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Original Contribution

INVESTIGATIONS ON ACID PHOSPHATASE ACTIVITY IN THE SEMINAL PLASMA OF HUMANS AND ANIMALS

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ABSTRACT
A comparative study on the concentration of the enzyme acid phosphatase in seminal plasma in the ejaculates of men, jacks and rams was performed.
After the semen collection, the following indicators were established: ejaculate volume, spermatozoa concentration, percentage of motile and *pathological spermatozoa*. Seminal plasma was separated through centrifugation, and acid phosphatase concentrations were determined by means of Acid Phosphatase Colorimetric Humazym-Test and Automatic Biochemical Analyzer BS-3000 P.
Significant differences ($p<0.05$) in the activity of acid phosphatase in the seminal plasma of humans, jacks, and rams were found. The concentration, motility of percentage of abnormal sperm in the ejaculate did not play a significant role in determining the enzyme activity in the semen plasma.
Our results could be used in forensic medicine to prove the species of semen specificity.

Key words: acid phosphatase, semen, human, animals

INTRODUCTION

Acid phosphatase is an enzyme (hydrolase), which catalyzes the hydrolysis of various phosphate esters with pH-optimum in the acid zone. It can be found in high concentrations in the prostate, bones, blood cells, the spleen and other organs. Several molecular variants of the enzyme are known (isoenzymes), and the one with the highest significance in diagnostics is the prostate isoenzyme (1).

Seminal plasma is one of the richest sources of acid phosphatase and is a basic substrate in the testing of this enzyme in order to assess the prostate's function (2, 3, 4).

The detection of acid phosphatase in human semen has a diagnostic value, not only in the treatment of prostate diseases, but also in forensic medicine when it is necessary to prove the presence of semen (5). According to Telisman et al. (2000) the average value of the enzyme is $(637\pm357)\times10^3$ UI and varies in a wider range from 144×10^3 UI to 1510×10^3 UI.

In animals, acid phosphatase in semen was detected for the first time in a bull (7). This enzymatic marker is primarily related to the metabolic function of spermatozoa in ruminants and odd-toed ungulates and prostate diseases in dogs. The enzyme concentration for different species vary significantly: 24.7±11.8 UI for bulls, 64.9±14.6 UI in rams, 680±304 UI in pigs, and depend on the method of quantitation as well (8, 9, 10).

The review on literature references made it clear that there aren't sufficient studies on the concentration of acid phosphatase in the seminal plasma of jacks and rams. There are no comparative studies on the concentration of this enzyme in the seminal plasma of humans and the abovementioned animal species.

Aim

The aim of the current study was to perform a comparative research on the concentration of acid phosphatase in the seminal plasma of humans, jacks, and rams.

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MATERIALS AND METHODS

We performed comparative investigations on semen from human (n = 4), donkey jack (n = 3), and ram (n = 3) *ejaculates*. The semen was assessed immediately after obtaining it. The ejaculate volume (ml), spermatozoa concentration (10^6/ml), spermatozoa motility (%), and amount of pathological spermatozoa in the ejaculate (%) were measured.

The determination of the volume was performed with a graduated pipette, and the concentration – by counting in a chamber (11). Progressive sperm motility was determined in a native preparation, and the percentage of abnormal ones – using the method described by Fourie et al. (2004).

Microscopic study of the semen was performed using the computer program Motic Image Plus (Motic China Group Ltd, 2001-2004), including a phase contrast microscope with warming table, digital camera, and computer software.

After the semen was obtained, it was centrifuged at 5000×g, and seminal plasma was separated for determination of acid phosphatase activity.

To measure the concentrations of acid phosphatase, we used Acid Phosphatase Colorimetric Humazym-Test Orthophosphoric - Monoester Phosphohydrolase (Acidic Optimum) manufactured by Human Gesellschaft für Biochemica und Diagnostica mbH, as per the methods of Hillman (1971) and Junge et al. (1993).

The seminal plasma samples were tested on the automated biochemical analyzer BS-3000 P (Synova Medical Science & Technology, Co. Ltd, Nanjing, China).

The results were processed using the statistical software StatSoft (Microsoft Corp. 1984-2000 Inc.) by means of ANOVA and non-parametric analysis for comparison of mean average values and proportions.

