

นิพนธ์ต้นฉบับ

A study of acid phosphatase and fructose in normal Thai fertile men's semen.

Suthat Kolkijkovinda*

Yuwasarn Nitichai* Yenchitt Chanprasitr*

Boontharika Wongwaitayangkoon* Wisut Boonkasemsanti*

Kolkijkovinda S, Nitichai Y, Chanprasitr Y, Wongwaitayangkoon B, Boonkasemsanti W. A study of acid phosphatase and fructose in normal Thai fertile men's semen. Chula Med J 1989 Mar;33(3): 207-211

Acid phosphatase and fructose determinations were performed on the seminal plasma of the normospermic fertile men (n=74) to determine the normal value of these substances. Semen analysis was performed by World Health Organization conventional method. Acid phosphatase and fructose were determined by spectrophotometry measurement. The count of normal sperm was 57.0 (\pm 48.5) million/ml. The value for acid phosphatase in seminal plasma was 50.74 (\pm 30.70) $\times 10^3$ IU/ml, and the value for fructose in seminal plasma was 14.8 (\pm 6.4) mmol/L. These values agree with previously published results. The method used was appropriate for both research and clinical use.

Reprint request: Kolkijkovinda S, Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, Bangkok 10500, Thailand.

Received for publication. February 1, 1989.

สุทัศน์ กลกิจโกวินท์, ยุวสาร นิตชัย, เย็นจิต จันทรประสิทธิ์, บุญชริกา วงศ์ไวทยางกูร, วิสุทธิ บุญเกษมสันติ. การวิเคราะห์เชื้ออสุจิ ปริมาณแอสิด ฟอสฟาเตส และฟรุคโตส ในน้ำอสุจิผู้ชายไทยปกติ. จุฬาลงกรณ์เวชสาร 2532 มีนาคม; 33(3): 207-211

ได้ทำการวิเคราะห์เชื้ออสุจิ หาปริมาณของ *Acid Phosphatase* และ *Fructose* ในผู้ชายไทยปกติจำนวน 74 ราย เพื่อหาค่าปกติของสารต่าง ๆ เหล่านี้ ได้ตรวจวิเคราะห์เชื้ออสุจิหาปริมาณของ *Acid phosphatase* และ *Fructose* ด้วยเครื่องมือ *Spectrophotometer* ผลปรากฏว่าจำนวนของตัวอสุจินับได้ 57.0 ± 48.5 ล้าน/มล. ปริมาณของ *Acid phosphatase* ในน้ำอสุจิมีค่าเท่ากับ $(50.74 \pm 30.70) \times 10^3$ IU/มล. และหาปริมาณของ *Fructose* ในน้ำอสุจิมีค่าเท่ากับ 14.8 ± 6.4 mmol/ลิตร ค่าปกติที่ได้ในการศึกษารังนี้มีค่าสอดคล้องกับค่าต่าง ๆ ที่ได้มีผู้หาไว้ในรายงานจากต่างประเทศ ค่าที่ได้นี้อาจสามารถใช้เป็นค่าอ้างอิง (*reference value*) สำหรับชายไทย วิธีการทำเป็นวิธีที่เหมาะสมใช้ได้ทั้งในการวิจัย และการบริการทางคลินิก

Infertility is one of the major health problems in Thailand which requires meticulous investigations, care and treatments from medical personnels, especially, gynecologists. A lot of studies have been carried out in this field for the purposes of both infertility solving and fertility regulation. The assessment and evaluation in male infertility, is still not substantially clear. In general, semen analysis is routinely used to assess male fertility status. We are not only interested in the amount of sperms, but also in their movement, morphology and viability. The major enzyme, "acid phosphatase", and the specific nutrient, "fructose", in seminal plasma are believed to be two main factors that facilitate semen quality. There have been for the normal values of acid phosphatase and fructose in seminal plasma^(1,2), but so far not in Thailand or in Thai males. The purpose of this study was to establish a normal values of fructose and acid phosphatase in normal Thai fertile men's semen, which could serve as reference values for evaluating semen quality in infertile cases. Fructose is synthesized and secreted in seminal vesicles in the normal range of 120-450 mg/dl or 6.67-25.00 mmol/l.⁽¹⁾ Values below 6.67 mmol/l are commonly related with inflammation and abnormality of seminal vesicles which will affect the sperm. Eliasson⁽²⁾ reported the normal value of acid phosphatase in semen of at $(20-60) \times 10^3$ IU/ml and that the enzyme decreases in cases of prostatitis which can return to normal value after the prostatitis has been cured.

