

## PROSTATE-SPECIFIC ANTIGEN IN HUMAN AND CANINE SEMEN – A COMPARATIVE STUDY

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*Із звітами про содомію (скотозлягання) іноді стикаються в судовій медичній спеціалізованій літературі. Мета дослідження – визначення видової специфіки собачої та людської сперми. Для встановлення видової приналежності простат-специфічного антигену (ПСА), використовували спеціальний мембранний тест, SERATEC® PSA Semiquant, SERATEC, Gesellschaft für Biotechnologie GmbH, Göttingen, Germany та якісне визначення кислої фосфатази. Результати можуть бути використані у судовій медичній практиці для визначення походження сперми.*

**Сперма, ПСА, кисла фосфатаза, людина, собака.**

Biological materials (blood, semen etc.) are common subjects of forensic medical expertise in cases of sexual abuse of children, women and men. The expert has to establish the species affiliation of semen – whether it is human or not [1]. Apart morphological examination of semen, a variety of methods and express tests are used for this purpose. During the last years, rapid immunological tests are used in many countries (Germany, France, USA, Japan, Thailand) for detection of semen in cases of sexual assaults. PSA Semiquant test has proven highly specific in the examination of semen and gives categorical negative results for semen from stallion, boar, bull, tom cat and dog [5], as well as ram and jack [4].

It is acknowledged that acid phosphatase concentrations in human semen are very high – over 400 units/ml [6]; for comparison, in animal semen the levels are up to 24 units/ml. Acid phosphatase could be assayed qualitatively in the practice by means of rapid diagnostic tests [2].

**Aim.** The aim of the present study was to determine the species-specificity of prostate-specific antigen in canine and human semen.

**Material and methods.** Semen was collected from seven men-volunteers and three male German shepherd dogs by masturbation in sterile 10-ml tubes. Specimens of mixed equal amounts of canine and human semen were also prepared. According to the routine analysis established by the World Health Organisation (WHO), 1992 the volume, concentration, motility, pathological spermatozoa, pH, were individually determined by light microscopy computer system Motic

Image Plus (Motic China Group Ltd, 2001–2004). The results were statistically processed with the Stat Soft software (Microsoft Corp. 1984–2000 Inc).

The qualitative determination of acid phosphatase (AP) activity of samples was done with analytical test strips Phosphatisimo KM, MACHEREY–NAGEL, Germany. For determination of species specificity of PSA in human and canine semen, the specific membrane test SERATEC® PSA Semiquant, SERATEC, Gesellschaft für Biotechnologie GmbH, Göttingen, Germany was utilised.

The results from semen examinations were photodocumented and compared.

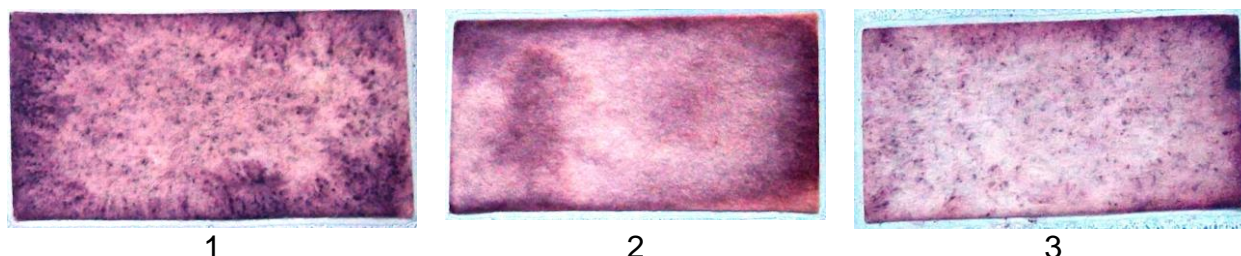
**Results and discussion.** The results from analyses were as followed:

Semen: Spermogrammes are presented in Table 1. There were no deviations from the reference values. Only one of human semen samples showed reduced number of spermatozoa.

**Biological parameters of semen (Mean±SEM)**

Biological parameters of semen	Volime, ml	Concentration ( $\times 10^6$ /ml)	Motility, %	Dead and abnormal spermatozoa, %
Man (n=7)	3.58±0.42	35.7±5.31	60.5±9.31	10.43±2.43
Dog (n=3)	6.83±1,87	246±0.5	76.54±3.48	4.66±1.32

Acid phosphatase: The results are shown on Fig. 1, photos 1, 2, 3. It could be seen that all studied semen samples from men, dogs and mixed semen specimens showed a positive reaction.

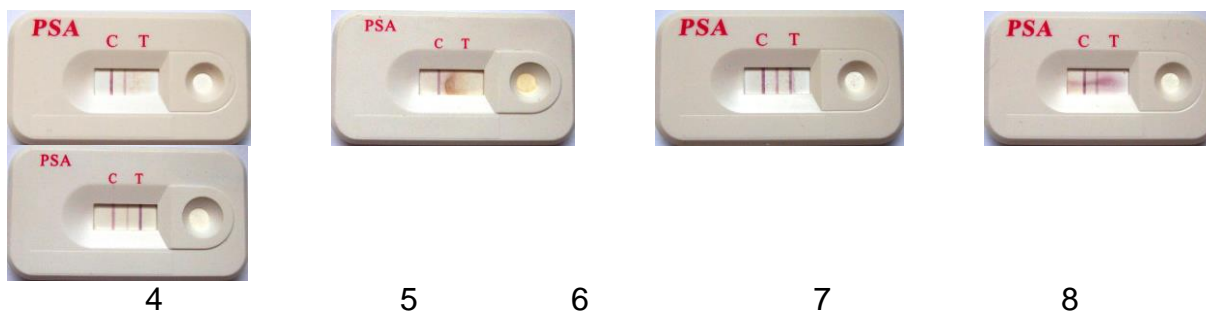


**Fig. 1. Acid phosphatase tests:** 1 – human semen; 2 – canine semen; 3 – mixed human and canine semen

PSA: Prior to semen tests, the dilution buffer used was tested and there was no positive reaction (Fig. 2, photo 4).

