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BOVINE SEMINAL PLASMA INHIBITION OF IMMUNE COMPLEX PRECIPITATION

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The influence of bovine seminal plasma (SP) on the antigen-antibody precipitating complexes was investigated using kinetic nephelometric procedure. Formation of OVA/anti-OVA (ovalbumin) immune complexes was inhbited by bovine seminal plasma in a dose dependent manner. Partial characterization of the active substance(s) was performed after chromatolography and gel- filtration separation procedures.

Key words: Bovine seminal plasma, Immune precipitation, immune complex

INTRODUCTION

One of the major paradoxed of reproductive immunology is certainly the physiological absence of an immune responce to sperm and/or seminal antigens, both in males and females. For a long time it was thought that the main reasons for this phenomenon were: (1) the presence of the blood-testis barrier in males (Johnson and Setchell, 1968); (2) masking of sperm surface antigens by low antigenicity components from the seminal plasma in females (Weil and Rodenburg, 1962). In the mid-70's it became clear that therelationship between the immune system and gametes is more complex. During the following years numerous scientists demonstrated that mammalian seminal plasma can influence many cellular and molecular functions of the immune system (reviewed by James and Hargreave 1984., and Witkins 1988.)

Up to now much more attention has been paid to the effects of seminal plasma on the cells than on the effector molecules of the immune system. It is our opintion that the latter influences are also of great importance because they may prevent sperm destruction even if the immune responce has been evoked for some reason.

It is well documented that human seminal plasma inhibits hemolytic activity of the complement system (Peterson et al. 1980., Tarter and Alexander 1984., Jane and Tschopp 1989.) Simmilar effects were observed for the seminal

vesicles and prostate secretions in mice (Peitz and Bennet 1981, Anderson and Tarter 1982.). Witkin and colleagues (1983) demonstrated decreased binding of IgG molecules to the Fc receptors on the cells in the presence of seminal plasma.

While investigating the effects of bovine seminal plasma on activation of the complement system we noticed that seminal plasma itself can influence the precipitation of artificial antigen-antibody complexes. Until now, this phenomenon was attributed to the inhibitory actions of the complement system or stimulatory activity of antiimmunologlobulin antibodies (Czop and Nussenzweig, 1976, Shifferly et al. 1982).

The objestive of this study was to investigate the influence of bovine seminal plasma on immune complex precipitation and to characterize partialy the active substance(s).

MATERIAL AND METHODS

Semen samples were collected from black and white spotted bulls (Holstein breed) by means of an artificial vagina in the Regional Center For Artificial Insemination. The semen possessed normal characteristics of motility, morphology and concentration. After collection ejaculates were cooled at + 4° C over 3 hrs. The SP was separated after three sequential centrifugations for 15 min. at 400, 800 and 1200 g at + 4°C and stored at -20° C.

The pool of SP was created by mixing equal volumes (1 ml) of seminal plasma from ten bulls. Pooled seminal plasma (10ml) was diluted in 3 mM phosphate buffered saline (pH 7.2) up to 80 ml and chromatographed on a DEAE-Sephacel column (26 x 80 mm) (Pharmacia), previously equilibrated with the same buffer.

Fractions (10 ml) were eluted by linear gradient up to 0,4 M NaCl in 3 mM phosphate buffered saline (200 + 200 ml) and collected using an automatic collector (Multirac 2111, LKB-Bromma, Sweden). The elution curve was recorded on a spectrophotometer (Uvicord 2138, LKB-Bromma, Sweden) at 278 nm. According to the elution curve fractions were pooled into 8 groups and concentrated using a (PM 30) Amicon-Diaflo membrane (cut off 30 kDa) with N2 pressure of 50 Bars.

Gelfiltration of the active SP fractions was performed using an Ultrogel AcA 34 (LKB) column (16 x 580 mm) and the same apparaturs as previously described. After the gel-filtration fractions were pooled into 4 groups according to the elution curve and concentrated using a YC 5 Amicon-Diaflo membrane (cut off 500 Da) with N2 pressure of 50 Bars.

Protein concentration in seminal plasma fractions was determined using BCA-assay (Sigma No TPRO - 562).

