The diagnostic value of seminal α-glucosidase enzyme index for sperm motility and fertilizing capacity

Dawoud A. Yassa, PhD, Walid K. Idriss, MD, FACOG, Majed E. Atassi, MD, Sambasiva K. Rao, PhD.

ABSTRACT

Objectives: To find out the diagnostic value of seminal α-glucosidase enzyme index in the ejaculated semen samples of proven-fertility and primary infertility patients, for the sperm motility and fertilizing capacity.

Methods: The study which lasted approximately 2 years was carried out in the Andrology Department of the Arab Fertility Center, Jeddah, Kingdom of Saudi Arabia. Eighty-five healthy male patients (age 25-38 years) were admitted into the study. They were divided into 2 main groups as proven-fertility group and primary infertility group. Each group was again subdivided into 4 classes based on the sperm concentrations. The sperm motility assessment was carried out quantitatively by Semi automated Computer assisted Semen analysis using Autosperm analyzer. Simultaneously, α-glucosidase enzyme index was determined in all the semen samples.

Results: The results were tabulated as per the standard sperm motility grades of the World Health Organisation, under the classified sperm concentrations of the study. The progressive motility grades were compared with the enzyme index of α-glucosidase in both the groups. The total progressive motility was found higher in the high-density (classes I and II) than the low-density (classes III and IV) semen samples. The enzyme index readings of the various classes of sperm concentrations were significant ($p<0.05$) and showed a stepwise fall from 89% +/-9% to 52%+/-3% in the proven fertility group. Although, a similar trend has been observed (48%+/-5% to 20%+/-2%) in the primary infertility group, the enzyme index class II sperm concentration (44%+/-6%) was not found significant with class 1 (48%+/-5%) sperm concentration, but the significance was achieved with the other 2 classes. The readings of enzyme index between the groups were found highly significant (p, 0.001).

Conclusions: The enzyme index of α-glucosidase estimations in semen samples may indicate a positive correlation with progressive motility and fertilizing capacity of spermatozoa and may serve as diagnostic value in individuals showing normal levels of sperm concentration and serum androgen.

Keywords: α-glucosidase, sperm motility, male fertility.

Saudi Med J 2001; Vol. 22 (11): 987-991

The epididymis is believed to provide a favorable milieu for spermatozoa to attain maturity and fertilizing ability.[1,2] The epididymal maturity also contributes sperm membrane modifications required to fulfil future functional activity during capacitation, acrosome reaction and sperm-egg binding in the female genital tract.[3,4] In addition, spermatozoa are protected in the epididymis from any harmful effects.[5] The biological significance of epididymal secretions is studied and correlated positively with conventional semen parameters.[2] Epididymal secretions play a crucial role for the acquisition of progressive motility of spermatozoa.[6-8] Progressive motility is one of the important contributing factors for reaching the fertilization goal in the natural cycles. The neutral α-glucosidase, carnitine and glyceryl phosphocholine are the important markers of epididymis to be estimated in the seminal fluid.

From the Arab Fertility Center, Khalid Idriss Hospital, Jeddah, Kingdom of Saudi Arabia.

Received 28th February 2001. Accepted for publication in final form 30th April 2001.

Address correspondence and reprint request to: Dr. Dawoud A. Yassa, Arab Fertility Center, Khalid Idriss Hospital, PO Box 75, Jeddah 21411, Kingdom of Saudi Arabia. Tel. +966 (2) 6423555. Fax. +966 (2) 6426844. E-mail: Kih@sps.net.sa

The enzyme neutral α–glucosidase has a specific significance and proved to be superior to assess the function of the epididymis.[9,10] The presence of total enzyme in semen has been found directly proportional to the secretory capability of the epididymis.[9] However, interpretation of total α–glucosidase levels may not be very accurate to assess the epididymal activity since the semen contains the contributions of acid isoenzyme by prostate gland.[11] The low levels of the enzyme observed in the semen even after complete epididymal failure is mostly due to the negligible contributions by the seminal vesicle and prostate gland.[12,13] The reduced concentration of neutral α-glucosidase in semen is attributed to infertility[14] in normozoospermics. In other words, the failure in the function of the epididymis may lead to infertility.[15,16] On the other hand, very low levels of the neutral isoenzyme are present in vasectomy,[5] agenesis of vasdeferens, and obstructive azoospermia.[17,18] The quantitative readings of neutral isoenzyme in most of the cases also can indicate the location of obstruction and can differentiate between obstructive and non-obstructive azoospermia. The estimations of neutral α-glucosidase in semen act as a reliable marker of epididymal contribution to ejaculate of persons with normal androgen levels. But, the role of this enzyme on the sperm motility parameters is not well studied. The percentage of neutral α-glucosidase to that of total enzyme activity was thought to be more ideal to express the enzyme activity of the epididymis. Therefore, the present study was mainly aimed at finding out the relationship between the enzyme index of α-glucosidase and the sperm progressive motility in the ejaculated semen samples of proven-fertility and primary-infertility patients.

