



Original Contribution

DETERMINATION OF SPECIES SPECIFICITY OF PROSTATE-SPECIFIC ANTIGEN (PSA) IN SEMEN

R. Miteva^{1*}, S. Yotov², P. Georgiev², I. Fasulkov³

¹Faculty of Medicine, ²Faculty of Veterinary Medicine, ³ student of Veterinary Medicine
Trakia University, Stara Zagora, Bulgaria

ABSTRACT

The aim of the present investigation was to determine the species specificity of prostate-specific antigen in the semen of ram, jack and human. A comparative study of undiluted and diluted semen from ejaculates of rams, male donkeys (jacks) and human, was performed. The species specificity of PSA was done with the specific membrane test SERATEC® PSA Semiquant, SERATEC, Gesellschaft für Biotechnologie mbH, Göttingen, Germany. Our results showed the lack of cross reactivity between PSA in semen from ram, jack and human. The SERATEC® PSA Semiquant test is human-specific and not appropriate for detection of prostate-specific antigen in either ram or jack semen.

Key words: semen, PSA, human, animals

INTRODUCTION

The prostate specific antigen - PSA was discovered by Hara et al (1) and was named γ -seminoprotein. Later, Li and Beling (2) described the same protein and called it E1. It could be detected in various biological fluids and tissues, including mammary gland, mammary gland tumours and milk (3, 4, 5, 6), accessory sex glands (7), amniotic fluid (6), female urine (8) and endometrium (9).

An attempt at complete biochemical analysis of the protein, isolated from human semen, was performed by Sensabaugh (10) who determined it as a protein with molecular weight of about 30 kDa and called it p30. The most recent studies showed that PSA is an organ-specific enzyme, a 38 kDa protease, produced by the prostate gland. It is one of the most important proteins in semen and its primary function is to liquefy the semen. The activity of this enzyme in other biological fluids is limited (about 15 min) due to its binding to the respective protein C inhibitor (11, 12).

The detailed investigation of PSA is a prerequisite for production of antiserum and

express diagnostic kits from various laboratories (13, 14, 15). They are primarily used in human medicine for diagnostics of prostate tumours (16) and in forensic medical practice for detection of semen stains (17, 18). Aside the rapidity, high precision and easy interpretation, another advantage of tests based upon PSA detection is evidencing of semen fluid in vasectomized or azospermic individuals. The specificity of the test for human semen varies from 88.7% to 100% (15, 19).

Most authors using PSA tests work with human semen (1, 14, 18). There are single reports about the use of the express PSA test in semen from other mammalian species. In his studies, Hochmeister et al. (19) did not report a cross reactivity of the test when used on semen from dogs, cats, horses, bulls and boars. There are however no information with regard to either ram's or jack's semen.

AIM

The aim of the present investigation was to determine the species specificity of prostate-specific antigen of ram, jack and human semen.

MATERIAL AND METHODS

A comparative study of semen from ram (n=3), jack (n=3) and human (n=6) ejaculates

* **Correspondence to:** Dr. Radostina Miteva, Department of Pathoanatomy, Forensic Medicine and Deontology, Faculty of Medicine, Trakia University, Bulgaria, 6000 Stara Zagora; E-mail: drmiteva72@abv.bg

was performed. Immediately after obtaining the semen, it was evaluated and placed in a water bath at 35^o C. The volume of ejaculate (ml), spermatozoa concentration (10⁹ or 10⁶ depending on the species), spermatozoa motility (%) and dead spermatozoa (%) were determined. Spermatozoa counts were counted in a chamber, the motility – in a native preparation and dead spermatozoa were quantified by the method of supravital staining of spermatozoa (20).

For determination of the species specificity of PSA, a specific membrane test SERATEC® PSA Semiquant, SERATEC, Gesellschaft für Biotechnologie mbH, Göttingen, Germany, was used. The Seratec PSA test is a chromatographic immunoassay (CIA) for the rapid determination of PSA.