Material and Method

The recruitment of volunteers was carried out at the antenatal clinic, Chulalongkorn Hospital. They were healthy men whose wives were pregnant at 12-24 weeks of gestation. They did not take any drugs at least 1 week before masturbation after 2-7 days of abstinence. The 74 volunteers cooperated well with the instructions of the study. They were 19-49 years of age. (Mean \pm S.D. = 27.9 ± 5.1)

The semen was thoroughly analysed by the conventional method⁽²⁾ for general appearance, volume, sperm count, grading of motility⁽³⁾, viability and morphology. The analysis of fructose and acid phosphatase in the seminal plasma were performed according to Karvonen and Malm Method⁽⁴⁾ and Sigma Reagent Method⁽⁵⁾ respectively after centrifugation at 2000 g, 4°C for 15 minutes.

The standardization of both methods for fructose

and acid phosphatase was performed by measuring pool semen, 10 specimens for each batch of reagents. The standard deviation and coefficient of variation were calculated afterwards.

Fructose analysis in seminal plasma

One ml of diluted seminal plasma, 1:50 in distilled water, was mixed with 0.3 ml of 0.045 M. cadmium sulphate, before 0.2 ml of 0.55 N.NaOH was added and mixed. The mixture was incubated in a water bath at 50°C for 20 minutes then placed in an ice bath for 15 minutes when the absorbance was measured with a spectrophotometer at OD 470 nm, compared to standard fructose solution of concentrations 0.14 mmol/l and 0.28 mmol/l.

Acid phosphatase analysis

A 0.5 ml of 0.011 M. p-Nitrophenyl phosphatase disodium salt solution was mixed with a 0.5 ml of 0.09 M. citrate buffer at pH 5.5 and incubated at 37°C for 5 minutes before being added to a 0.2 ml of diluted seminal plasma, 1:50,000 in 0.3 gm % bovine serum albumin (BSA) in normal saline solution. The mixture was then incubated at 37°C, for 30 min, before being added to 4 ml of 0.1 N.NaOH, and measured for absorbance with a spectrophotometer at OD 450 nm. One OD 450 nm equalled to 2.81 IU/ml of acid phosphatase.

Result

The semen samples from these 74 volunteers showed normal general appearance, liquifaction within 30 minutes and volume, of 2.50 ± 1.12 ml (mean \pm S.D.). The sperm counts were 57.4 ± 48.5 millions/ml with the percentage viability of 65.0 ± 10.6 % and percentage of motile sperms at 54.3 ± 9.6 % (excellent or grade 3 movement 26.4 %; good or grade 2 movement 55.6 % and poor or grade 1 movement 18.1 %). The normal sperm morphology was found in 52.1 ± 12.7 %. The fructose level in these seminal plasma was 14.8 ± 6.9 mmol/l with the coefficient of variation within batch and between batches assayed at 1.10 % and 1.33 % respectively as table 1.

The acid phosphatase level was $(50.74 \pm 30.70) \times 10^3$ IU/ml by the method described and the coefficient of variation within batch and between batches assayed were 1.62 % and 1.59 % respectively, as in table 2.

Table 1. Accuracy of the method.

Fructose	Within batch assay variation		Between batches assay variation
	batch 1 (n=10)	batch 2 (n=10)	n = 20
Mean (mmol/l)	21.60	21.92	21.76
S.D.	0.27	0.21	0.29
C.V. (%)	1.25	0.95	1.33

Table 2. Accuracy of the method.

Acid Phosphatase	Within batch assay variation		Between batches assay variation
	batch 1 (n=10)	batch 2 (n=10)	n = 20
Mean (IU/ml)	32947	33059	33003
S.D.	526.8	545.2	525
C.V. (%)	1.59	1.64	1.59

Discussion

Studies on male fertility have been limited and not widely done compared to studies on female fertility. However, the subject had become increasingly interesting and studied especially concerning fructose and acid phosphatase levels in seminal plasma. Several reports confirmed that the fructose and acid phosphatase in seminal plasma are related to male infertility.^(2,6,7,8)

Biswas⁽⁸⁾ found reduced fructose level in hyper-spermic semen which suggested that fructose was used by sperm. We believe that fructose is an essential carbohydrate for sperm metabolism.