Methods. Eighty-five healthy male patients (age 25-38 years) who attended our fertility center and hospital were admitted into the study with their consent. All the patients showed normal serum androgen levels and normal sperm concentrations as per World Health Organization standards.[19] They were divided into 2 main groups based on the previous fertilizing ability of persons, known as proven fertility group and primary-infertility group. All the males in the proven fertility group were males who had fathered a child in the last 8-13 months period. The males in the primary infertility group could not become fathers even after 2-3 years of uninterrupted sexual life with the wives. Each group is again subdivided into 4 classes based on the sperm concentration, such as class I (>80 mill/ml), class II (60-79 mill/ml), class III (40-59 mill/ml) and class IV (20-39 mill/ml). The semen samples were collected by masturbation at regular intervals after sexual abstinence of 3-5 days. Semen analysis and α-glucosidase estimations were made with every sample. Sperm motility assessments were determined by a computer programmed Autosperm Analyser.

Sperm motility assessment. Sperm motility was assessed quantitatively by determining the percentage of linear progressive, slow progressive, non-progressive and immotile spermatozoa by Semi automated computer assisted semen analysis using an Autosperm Analyser. A simple and accurate objective assessment of motility of spermatozoa was conducted based on the observation of sperm movement through a microscope and tracking the movement in a digitizing tablet in accordance with World Health Organization[19] standards. An aliquot (11.5 μl) of undiluted fresh semen sample was placed on a glass slide covered with a cover slip of 24x24 mm size to give a uniform depth of 20 μm.[20] The movement of each spermatozoon was tracked by cursor and analyzed not less than 50 spermatozoa in different fields. The computer was programmed to give final results of velocity and classified into 4 groups as follows. Grade A: linear velocity >22 μm/second, Grade B: velocity between 22 and 5 μm/second, Grade C: velocity <5 μm/second, and Grade D: immotile.

Estimation of α-glucosidase in semen. The secretory activity of epididymis can be assessed by estimating the total and neutral α-glucosidase in semen of patients[21] with intact genital tract communications and with normal androgen levels. An aliquot of 125 ml of seminal plasma was incubated in duplicate for 4 hours at 37°C with an equal amount 125 ml of phosphate buffer containing 20 mM p-nitrophenyl-glucopyranoside. The reaction was stopped by the addition of 3 ml 0.1M sodium carbonate. The sample was centrifuged for 6 minutes at 300g and the supernatant layer was aspirated into a cuvette. Finally, the reading was taken in a photometer at a wave- length of 405 nm. The results were expressed in milli units per ml concentration. For determining the neutral α-glucosidase, the acid isoenzyme was inhibited by the addition of 1% sodium dodecyl sulphate with phosphate buffer (pH 6.8) and the neutral isoform was measured.[14,21] The enzyme index (EI) of a-glucosidase was determined by calculating the percentage of the neutral isoform to that of total enzyme content.

Statistical analysis. The results were expressed as mean +/- SE (standard error of the mean). Student t-test was conducted to determine the level of significance between the parameters.

Results. The results are shown in Tables 1 and 2 as per the standard sperm motility grades of the World Health Organisation (WHO) (A-D) under the classified sperm concentrations (Class I-IV) of the study. The progressive motility grades A and B of the different sperm concentration-classes were compared with EI of α-glucosidase in the proven-fertility group

Table 1 - Motile and immotile sperm percentage (mean ± SE %) and the enzyme index of α-glucosidase in the proven fertility Group (Group 1).