Anti-PSA antibodies, immobilized at the test region on a membrane, are used. The prostate-specific antigen in samples is bound to the antibody and forms a PSA-anti-PSA-antibody-complex. In this case, the complex binds to anti-mouse antibody, and thus, two red lines develop. These lines are independent of the presence of PSA in the sample and indicate only the correct performance of the test. The positive sample is manifested by the formation of an additional line (21).

Analyses of buffer, undiluted semen and semen, diluted 1:500 and 1:1000, were

made. For this purpose, a standard buffer solution with neutral pH, recommended by the manufacturer, was used.

The detection of results was done according to the instructions and procedures given by the manufacturer (<http://www.seratec.com>). The negative results were based upon detection of the control line (C) of the test and the internal standard line in the result window.

Results, where the control line of the test (C), the internal standard line and the test line (T) were detected, were accepted as positive.

Incorrectly analysed samples were present when there was no line in the result window. The time for appearance of the test line was determined.

The results from the analysis of seminal fluid in the different species were photographed and compared.

RESULTS

The data obtained from the analysis of semen in the different species are presented on **Tables 1** and **2** and **Figs. 1-4**.

The biological parameters of semen in the different species (**Table 1**) were within the reference range.

Table 1. Biological parameters of semen (Mean±SEM)

<i>Biological parameters of semen</i>	<i>Ram n=3</i>	<i>Jack n=3</i>	<i>Human n=6</i>
Volume, ml	1.2±0.12	38±8	3.48±0.52
Concentration 10 ⁹ / 1 ml for rams and jacks 10 ⁶ / 1 ml for men	2.24±0.39	0.45±0.18	34.7±5.22
Motility, %	80±5.77	70±15	57.5±10.31
Dead spermatozoa, %	21±2.92	4.46±1.28	11.43±2.58

Initially, the buffer solution used for semen dilution was tested and no reaction was detected (**Figure 1**).

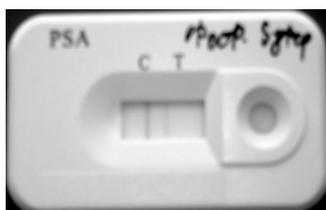


Figure 1. PSA test of phosphate buffer

The results from the analysis with SERATEC® PSA Semiquant (**Table 2**) of undiluted seminal fluid from ram and jack, showed invalid sample for the undiluted ram semen (**Figure 2a**) and negative results for that of jacks (**Figure 3a**). After dilutions of 1:500 and 1:1000, there was no positive reaction for both species (**Figure 2b, c** and **Figure 3 b, c**), but unlike undiluted semen, the control line and that of the internal standard were detected. The appearance of reaction in diluted ram semen was observed

on the average 9 ± 3 min after adding seminal fluid drops in the sample window, but a positive result was not obtained. In the jack, the average time was 5 ± 2 min, and in all three cases, the result was negative.

The study of undiluted human semen gave also negative reaction (**Figure 4a**). The analysis of diluted human sperm were, however, all positive (**Figure 4b, c**). The time for appearance of the test line was 6 ± 3 min on the average, the intensity of the test line being clearer in three out of all samples.

Table 2. Analysis of semen samples from rams, jacks and human via the SERATEC® PSA Semiquant test

Semen type	Species		
	Ra m	Jack	Ma n
Undiluted	(0)	(-)	(-)
Diluted	(-)	(-)	(+)
1:500	(-)	(-)	(+)
1:1000	(-)	(-)	(+)

(0) - no reaction

(+) - positive result

(-) - negative result



Figure 2. PSA test of ram semen
a) undiluted semen b) dilution 1:500 c) dilution 1:1000



Figure 3. PSA test of jack semen
a) undiluted semen b) dilution 1:500 c) dilution 1:1000

