may indicate an efficient secretory activity of the accessory sex glands. The age-related and season-dependent changes observed in PAF-AH activity suggest that the enzyme may play a role in boar reproductive functions. Additional studies are needed to elucidate the physiological role of PAF-AH in boar seminal plasma relative to reproductive functions.

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Aktywność acetylohydrolazy płytkowego czynnika aktywującego (PAF-AH) oraz zawartość jonów Ca^{2+} w plazmie nasienia knura zależnie od wieku i pory roku

S t r e s z c z e n i e

Określono aktywność PAF-AH oraz zawartość jonów Ca^{2+} w plazmie nasienia knura z uwzględnieniem rozwoju osobniczego i pory roku. Aktywność PAF-AH kształtowała się na poziomie 334-524 nmoli PAF/min/mg białka i wykazywała zależność od wieku knurów oraz pory roku. Zawartość jonów Ca^{2+} wynosiła średnio 2,90 mg/100 ml (wahania od 2,41 do 3,38 mg/100 ml plazmy), zmieniała się z wiekiem knurów i zależała od pory roku. Wykazano korelację między aktywnością PAF-AH, a zawartością jonów Ca^{2+} w plazmie nasienia w czterech grupach wiekowych i sezonowych. Fluktuacje aktywności PAF-AH wskazywać mogą na związek enzymu z procesami reprodukcyjnymi.