The influence of whole SP and SP fractions on the immune complex precipitation was investigated using purified OVA (ovalbumin) and anti-OVA (anti-ovalbumin) obtained by rabbit immunization (Rodić 1986). Purified OVA

and anti-OVA solutions were diluted with VBS[1] in the rations 1:32 and 1:5, respectively before testing. Tests were performed with 75 μl of SP (or its fractions), 25 μl of VBS, 100 μl of OVA and 100 μl of anti-OVA in the test tube. In cases when a volume of the SP (or its fractions) was changed an appropriate volume of VBS was added to reach the final volume of 300 μl.The negative control sample contained only 100 μl of VBS, OVA and anti-OVA while the positive control contained 50 μl of NHS (Normal Human Serum), 50 μl of VBS and 100 μl of OVA and anti-OVA.

Immune compllex precipitation was measured by a kinetic nephelometric procedure over 20 min. The percent of total precipitation was calculated from the laser diffraction values (LS,V) by planimetry. We used a Helium-neon laser, 4mV, 638,8 nm and Pye Unicam linear recorder AR 55 (Behring).

Immunoelectophoretic chararacterization (IEF) characterization of protein molecules in the active fractions of polyspecific anti-SP and anti BS (bovine serum) sera obtained by rabbit immunization.

RESULTS

The influence of bovine seminal plasma on the OVA/Anti-OVA precipitating complex was investigated kinetically during a 20 min. period. Pooled SP inhibited immune complec precipitation in a dose dependent mode (Figure 1) showing almost linear correlation (r=0.997).

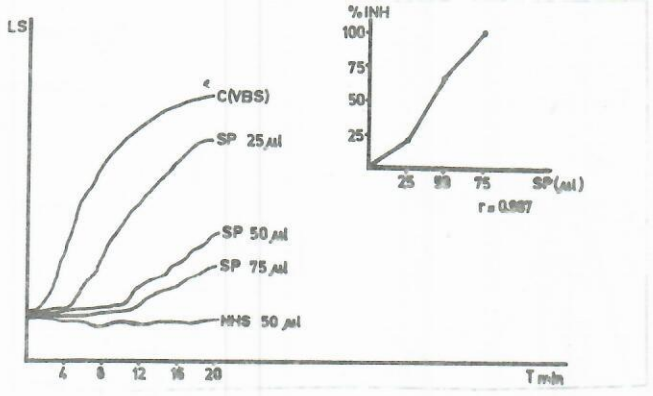

Figure 1. The influence of bovine seminal plasma (SP) on the immunoprecipitation kinetics
NHS — Normal Human Serum
VBS — Veronal Buffer Saline

1 Veronal buffer saline (VBS), pH 7.2

NaCl	17.0	g
Na-veronal	0.37	g
Veronal	1.15	g
MgC12	0.7167	g
CaC12	0.056	g
NaN3	0.4	g
H_2O dest.	ad 2000	ml

pH was adjusted with 1M Na-veronal or 1M Veronal solutions.

When ten individual bovine seminal plasma samples were tested for their influence on antigen-antibody precipitation, it was demonstrated that inhibitory activity is their common feature. (Table 1.)

Table 1. The inhibition of OVA anti-OVA immune complex precipitation by bovine seminal plasma (25 μl)

Sample No	% Inhibition	Protein conc (mg/ml)
1	66.78	78.57
2	45.67	61.60
3	47.06	50.00
4	44.29	50.89
5	39.79	40.90
6	20.42	4147
7	24.22	67.85
8	33.91	34.09
9	30.80	101.53
10	32.18	96.42

Fractions of bovine seminal plasma, obtained by chromatography on a DEAE-Sephacel column, were tested in the same way and only fraction SP 1/1 clearly inhibited immune complex precipitation. Fractions SP 1/2 and 1/8 caused moderate enhancement (not shown here) of LLS (Laser Light Scattering) (Figure 2). The influence of fraction SP 1/1 was also dose dependent (r=0.90) (Figure 3).