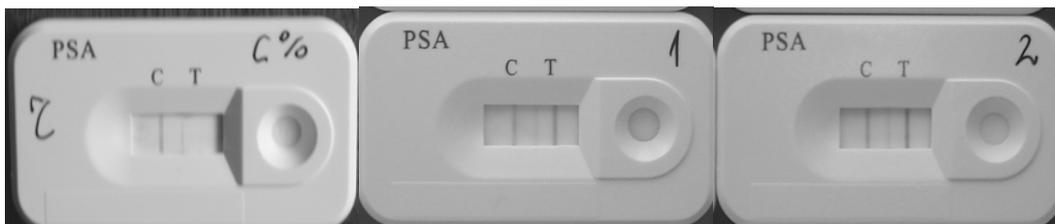


Figure 4. PSA test of human semen
a) undiluted semen b) dilution 1:500 c) dilution 1:1000

DISCUSSION

The values of biological parameters of ram and jack semen showed that there were no diseases of the genital tract.

The negative test of the buffer solution showed that there was no reaction following mixing the buffer ingredients with those of the test and that it was appropriate for dilution of the sample.

The negative result in the study of undiluted ram semen according to us was due to the high density (2.24 ± 0.39) of semen that

did not allow the penetration of seminal fluid through the capillary membrane and the reaching of the control line, the test line and the internal standard line. A similar effect was described by Laux and Custis (13) throughout the analysis of male urine. In such cases, Sato et al. (14) recommended dilution of 1:200 or higher for prevention of diagnostic errors.

The appearance of control line and the internal standard line in undiluted donkey semen and after dilutions 1:500 and 1:1000 of semen from both species was an indication of the correct execution of the test. The absence

of a time-dependent effect upon the intensity of colour development of the control line and that of the internal standard confirmed once again the negative results in rams and jacks.

The absence of positive reaction in the analysis of undiluted human sperm, although less concentrated ($34.7 \pm 5.22 \times 10^6$) (2) according to us, was due to the high amounts of PSA. This confirms the assumptions of Sato et al. (14) about the need of additional dilution in these cases. Our data from the analysis of diluted human semen also support this hypothesis.

The differences in the time of appearance of the test line and the intensity of the colour could be explained by the effect of the PSA amount in the different ejaculates. Individual differences in the amount of this protein have been communicated by Sensabaugh (10). The weaker intensity of the test line colour when a higher dilution was performed attests to this fact.

Similar results with the use of the same method were reported by Maher et al. (17), whereas, according to Laux et al. (21), the test could serve for quantitative determination of PSA, comparing the test line coloration with that of the internal standard, the latter corresponding to 4 ng PSA/ml. According to Sato et al. (14), PSA concentrations in cases of prostate diseases reach 200 ng/ml that was manifested by a thick, intensively coloured test line and is indication for performance of more detailed investigations. The lack of significant deviations from the normal spermogram and the appearance of intensely coloured thick test line in human ejaculates studied by us, were indicative of the lack of such pathology.

Our results evidenced the lack of cross reactivity between PSA from seminal fluids obtained from rams, jacks and humans and supported the data of Hochmeister et al. (19) from their comparative study of human and animal PSA.

CONCLUSION

The prostate-specific antigen is human-specific. The SERATEC® PSA Semiquant test could not be used for detection of prostate-specific antigen in either ram or jack semen.

REFERENCES

1. Hara M., Inorre T., Fukuyama T. Some physico-chemical characteristics of gamma-seminoprotein, an antigenic component specific for human seminal

