Journal of Andrology, Vol. 27, No. 1, January/February 2006

Seasonal Changes of Neutral α-Glucosidase Activity in Human Semen

RALF HENKEL,* GESA MAAß,† HANS-CHRISTIAN SCHUPPE,† ANDREAS JUNG,† JÖRG SCHUBERT,* WOLF-BERNHARD SCHILL†

*From the *Department of Urology, Friedrich Schiller University, Jena, Germany; and the †Centre for Dermatology and Andrology, Justus Liebig University, Giessen, Germany.*

ABSTRACT: Although there are contradictory reports, the biochemical evaluation of the seminal activity of neutral α-glucosidase (NAG) has repeatedly been described as an important parameter to test epididymal patency and is recommended by the WHO. Because, for a number of diagnostic parameters, seasonal variations have been described even in the human, it was the aim of this study to investigate possible circannual changes of NAG. This is an important aspect of andrological diagnosis, as seasonal changes of specific diagnostic parameters might have an impact on the accuracy and predictive power of these parameters, which in turn might have an effect on the therapeutic concept for the patients. In a total of 473 patients, sperm concentration, volume of the ejaculate, total motility, progressive motility, pH value, number of peroxidase-positive cells, concentration of fructose, and NAG as functional markers of the seminal vesicles and the epididymis, respectively, were analyzed according to standard procedures. Seminal activity of NAG was significantly correlated with the sperm concentration ($P < .0001$), ejaculate volume ($P < .0001$), and the pH ($P = .0025$). Moreover, significant ($P = .0008$) seasonal changes in the activity of seminal NAG with the maximum in spring (76.87 mU/ejaculate) and the minimum in autumn (58.55 mU/ejaculate) were found. The incidence of low-ranged activity of the enzyme was 9.2% in spring, while it was 20.3% in autumn. Thus, our data clearly demonstrate circannual changes of the seminal activity of neutral α-glucosidase. This in turn has clinical impact as the predictive power of the test system changes throughout the year.

Key words: Human spermatozoa, seasonality, ejaculate, epididymal patency.

J Androl 2006;27:34–39

Within the scope of the andrological diagnostic, a series of biological and biochemical tests are performed. One of the parameters taken is the activity of seminal α-glucosidase (EC.3.2.1.20), of which two isoforms, a neutral and an acidic, exist. From these, the neutral α-glucosidase (NAG) is reported to originate almost exclusively from the epididymis, while the acid isoenzyme is secreted by the prostate (Paquin et al, 1984; Yeung et al, 1990; WHO, 1999). On the one hand, there are reports that NAG is possibly involved in sperm-zona pellucida interaction (Ben Ali et al, 1994) or are correlated with human sperm motility (Viljoen et al, 1990). On the other hand, the success after intrauterine insemination (Spiessens et al, 1998) and seminal visco-elasticity (Elzanaty et al, 2004), the actual function of this enzyme in the ejaculate and its importance for sperm function is still unknown. However, despite few contradictory reports (Krause and Bohring, 1999), biochemical evaluation of the seminal activity of NAG has repeatedly been described as an important parameter to test epididymal patency (Mahmoud et al, 1998; Zöpfgen et al, 2000), which is a test recommended by the World Health Organization (WHO, 1999).

Because, for a number of diagnostic parameters, like sperm concentration, motility, acrosin activity, or chromatin condensation, seasonal variations have been described even in the human (Henkel et al, 2001; Krause and Krause, 2002), it was the aim of this study to investigate possible circannual changes of other parameters. This is an important aspect of andrological diagnosis, as seasonal changes of specific diagnostic parameters might have an impact on the accuracy and predictive power of these parameters, which in turn might have an effect on the therapeutic concept for the patients.