Motility Grades by Velocity	Sperm Concentration Classes			
	Class I (>80 mill/ml)	**Class II (60-79 mill/ml)**	**Class III (40-59 mill/ml)**	**Class V (20-39 mill/ml)**
Grade A (>22μm/sec)	28 ± 8	26 ± 9	22 ± 5	19 ± 2
Grade B (5-22μm/sec)	36 ± 6	40.5 ± 8	34 ± 9	27 ± 5
Grade C (<5μm/sec)	21 ± 4	1 ± 2	26 ± 5	25 ± 6
Grade D (Immotile)	14 ± 3	23 ± 6	18 ± 2	28 ± 6
Enzyme Index (EI)	89 ± 9*	76.5 ± 5*	60 ± 4*	52 ± 3*
Number of Patients	6	5	7	8

SE=standard error
*Statistically significant ($p<0.05$) with each other in the same group (t-test)
*Statistically significant ($p<0.001$) between groups of the same class (t-test)

(Group I) and in the primary-infertility group (Group II). It has been observed that the total progressive motility Grades A and B together was higher in the high-density semen samples (Classes I and II) than the low-density samples (Classes III and IV) in both the groups. However, the readings were not statistically significant with each other in the same group. Interestingly, significant ($p<0.05$) variations in sperm motility were observed between the groups. Proven fertility group (Group I) yielded the semen samples of higher linear progressive motility (28%+/-8% to 19%+/-2%) than the primary-infertility group (Group II) values (20%+/-8% to 12%+/-3%), when comparing the respective classes. The immotile spermatozoa were found in higher percentage as 28%+/-6% and 47.5%+/-2% in the lowest concentration (Class IV) than the other 3 classes in both the groups, indicating a significantly ($p<0.001$) high number of immotile spermatozoa in the primary-infertility group. The linear-progressive

Table 2 - Motile and immotile sperm percentage (mean ± SE %) and the enzyme index of a α-glucosidase in the primary infertility group (Group-2).

Motility Grades by Velocity	Sperm Concentration Classes			
	Class I (>80 mill/ml)	**Class II (60-79 mill/ml)**	**Class III (40-59 mill/ml)**	**Class V (20-39 mill/ml)**
Grade A (>22μm/sec)	20 ± 8	10 ± 7	14 ± 3	12 ± 3
Grade B (5-22μm/sec)	26 ± 7	30 ± 10	20 ± 8	16 ± 7
Grade C (<5μm/sec)	17 ± 3	28.5 ± 4	31 ± 9	25 ± 2
Grade D (Immotile)	36 ± 9	31 ± 0	34 ± 4	47.5 ±2
Enzyme Index (EI)	48 ± 5*	44 ± 6**	29 ± 8*	20 ± 2*
Number of Patients	12	17	12	18

SE=standard error
*Statistically significant ($p<0.001$) with each other in the same group (t-test)
**Statistically significant ($p<0.001$) with each in the same group except with class-1 (t-test)

motility with highest velocity Grade A showed a steady-fall from 28%±8% to 19%±2% (Class I to Class IV) in the proven-fertility group while the steady-fall was disrupted by a steep fall at Class II sperm concentration (10%±7%) in the primary-infertility group. The EI readings of the various classes of sperm concentrations were found statistically significant ($p< 0.05$) with each other and showed the stepwise fall from 89%±9% to 52%±3% in the proven-fertility group. Although, a similar trend has been observed (48%±5% to 20%±2%) in the primary infertility group, the EI of Class II sperm concentration (44%±6%) was not found significant with Class I (48%±5%) sperm concentration, but the significance was achieved with the other 2 classes. However, the readings of EI between groups were significant ($p<0.001$) against each class of sperm concentrations. The prominent higher values of EI in all the classes of the proven-fertility group were noteworthy for the discussion.