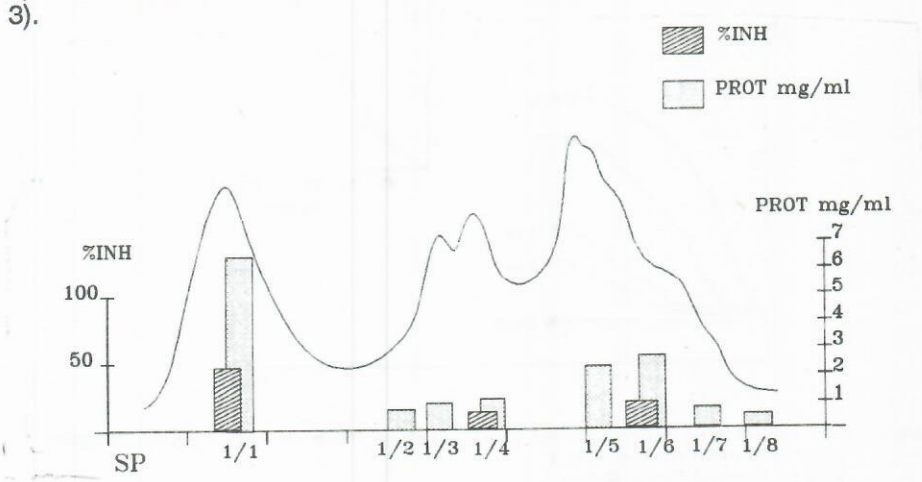

Figure 2. Inhibition of immune complex precipitation (%) by bovine seminal plasma fractions (obtained by ion exchange chomatography on a DEAE-Sephacel column)

After gel-filtration of fraction SP 1/1 only one subfraction (SP 2/1.1.) inhibited immune complex precipitation while subfractions SP 2/1.3. and 2/1.4. caused enhancement (not shown here) of LLS (Figure 4). Wishing tc confirm the dose-dependent mode of the inhibitory influence of subfraction SP 2/1.1.

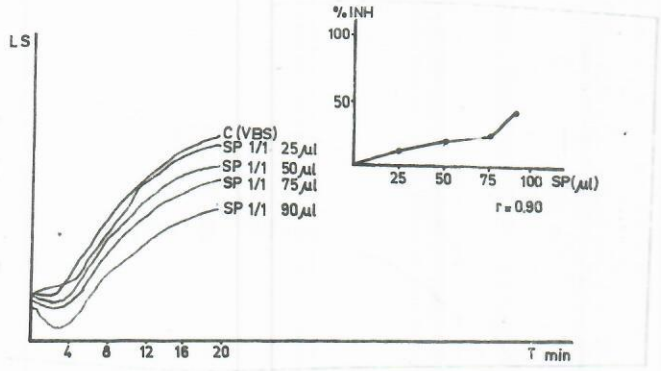

Figure 3. The influence of the bovine seminal plasma fraction SP 1/1 on the immunoprecipitation kinetics
VBS — Veronal Buffer Saline

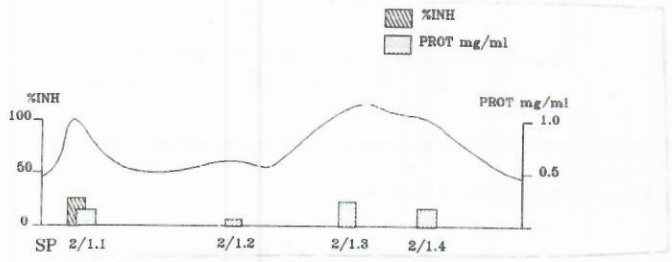

Figure 4 Inhibition of immune complex precipitation (%) by bovine seminal plasma subfractions (obtained by gelfiltrtion of fraction Sp 1/1 on an Ultrogel AcA 34 column)

we added 150 µl to a test tube containing smaller smounts of OVA and anti-OVA (but less diluted) The results shown in Figure 5. clearly indicate the higher degree od inhibition.