Materials and Methods

In this retrospective study, a total of 473 consecutive patients attending the outpatient clinic for fertility problems between January 1998 and January 2000 (age: 17–66 years) were analyzed for sperm concentration, volume of the ejaculate, total motility, progressive motility, pH value, number of peroxidase-positive cells, concentration of fructose, and NAG as functional markers of the seminal vesicles and the epididymis, respectively. All parameters were assessed according to WHO guidelines (1999).

Correspondence to: Dr Ralf Henkel, University of the Western Cape, Department of Medical Biosciences, Private Bag X17, Bellville, 7530, South Africa (e-mail: rhenkel@uwc.ac.za).

Received for publication May 10, 2005; accepted for publication July 14, 2005.

DOI: 10.2164/jandrol.05064

Table 1. *Summary statistics of the parameters analyzed in the study*

Parameters	n	Mean ± SD	Range	Median
Age (years)	383	33.3 ± 5.8	22–55	32
Sperm concentration (million/mL)	470	30.5 ± 67.1	0–740	4.0
Volume of ejaculate (mL)	475	3.9 ± 1.9	0.2–11.5	3.8
Total motility (%)	287	37.1 ± 18.4	0–85	40
Progressive motility (%)	287	19.8 ± 15.3	0–57	17
Number of peroxidase-positive cells (million/mL)	152	1.3 ± 1.9	0–14.7	0.5
pH value	475	7.43 ± 0.27	6–8	7.5
Fructose (μmol/mL)	344	16.5 ± 7.4	0.6–35.6	16
α-Glucosidase (mU/ejaculate)	475	67.0 ± 51.2	0.35–306.6	56.2

For the assessment of seasonal variations, data were grouped according to the calendar month of obtaining the ejaculate and according to the season (spring: March, April, May; summer: June, July, August; autumn: September, October, November; winter: December, January, February) and averages were compared.

In brief, the concentration of fructose in seminal plasma was measured in duplicate after centrifuging the semen sample for 10 minutes at 1000 × *g*. The seminal plasma was removed and frozen at −20°C until analysis. After thawing, the samples were mixed and diluted (5 μL seminal plasma plus 50 μL distilled water) in an Eppendorf cup. Afterward, 12.5 μL 63 μM $ZnSO_4$ and 12.5 μL 0.1 M NaOH were added, mixed, incubated at room temperature for 15 minutes, and then centrifuged for 5 minutes at 8000 × *g*. Fifty microliters of the supernatant were removed, mixed with 50 μL indole reagent (2 μM indole in 16 μM benzoic acid), 500 μL 32% HCl added, covered with Parafilm, and heated for 20 minutes at 50°C in a water bath. After all, the samples were cooled in ice water for 15 minutes. Finally, 250 μL of the solution were pipetted into a 96-well microtiter plate and read at 470 nm in a spectrophotometer (EAR400 ATC, SLT LAB-Instruments, Vienna, Austria). Subsequently, the fructose concentrations were calculated.

For the determination of the activity of NAG, 300 μL ejaculate were centrifuged for 10 minutes at 1000 × *g* and the supernatant was frozen at −20°C until analysis. After thawing the sample, seminal plasma was diluted 1:5 with 100 mM phosphate buffer, pH 6.8, and centrifuged for 15 minutes at 10 000 × *g*. Then 10 μL diluted seminal plasma were placed into a microtiter plate well and 100 μL of 0.5% (w/v) 4-nitrophenyl-α-D-glucopyranoside (PNPG) and 1% (w/v) SDS in 100 mM phosphate buffer, pH 6.8, were added, mixed well, and incubated at 37°C for 4 hours. Finally, 100 μL of 0.2 M Na_2CO_3 were added to each well, including the blanks, and the samples were measured in duplicate against standards of p-nitrophenol (0, 10, 20, 40, 60, 80, 100 μM), which are equivalent to an activity of NAG (0, 4.37, 8.75, 17.5, 26.3, 35.0, 43.8 μU/mL), at 405 nm using an EAR microtiter plate reader (EAR 400 ATC).