Discussion. The epididymal secretions contain a number of active principles, which are associated with motility and fertilizing ability of spermatozoa. In particular, the glucosidase enzymes are expected to participate in sperm surface modifications[22] and may help in acquiring sperm motility after ejaculation. The estimation of neutral isoenzyme of α-glucosidase in semen is a very sensitive non-invasive method to localize not only the site of obstruction but also to assess the status of the epididymis.[13,23] In the present study, the EI of α-glucosidase was recorded at higher levels as 89%+/-9% and 48%+/-5% in the higher sperm motility grades of class-1 sperm concentration and at lower levels as 52%+/-3% and 20%+/-2% in the lower sperm motility grades of class IV sperm concentration in both the groups. These levels clearly indicate the gradual decline as the sperm motility percent falls in both the groups. The trend, therefore represents a positive correlation between progressive sperm motility and EI as an epididymal marker, which was statistically significant. Low levels of neutral α-glucosidase enzyme may represent either the inflammatory condition of the epididymis[24] or improper sperm maturation,[25] whereas high level is an indication of good fertilizing ability of sperm cells.[26,27] The high levels of EI (89%±9% to 52%±3%) were also found in the proven-fertility group of our study when compared with the levels (48%±5% to 20%±2%) observed in the primary infertility group. These results support the above statement of good fertilizing ability of sperm cells. Incubation of spermatozoa in-vitro with epididymal cells increased the sperm motility[28-30] and improved the fertilizing capacity.[31,32] We also agree with the concept that the increased sperm motility acquired in the epididymis would contribute to the fertilizing capacity of spermatozoa. Our results substantiate the concept that the EI of α-glucosidase is a reliable marker to predict the sperm motility in the ejaculated sperm samples when the other conditions are satisfied. The sperm motility and the fertilizing ability are the time bound factors, usually acquired in the epididymis in[10] approximately 2 weeks of time, depending on the species.[33] But, the acquired motility and fertilizing ability are lower in the case of testicular spermatozoa.[34] A further study has been planned in continuation of the present work to identify the extent of acquisition of motility when spermatozoa pass from one part to another in the epididymis in a period of 2 weeks.

The EI of α-glucosidase estimation in semen samples of patients, showing normal sperm concentrations and normal serum androgen levels, may indicate a positive correlation with the progressive motility and fertilizing capacity of spermatozoa.

Acknowledgment. The authors would like to thank Ms Munira Haj Omar, Arab Fertility Center of Khalid Idriss Hospital, Jeddah, Kingdom of Saudi Arabia, for her help in the typographic preparation of the Arabic translation of the abstract.

References

1. Blaquier JA, Cameo MS, Cuasnicu PS. On the role of the epididymal factors in sperm fertility. Reprod Nutr Dev 1980; 28: 1209-1217.
2. Cooper TG, Yeung CH, Nashan D. Epididymal markers in male infertility. J Androl 1988; 9: 91-101.
3. Fleming AD, Yanagimachi R. Effects of various lipids on the acrosome reaction and fertilizing capacity of guinea pig spermatozoa with special reference to the possible involvement of lysophospolipid in the acrosome reaction. Gamete Res 1981; 4: 253-273.
4. Benoff S, Hurley I, Cooper GW. Fertilization potential in-vitro is correlated with head-specific mannose-ligand receptor expression, acrosome status and membrane cholesterol content. Hum Reprod 1993; 8: 2135-2166.
5. Hinton BT, Palladino MA, Rudolph D. The role of the epididymis in the protection of spermatozoa. Curr Top Dev Biol 1996; 33: 61-102.
6. Cooper TG. Physiology and evaluation of epididymal function. In: Comhaire FH, editor. Male fertility. Chapman & Hall: London; 1996. p. 97-119.
7. Mahmoud AM, Geslevich J, Kint J, Depuydt C, Huysse L, Zalata A, et al. Seminal plasma a-glucosidase activity and male infertility. Hum Reprod 1998; 13: 591-595.
8. Haidl G, Schill WG. Is research on epididymal functions still of clinical relevance? Andrologia 1997; 29: 117-119.
9. Cooper TG, Weidner W, Nieschlag E. The influence of inflammation of the human genital tract on secretion of seminal markers alpha-glucosidase, glycerophospocholine, carnitine, fructose and citric acid. Int J Androl 1990; 13: 329-336.
10. Purvis K, Egdetveit I. Segmental distribution of alpha glucosidase, ornithine decarboxylase and polyamines in the human epididymis. J Reprod Fertil 1993; 97: 575-580.
11. Paquin R, Chapdelaine P, Dube JY, Tremblay RR. Similar biochemical properties of human seminal plasma and epididymal alpha glucosidase. J Androl 1984; 5: 277-281.
12. Tremblay RR, Chapdelasine P, Mailhot J. Alpha-1,4-glucosidase activity in human semen: variations with number and motility of spermatozoa. Fertil Steril 1979; 31: 592-593.