IEF analyses of active bovine seminal plasma fraction SP 1/1 with polyspecific antisera against proteins of bovine seminal plasma (a -BSP) raised in rabbits revealed the presence of one precipitation line, on the anode side, in the zone of alpha globulins. On the cathode side it was possible to demonstrate 4 precipitation lines in the beta-gamma globulin zone. When antisera against bovine serum proteins were used (a-BS) only one precipitation line was demonstrated at the cathode and in the gamma 1 zone. The same antisera (a-BSP and a-BS) were used to determine the presence of the proteins in the active seminal plasma subfraction SP 2/1.1. but no precipitation lines could be demonstrated (Figure 6).

DISCUSSION

One of the biological functions of the complement system is its ability to prevent formation of large antigen-antibody aggregates. This phenomenon is

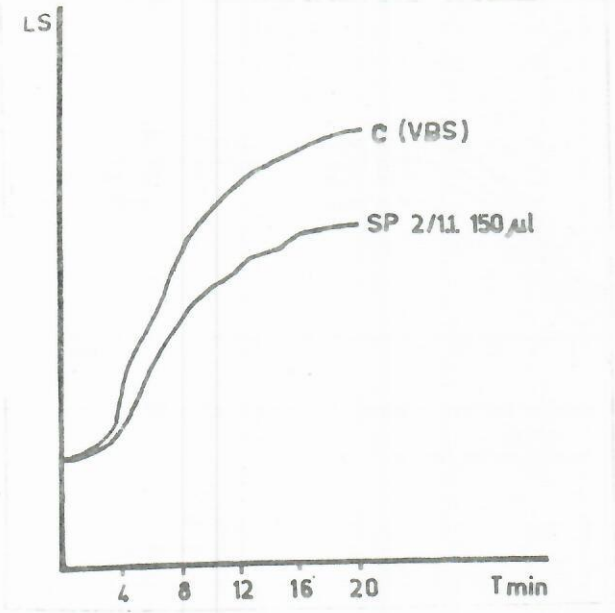

Figure 5. The influence of the bovine seminal plasma subfraction SP 2/1.1 on the immunoprecipitation kinetics
VBS — Veronal Buffer Saline

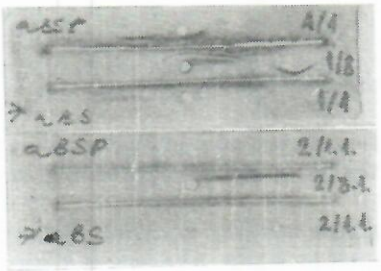

Figure 6. Immunoelectropherograms of the active bovine seminal plasma fraction (SP 1/1) and subfraction (SP 2/1.1.)
SP 1/8 and SP 2/8.1. — control fraction and subfraction with no significant influence on immune complex precipitation
a-BS — rabbit anti Bovine Serum
a — BSP rabbit anti Bovine Seminal Plasma serum

called complement mediated in hibition (CMI) and has a protective role in preventig the deposition of such aggregates in the tissue. CMI is mainly due to the activation of classical pathway (Shifferly et al. 1982).

The above results, demonstrate that bovine seminal plasma also has the ability to inhibit antigen-antibody complex precipitation in dependent of complement. This conlusion can be supported by two criteria: (1) contrary to the

complexity of the complement system, the inhibitory component of bovine seminal plasma seems to be homogenous and located in a single DEAE-Sephacel and Ultrogel AcA Fraction, and (2) contrary to the inhibitory influence of repeated freezing-thawing and thermal inactivation on complement activity, the inhibitory component in bovine seminal plasma is thermostable and not affected by the conservation procedure.

Immunoelectrophoretic analysis of Ultrogel AcA 34 fraction (BSP 2/1.1.) with anti-bovine seminal plasma and anti-bovine serum antisera did not confirm the presence of reactive protein molecules . That leads us to three possible conclusions:

1. The antisera used in this experiments did not contain antibodies against active (inhibitory) protein molecules.

2. Inhibitory protein molecules had weak antigenic reactivity.

3. Protein molecules that expressed inhibitory activity towards the OVA-anti OVA precipitation were present in this fraction but in low concentrattions.

In our opinion, last possibility is the most reasonable because of the very low protein concentration in subfraction SP 2/1.1.At the same time the presence of protein molecules in the active seminal plasma fraction (SP 1/1) and their absence in the active subfraction (SP 2/1.1.) lead to the conclusion that these proteins are not responsible for the inhibitory activity.