RESULTS

The results are presented in **Table 1**. The semen's biological parameters for all studied species involved were within the reference ranges.

Table 1. *Acid phosphatase activities in seminal plasma and biological parameters of semen from man, jack and ram.*

Semen	Acid phosphatase (U/l)	Volume (ml)	Concentration (10^6/ml)	Motility (%)	Pathological spermatozoa (%)
Men n=4	1031.9±536.5a	3.88±0.52a	35.8±5.44a	68.5±11	10.44±2.40a
Jacks n=3	11.5±3.6b	46±0.55b	48.3±2.25a	75±12.5	5.68±1.38b
Rams n=3	18.7±1.7b	1.4±0.15c	2034±10.59c	80±5.8	20±3.6c

Values with different letters in the same column are statistically significantly different at $P<0.05$.

There were significant variations in the concentration of acid phosphatase in humans (1031.9±536.5 U/l). Still, the values were significantly ($p<0.05$) higher than those in jacks (11.5±3.6 U/l) and rams (18.7±1.7 U/l).

The ejaculate volume varied within the acceptable norms for the species. The highest volume was observed in jacks, and the lowest – in rams (1.4±0.15 ml).

The concentration of spermatozoa in rams (2034±10.59 x 10^6/ml) was significantly ($p<0.05$) higher than the values for humans (35.8±5.44 x 10^6/ml) and jacks (48.3±2.25 x 10^6/ml).

As to the motility, no significant variations between the different ejaculates were found out.

The percentage of abnormal sperm was significantly different ($p<0.05$) for the three species, with highest value found in rams (20±3.6%).

DISCUSSION

Seminal plasma plays a major role in the metabolism of spermatozoa, their function, and their transport (15).

Enzyme concentration in the seminal plasma can be used as a biochemical marker for the semen's origin. The obtained values for the enzyme acid phosphatase are close those established by Telisman et al. (2000) in humans and by Roussel and Stallcup (1966) for rams. The significantly higher ($p<0.05$) acid phosphatase concentration in human seminal plasma, compared to donkey studs, indicated that this assay was suitable for comparative studies of the enzyme and proving the origin of semen. The results confirmed the statement by Stallcup (1965) that the activity of acid phosphatase is higher in humans and lower in animals.

In cases of proven sexual assault, acid phosphatase can be used as an indicator for the presence of semen, even if no spermatozoa had been found, and it can also indicate the time of the event. In this relation, it is true that the assay of acid phosphatase is a better method than the cytological one, and that it also provides information on the presence of semen in the vaginal fluid in cases of azoospermia, spermias of unknown origin, or oligospermia (17). This was confirmed by our study, in which we could observe single spermatozoa in the seminal plasma separated after centrifugation.

We accepted that the differences in the concentration of the enzyme can be explained primarily with the anatomical and functional peculiarities of the reproductive systems of humans and animals.

In humans, the accessory sex gland, especially the prostate gland, are well-developed and play a key role in the production of seminal plasma (18), while in animals the prostate is well-developed in dogs, but not clearly developed in rams and jacks (19).

Reid et al. (1948) reported on the influences of food intake, testicular degeneration, and testicular hypoplasia on enzyme activity. Regardless of this, determination of acid phosphatase concentration in seminal plasma can serve to prove the species and origin of the semen.

Our results show that the significant ($p<0.05$) variations in the concentrations of spermatozoa between the species can play a significant role in fertilization, yet do not have an influence on seminal plasma enzymatic activity. In this case, we confirm the statement of Singer et al. (2) on the lack of influence of the studied parameter on the activity of acid phosphatase.

With regard to sperm motility, we did not prove a significant correlation between the activity of acid phosphatase and the percentages of motile and abnormal sperm in the ejaculates of humans and animals, as indicated by Roussel and Stallcup (1966). This gives us reason to accept that they do not have a significant influence on the studied enzyme.

In conclusion, the activity of the enzyme acid phosphatase in the semen of humans was significantly ($p<0.05$) higher than it was in donkey jacks and rams. These results could be also used in forensic medicine to evidence the presence of semen and species semen specificity determination.

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