Table 2. *NAG activity in normozoospermic, oligozoospermic, severe oligozoospermic, and azoospermic patients*

Group	Mean ± SD NAG Activity (mU/ejaculate)	n
Normozoospermia*	86.7 ± 57.4	150
Oligozoospermia†	74.6 ± 54.1	78
Severe oligozoospermia‡	63.5 ± 40.9	132
Azoospermia§	39.4 ± 38.1	110

* vs †: $P = .1250$.
* vs ‡: $P = .0001$.
* vs §: $P < .0001$.
† vs ‡: $P = .0958$.
† vs §: $P < .0001$.
‡ vs §: $P < .0001$.

All statistical calculations (mean, median, Spearman's rank correlation, Wilcoxon test, *H* test according to Kruskal-Wallis and Fisher's exact test) were performed after checking normal distribution of the data by means of the Kolmogorov-Smirnov test. For the receiving operating characteristic (ROC) curve analysis, azoospermia and α-glucosidase (20 mU/ejaculate) have been used as discriminator, respectively. All calculations including ROC curve analysis were carried out with MedCalc (v7.5, MedCalc Software, Mariakerke, Belgium). A value of $P < .05$ was considered as significantly different.

Results

The summary statistics of all analyzed parameters are summarized in Table 1. The high variation of the values for sperm motility and concentration, which can be seen in the high standard deviation, is obvious, as is normal for these biological parameters. While strong significant positive correlations were observed for sperm concentration ($r = .399$; $P < .0001$) and the ejaculate volume ($r = .497$; $P < .0001$), there was a weaker inverse correlation with the pH ($r = -.139$; $P = .0025$). No correlation of NAG activity was found with the patient's age (*H* test: $P = .1658$), seminal concentration of fructose, the number of peroxidase-positive cells, and sperm motility ($r = .083$; $P = .1589$), though the significance level for progressive motility was close to significance ($r = .110$; $P = .0621$). As expected, significant differences in NAG activity were observed between normozoospermic, severe oligozoospermic (sperm count <5 million/mL) and azoospermic patients (Table 2). No differences in seminal NAG activity were observed between normozoospermia and oligozoospermia or oligozoospermia and severe oligozoosper-

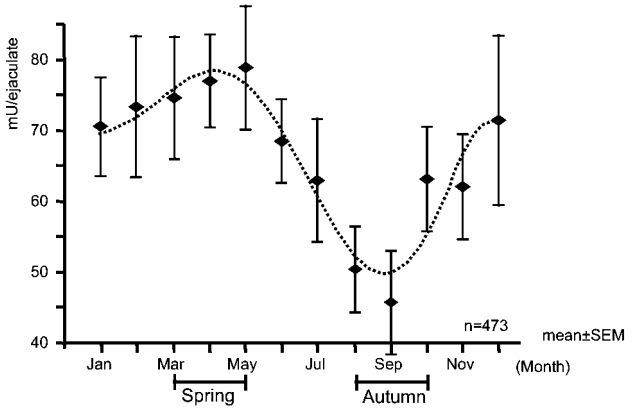

Figure 1. Seasonal variation of seminal activity of the neutral α-glucosidase in 473 patients in central Germany. A maximum of neutral α-glucosidase (NAG) activity can be seen in April/May, while the nadir is in August/September.

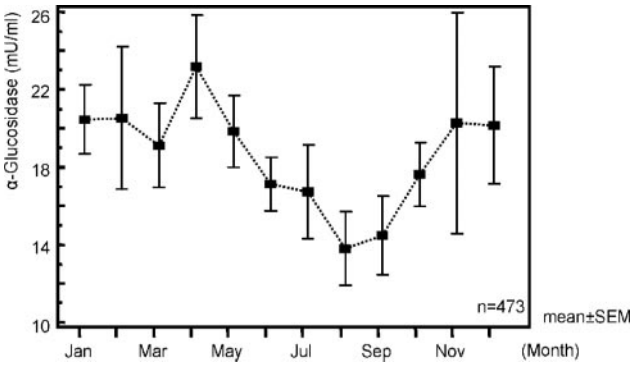

Figure 2. Seasonal variation of seminal concentration of the neutral α-glucosidase in 473 patients in central Germany. The maximum concentration of 23.16 mU/mL can be seen in April, while the minimum of 13.80 mU/mL is in August. The difference between these 2 months is significant (P = .0016).