13. Guerin JF, Ben Ali H, Roller J. Alpha glucosidase as a specific epididymal enzyme marker: its validity for the etiologic diagnosis of azoospermia. J Androl 1986; 7: 156-162.
14. Zopfgn A, Priem F, Sudhoff F, Jung K, Lenk S, Loeming SA et al. Relationship between semen quality and seminal plasma components carnitine, alpha-glucosidase, fructose, citrate and granulocyte elastase in infertile men compared with a normal populations. Hum Reprod 2000; 15: 840-845.
15. Guerin JF, Bin Ali H, Cottinet D. Seminal alpha-glucosidase activity as a marker of epididymal pathology in normozoospermic men consulting for infertility. J Androl 1990; 11: 240-245.
16. Mathieu C, Guerin JF, Cognat M. Motility and fertilizing capacity of epididymal spermatozoa in normal and pathological cases. Fertil Steril 1992; 57: 871-876.
17. Thakur AN, Sheth AR, Rao SS, Thacker PV. Effect of vesectomy on the prostatic function as indicated by seminal maltose activity. Contraception 1975; 11: 155-159.
18. Casano R, Orlando C, Caldini AL. Simultaneous measurement of seminal L-canitine, alpha glucosidase and glyceryl phosphorylcholine in azoospermic and oligospermic patients. Fertl Steril 1987; 47: 324-327.
19. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. Cambridge (UK): World Health Organization; 1999. p. 85-87.
20. Overstreet JW, Katz DF, Hanson FW, Fonseca JR. A simple inexpensive method for objective assessment of human sperm movement characteristics. Fertil Steril 1979; 31: 162-172.
21. Cooper TG, Yeung CH, Nashan D, Jocken H, Nieschlag E. Improvement in the assessment of human epididymal function by the use of inhibitors in the assay of a-glucosidase in seminal plasma. Int J Androl 1990; 13: 297-305.
22. Tulsiani DR, Skudlarek MD, Araki Y. Purification and charactirization of two forms of beta-D-galactosidase from rat epididymal luminal fluid: evidence for their role in the modification of sperm plasma membrane glycoprotein(s). Biochem J 1995; 305: 41-50.
23. Garcia Diez LC, Esteban Ruiz PE, Villar E. Enzyme and hormonal markers in the differential diagnosis of human azoospermia. Arch Androl 1992; 28: 181-194.
24. Vonder KH, Scheit KH, Weidner W, Cooper TG. The evaluation of markers of prostatic function. Urol Res 1991; 19: 343-347.
25. Haidl G, Badura B, Hinsch KD. Disturbances of sperm flagellae due to failure of epididymal maturation and their possible relationship to phospolipids. Hum Reprod 1993; 10: 1070-1073.
26. Ben AH, Guerin JF, Pinatel MC. Relationship between semen characteristics, alpha-glucosidase and the capacity of spermatozoa to bind to the zona pellucida. Int J Androl 1994; 17: 121-126.
27. Milingos S, Comhaire F, Liapi A, Aravantinos D. The value of semen characteristics and tests of sperm function in selecting couples for intra-uterine insemination. Eur J Obstet Gyn Reprod Biol 1996; 64: 115-118.
28. Smith CA, Hartman TD, Moore HDM. A determinant of Mχ 34,000 expressed by hamster epididymal epithelium binds specificity to spermatozoa in co-culture. J Reprod Fertil 1986; 78: 337-345.
29. Moore HD, Curry MR, Penfold LM. The culture of human epididymal epithelium and in vitro maturation of epididymal spermatozoa. Fertil Steril 1992; 58: 776-783.
30. Bongso A, Tounson A. Evaluation of motility, fertilizing ability and embryonic development of murine epididymal sperm after culture with epididymal epithelium. Hum Reprod 1996; 11: 1451-1456.
31. Moore HD, Hartman TD. In-vitro development of the fertilizing ability of hamster epididymal spermatozoa after co-culture with epithelium from the proximal cauda epididymidis. J Reprod Fertil 1986; 78: 347-352.
32. Klinefelter GR, Roberts NL, Suarez JD. Direct effect of ethane dimethane sulfonate on epididymal function in adult rats: an In-Vitro demonstration. J Androl 1992; 13: 409-421.
33. Cooper TG. The epididymis sperm maturation and fertilization. Heidelberg: Springer-Verlag; 1986.
34. Setchell BP. Possible physiological bases for contraceptive techniques in the male. Human Reprod 1994; 9: 1081-1087.