The test of undiluted human semen did not yield either a positive or a negative reaction (Fig. 2, photo 5).

All diluted human semen samples reacted positively (Fig. 2, photo 6) – the time for appearance of test lines was  $6\pm 3$  min on the average. All samples from undiluted and diluted canine semen were negative (Fig. 2, photo 7). A positive reaction of the SERATEC® PSA Semiquant test was exhibited by all mixed human and canine semen samples (Fig. 2, photo 8). A positive reaction was also observed after centrifugation of the samples with abnormally low spermatozoa counts. The average time for appearance of the test line was  $7\pm 3$  min.



**Fig. 2. PSA Semiquant test:** 4 – phosphate buffer; 5 – undiluted human semen; 6 – diluted human semen; 7 – diluted canine semen; 8 – mixed human and canine semen sample

**Discussion.** Only one of tested human samples showed reduced spermatozoa counts (oligospermia). The lack of deviations from the reference values of the other human and canine ejaculates excluded the presence of any abnormalities of the reproductive organs. According to Walther, G. 1971, acid phosphatase concentrations in human semen were higher than canine ones. Nevertheless, the canine semen has reacted positively to the rapid test – there was no species-related specificity. The data about the positive reaction of the sample of seminal fluid obtained after centrifugation of the ejaculate are interesting. They confirm the reports of Miteva et al. (2010), that the test was a reliable method for detection of semen in aspermia, oligospermia and unknown spermatozoa in tested specimens.

During the test, three lines appear in the diagnostic window of the original PSA test device. The first two indicate the proper performance of the test. The third line indicates a positive reaction. The absence of reaction when the buffer solution was tested denotes that it is appropriate for this type of test. The results with diluted human semen showed that the method could be used for quantitative determination of PSA, as the standard line corresponds to at least 4 ng/ml PSA in the sample. The data supported a previous report of ours [4], that SERATEC® PSA Semiquant test could be employed in forensic medical practice for detection of semen in biological material from exhibits.

## CONCLUSIONS

Despite the lack of data confirming sodomy with a dog, the experts could determine the species affiliation and distinguish between men and dogs. The tests is a reliable method with this regard.

## References

1. Ashizawa K, Y. Ozavwa, K. Okauchi. Comparative studies of elemental composition on ejaculate tedfowl. Lul, rat, dog and boar spermatozoa by electron probe X- ray micronal ysis. Сотр. Biochet. Physiol. 1987, 88A, 2, 269–272.
2. Khaldi N, Miras A, Botti K, Benali L, Gromb S. Evaluation of three rapid detection methods for the forensic identification of seminal fluid in rape cases. J Forensic Sci. 2004, 49(4), 749–53.
3. R. Miteva, Zapryanova D., Fasulkov Iv., Yotov S., Mircheva T., Investigations on acid phosphatase activity in the seminal plasma of humans and animals, Trakia Journal of Sciences, Vol. 8, No 2, pp 20–23, 2010.
4. Miteva R., S. Yotov, P. Georgiev, I. Fasulkov, Determination of species specificity of prostatespecific antigen (PSA) in semen, Trakia Journal of Sciences, Vol. 4, No. 3, pp 64–68, 2006.
5. Sato I, S. Morihisa, A. Ishiwari, H. Nishijima, E. Ito, T. Mukai. Use of the „SMITEST” PSA card to identify the presence of prostate –specific antigen in semen and male urine. Forensic Sci Int. 2002, 127, 71–74.
6. Walther, G. 1971. Acid phosphatase. Its significance in the determination of human seminal traces. J. Forensic Med. 18: 15–17.

*С отчетами о содомии (скотоложестве) иногда сталкиваются в судебной медицинской специализированной литературе. Цель исследования – определить видовую специфику собачьей и человеческой спермы. Чтобы установить видовую принадлежность простат-специфического антигена (ПСА), был использован специальный мембранный тест, SERATEC® PSA Semiquant, SERATEC, Gesellschaft für Biotechnologie GmbH, Göttingen, Germany и качественное определение кислой фосфатазы. Результаты могут быть использованы в судебной медицинской практике или для определения происхождения спермы.*

**Сперма, PSA, кислой фосфатазы, человек, собака.**

*Sodomy reports are sometimes encountered in forensic medical specialized literature. The aim of the present study was to determine the species-specificity of canine and human semen. To establish the species specificity of prostate-specific antigen (PSA), the specific membrane test SERATEC® PSA Semiquant, SERATEC, Gesellschaft für Biotechnologie GmbH, Göttingen, Germany and qualitative assay of acid phosphatase were performed. The results could be utilized in forensic medical practice or detection of semen origin.*

**Semen, PSA, acid phosphatase, man, dog.**