In our opinion the inhibitory activity of bovine seminal plasma towards immune complex lattice formation (i.e. precipitation) should be added to the list of important biological phenomena caused by mammalian seminal plasma. It will be of substantial interest to determine if seminal plasma either inhibits primary antigen-antibody reactions or the secondary large lattice formation-reaction only. This problem will be the subject of our future investigation.

Acknowledgement This work was supported by a grant from the Republic Fund for Science of Serbia and carried out in the Department for Tissue Typing and Immunochemistry, Blood Transfusion Institute, Beograd

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SADRŽAJ

Uticaj semene plazme bika na precipitaciju imunih kompleksa, ispitivan je kinetičkim nefelometrijskim postupkom. Semena plazma bika dovodila je do inhibicije nastanka precipitata ovoabumin — antiovoalbumin, u zavisnosti od doze. Delimična karakterizacija aktivnih supstanci izvršena je imunoelektroforezom posle hromatografske i gel-filtracijske separacije.

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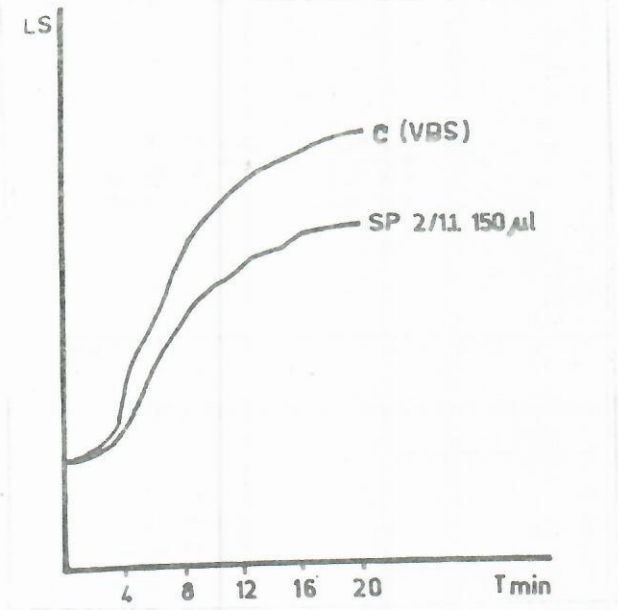

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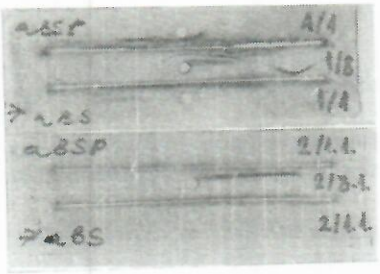

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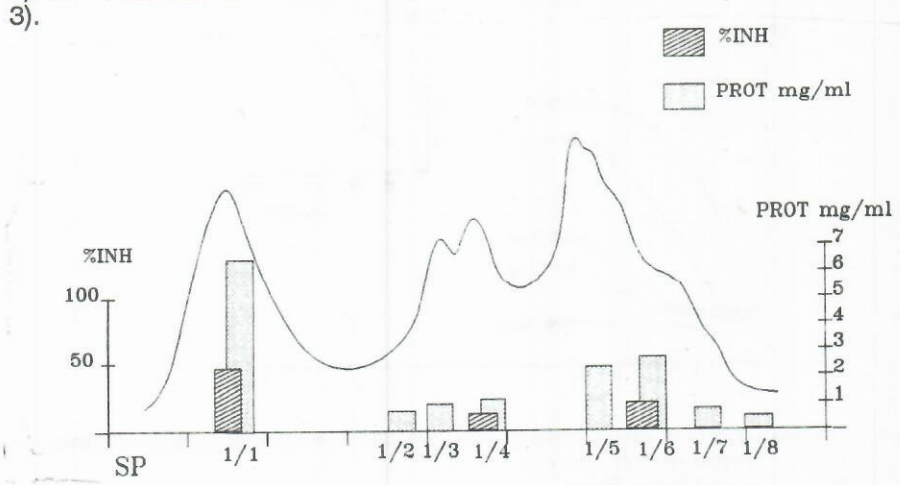

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