mia. For the azoospermic group of patients, both the mean (39.4 mU/ejaculate) and the median (27.6 mU/ejaculate) are higher than the cut-off value of 20 mU/ejaculate given by the WHO (1999).

Interestingly, the activity of NAG showed significant (P = .0008) seasonal changes, with its maximum in spring (76.87 mU/ejaculate; maximum value in May) and its minimum in autumn (58.55 mU/ejaculate; minimum value in September) (Figure 1). The differences between the actual maximum in May (78.78 mU/ejaculate) and minimum in September (45.73 mU/ejaculate) (P = .0009) were highly significant. In addition, the values of NAG in spring differ significantly from those in summer (P = .0107). This circannual variation was also obvious if the NAG concentration was plotted against the calendar month (Figure 2). The mean maximum concentration of 23.16 mU/mL was found in April and the minimum of 13.80 mU/mL in August. The difference was significant (P = .0016). All other matches showed no differences. Furthermore, with regard to sperm concentration, motility, number of peroxidase-positive cells, pH, fructose, and the ejaculate volume, no seasonality could be observed.

The incidence of a low-ranged NAG activity (<20 mU/ejaculate) in autumn (20.3%) was significantly (P = .0146) higher than in spring (9.2%). Moreover, while there was no difference (P = .4113) in the mean NAG activity of patients showing low-ranged NAG activity (<20 mU/ejaculate) in spring (mean: 11.69 mU/ejaculate) and autumn (mean: 10.11 mU/ejaculate), the mean NAG activity of those patients showing NAG activity above 20 mU/ejaculate differed significantly between spring (83.50 mU/ejaculate) and autumn (70.90 mU/ejaculate; P = .0247).

With the cut-off value NAG activity (20 mU/ejaculate) given by the WHO (1999), 91.9% of the ejaculates with spermatozoa and 39.1% of the azoospermic semen samples included in this study could correctly be identified. The optimized calculations after ROC-curve analyses are depicted in Tables 3 and 4. While the statistical values (prevalence, sensitivity, specificity, positive predictive value, and negative predictive value) for the assessment of azoospermia with the activity of NAG as variable to be calculated were quite similar for all data groups (all data, spring and autumn), the calculated cut-off values for the seminal NAG activity differed strikingly (Table 3).

Table 3. *Calculation of the statistical parameters of the determination of α-glucosidase (NAG) for azoospermia as differentiation criterion. For the calculation, a distinction was made between spring, autumn, and all data obtained throughout the year. The seasonal difference for the calculated cut-off value for NAG activity is obvious. The prevalence for summer and winter would be 25.9% and 26.5%, respectively*

Parameter	All Data	Spring	Autumn
Positive group (n)	110	28	27
Prevalence (%)	23.4	21.9	20.5
Area under ROC curve*	0.743	0.727	0.745
Calculated cut-off (mU/ejaculate)	≤33.0	≤50.5	≤24.5
Sensitivity (%)	57.3	67.9	59.3
Specificity (%)	81.1	75.0	82.9
Positive predictive value (%)	48.1	43.2	47.1
Negative predictive value (%)	86.1	89.3	88.8

* ROC indicates receiving operating characteristic.