Fructose is synthesized and excreted mainly by the seminal vesicles and partly by the ampullary glands. The normal range in seminal plasma is 6.67-25.0 mmol/l.⁽²⁾ Low fructose levels were found in cases of seminal vesicle or ejaculatory duct diseases; especially in case of absent seminal vesicles, the fructose level was very low.⁽⁹⁾

Acid phosphatase is synthesized and excreted by prostate gland in the range of $(20-60) \times 10^3$ IU/ml.⁽²⁾ Low acid phosphatase level is found in prostate gland diseases, especially prostatitis. In this study, the normal ranges of fructose and acid phosphatase in Thai fertile men's semen were 1.8-34.3 mmol/l (Mean \pm S.D. =

14.8 \pm 6.4 mmol/l) and 13.35-178.44 $\times 10^3$ IU/ml (Mean \pm S.D. = $(50.74 \pm 30.7) \times 10^3$ IU/ml) respectively. The results were similar to other studies^(2,6,9) with some wider ranges. We believed that the normal ranges were large because of poor homeostatic control mechanism in seminal fluid and prostatic fluid, compared to other secretae in blood or serum which had better homeostatic mechanisms. It was rather difficult to interpret any level of fructose or acid phosphatase which were in the wide normal ranges of values as abnormal, eventhough there were abnormal semen analysis and infertility. The findings of fructose and acid phosphatase in seminal plasma at lower levels than the normal ranges were significantly related to poor semen quality and infertility. They may also be useful in the follow up for improvement or deterioration of prostate and seminal vesicle diseases, as well as in assessing semen quality.

In this study, the sperm count of normal Thai fertile men was 57.4 ± 48.5 million/ml (Mean \pm S.E), which was similar to that reported by Nelson and Bunge⁽¹⁰⁾ the average of 48 million/ml. We found 17.56% of subjects (13 subjects) has sperm counts of lower than 20 million/ml; compared to Nelson and Bunge's report, about 20% of cases had sperm counts lower than 20 million/ml. As a matter of fact, these

17.56% of low sperm count semens had good sperm motility, high percentage of viable sperms and normal sperm morphology, high levels of fructose and acid phosphatase.

Summary

This study was the first to be conducted on Thai fertile men to establish the normal levels of fructose and acid phosphatase present in the seminal plasma of 74

subjects. The results of normal levels of seminal plasma fructose and acide phosphatase were $14.8 (\pm 6.4)$ mmol/l and $(50.74 \pm 30.70) \times 10^3$ IU/ml respectively. This wide range of results with high individual variations may be useful in the progression follow up of prostate gland or seminal vesicle abnormalities as well as for assessing sperm quality related to infertility problems. A further study concerning the male infertility related to seminal plasma fructose and acid phosphatase should be carries out.

อ้างอิง

1. Phaosawadi S, Chutivongse S, Reinprayoon D, Kolkijkovinda S. Gynecology. faculty of Medicine, Chulalongkorn University. Bangkok, Trio Ad, 1982.307-326
2. Sherins RJ, Howards SS. Male infertility. In: Walsh PC, Gittes RE, Perlmutter AD, Stamey TA, eds. Campbell's Urology. Vol.1 5th ed. Philadelphia : W.B. Saunder, 1986.640-674
3. Belsey MA, Eliasson R, Gallegos AJ, Moghisei KS, Paulsen CA, Preased MRN, eds. Laboratory manual for the examination of Human Semen and Cervical Mucus Interaction. World Health Organization. Press Concern, Singapore, 1980. 10
4. Karvonen MJ, Malm M. Fructose analysis in seminal plasma. Scand J Clin Lab Invest 1955; 7:305-307
5. Sigma Reagent Method. Sigma clinical Company 3500 De Kalb Street, St. Louis 18, Mo. USA, Sigma Technical Bulletin No. 104.
6. Eliasson R. Analysis of semen. In: Behrman SJ, Kistner RW,eds. Progress in Infertility. Boston : Little Brown, 1975. 691-713
7. Schoenfeld C, Amelar RD, Dubin I, Nameroff M. Prolactin Fructose and Zine levels found in human seminal plasma. Fertil Steril 1979 Aug; 32(2) : 206-208
8. Biswas S, Ferguson KH, Stednonska J, Baffoe G, Manfield MD, Kosbab MH. Fructose and hormone levels in semen : their correlations with sperm counts and motility. Fertil Steril 1978 Aug ; 30(2) : 200-204
9. Amelar RD. Coagulation liguifaction and viscosity of human semen. J Urol 1962 Feb; 87(2) : 187-190
10. Nelson CMK, Bunge R. Semen analysis: evidence for changing parameters of male fertility potential. Fertil Steril 1974 Jun; 25(6) : 503-507