- plasma. *Jap J Legal Med*, 25: 322-326, 1971.
2. Li T., Beling G. Isolation and characterization of two specific antigens of human seminal plasma. *Fertil Steril*, 24:134-144, 1973.
3. Papotti M., Paties C., Peveri V., Moscuza L., Bussolati G. Immunocytochemical detection of prostate-specific antigen (PSA) in skin adnexal and breast tissues and tumors. *Basic Appl Histochem*, 33 (1): 25-29, 1989.
4. Yu H., Diamandis P., Sutherland A. Immunoreactive prostate-specific antigen levels in female and male breast tumors and it's association with steroid hormone receptors and patient age. *Clin Biochem*, 27: 75-79, 1994.
5. Yu H., Diamandis P. Protease prostate specific antigen in milk of lactating women. *Clin Chem*, 41: 54-60, 1995.
6. Yu H., Diamandis P. Prostate specific antigen immunoreactivity in amniotic fluid. *Clin Chem*, 41: 204-210, 1995.
7. Iwakiri J., Grandbois K., Wehner N., Graves C., Stamey T. An analysis of urinary prostate specific antigen before and after radical prostatectomy: evidence for secretion of prostate specific antigen by the periurethral glands. *J Urol*, 149 (4):783-786, 1993.
8. Breul J., Pickl U., Hartung R. - Prostate-specific antigen in urine. *Eur Urol*, 26 (1):18-22, 1994.
9. Clements J., Mukhtar A. Glandular kallikreins and prostate-specific antigen are expressed in the human endometrium. *J Clin Endocrinol Metab*, 78: 1536-1539, 1994.
10. Sensabaugh F. Isolation and characterization of a semen-specific protein from human seminal plasma: a potential new marker for semen identification. *J Forens Sci*, 23:106-115, 1978.
11. Björk T. Metabolism of free and complexed prostate-specific antigen and their utility for diagnosis and prognosis of prostate cancer. Doctoral Dissertation. Malmö: Lund University, Malmö, 1998.
12. Eastham A., Riedel E., Scardino T., Shike M., Fleisher M., Schatzkin A., Lanza E., Latkany L., Begg B. Variation of serum prostate-specific antigen levels: an evaluation of year-to-year fluctuations. *JAMA*, 51: 2695-700, 2003.
13. Laux L., Custis S. Forensic detection of semen III. Detection of PSA using

- membrane based tests: Sensitivity issues with regards to the presence of PSA in other body fluids. Submitted MAFS Newsletter, 2003.
14. Sato I., Sagi M., Ishiwari A., Nishijima H., Ito E., Mukai T. Use of the "SMITEST" PSA card to identify the presence of prostate-specific antigen in semen and male urine. *Forensic Sci Int*, 127(1-2): 71-74, 2002.
 15. Williams R., Kelly A., Deluca J., Tambasco J. Validation of the AB Acard PSA membrane test for forensic applications, MAFS., Fall Meeting, Ann Arbor, Michigan, 1998.
 16. Scardino T. Early Detection of Prostate Cancer. *Hum Pathol*, 23: 211, 1992.
 17. Maher J., Vintiner S., Elliot D., Melia L. Evaluation of the BioSign PSA membrane test for the identification of semen stains in forensic casework. *N Z Med J*, 115 (1147): 48-49, 2002.
 18. Simich P., Morris L., Klick L., Rittenhouse-Diakun K. Validation of the use of a commercially available kit for the identification of prostate specific antigen (PSA) in semen stains. *J Forens Sci*, 44 (6): 1229-1231, 1999.
 19. Hochmeister N., Budowle B., Rudin O., Gehrig B., Thali M., Dirnhofer R. Evaluation of prostate-specific antigen (PSA) membrane test assays for the forensic identification of seminal fluid. *J Forens Sci*, 44: 1057-1060, 1999.
 20. Dimitrov S, G. Bonev, S. Georgiev, V. Atanassov. Methods for evaluation and control of semen fertility. *Con-Car Universe*: 5-29, 2000.
 21. Laux L., Tambasco A., Benzinger E. Forensic Detection of Semen II. Comparison of the Abacus Diagnostics OneStep ABACard, p30 Test and the Seratec PSA Semiquant Kit for the Determination of the Presence of Semen in Forensic Cases /<http://mafs.net/pdf/laux2.pdf/>, 2006.