Table 4. *Calculation of the statistical parameters of the determination of azoospermia for (NAG; α-glucoside 20 mU/ejaculate) as differentiation criterion. For the calculation, a distinction was made between spring, autumn, and all data obtained throughout the year. The seasonal difference for the prevalence of azoospermia and the positive predictive value of the test is obvious. The prevalence for summer and winter would be 17.6% and 15.7%, respectively*

Parameter	All Data	Spring	Autumn
Positive group (n)	74	12	27
Prevalence (%)	15.7	9.38	20.5
Area under ROC curve*	0.733	0.787	0.727
Calculated cut-off (million sperm/mL)	0	0	≤0.4
Sensitivity (%)	59.5	66.7	63.0
Specificity (%)	83.3	82.8	79.0
Positive predictive value (%)	40.0	28.6	43.6
Negative predictive value (%)	91.7	96.0	89.2

* ROC indicates receiving operating instructions.

The cut-off value for NAG activity in spring was 50.5 mU/ejaculate and that in autumn 24.5 mU/ejaculate.

For azoospermia as a variable to be calculated based on the cut-off for NAG activity (20 mU/ejaculate) given by the WHO (1999), all statistical values were quite similar, except for enormous low values for the prevalence (9.38%) and the positive predictive value (28.6%) in spring (Table 4). After calculation of the seminal NAG concentration (mU/mL) from its activity in the ejaculate (mU/ejaculate) and the ejaculate volume and subsequent ROC-curve analysis, a cut-off of 12.8 mU/mL for azoospermia was found. This value was 67.8% specific and 68.2% sensitive for the discrimination between ejaculates containing spermatozoa and those without. The positive and negative predictive values of this test were 39.3% and 87.5%, respectively.

Discussion

The epididymis is the main source of α-glucosidase in human seminal plasma (approximately 80%; neutral isoform; NAG) (Paquin et al, 1984; Yeung et al, 1990; Peña et al, 2004), while the remaining 20% of the α-glucosidase activity in human semen is attributable to the acidic isoform of α-glucosidase that originates in the prostate (Paquin et al, 1984). The evaluation of NAG is regarded as a reliable parameter to examine epididymal patency (Guerin et al, 1986; Cooper et al, 1990). Therefore, the World Health Organization (WHO, 1999) considered seminal NAG activity a useful parameter to determine the cause of azoospermia, obstructive or nonobstructive, with testicular origin and suggested a cut-off value of 20 mU/ejaculate. In general, the NAG activities obtained in this study were in the range of those obtained by others (Mahmoud et al, 1998; Krause and Bohring, 1999; Zöpfgen et al, 2000; Comhaire et al, 2002). However, a significant relationship with sperm motility, which was observed by Viljoen et al (1990) and Elzanaty et al (2002), could not be confirmed in this study. Yet, the correlation between NAG activity and progressive motility showed at least a tendency ($P = .0621$).

In this study, we observed a distinct seasonality of the neutral isoform of α-glucosidase with its maximum activity in spring (April/May) and a nadir in autumn (August/September). Minimum and maximum of seminal NAG activity coincided with the respective minima and maxima for sperm concentration (Henkel et al, 2001). Because the NAG activity is routinely used for andrological diagnosis in many centers, we performed ROC-curve analyses to assess the clinical value of the NAG determination as seasonal variations of the parameter might have an impact on its practical value. The calculated cut-off value for seminal NAG activity changed throughout the year, with higher values, reduced specificity, and increased sensitivity in spring. By setting the cut-off value of NAG activity in spring (55.5 mU/ejaculate) to the one of autumn (24.5 mU/ejaculate) or the one for all data (33.0 mU/ejaculate), specificity markedly increases (94.0 mU/ejaculate and 90.0 mU/ejaculate, respectively) and sensitivity decreases (39.3 mU/ejaculate and 46.4 mU/ejaculate, respectively). When applying the cut-off value of 20 mU/ejaculate given by the WHO (1999), the prevalence of azoospermia was markedly lower in spring than in autumn and is indicative of the generally elevated NAG levels in spring. Thus, our results indicate that the predictive power of the assay changes during the year, a fact of which clinicians should be aware of when counseling patients.

The reasons for the seasonality of seminal NAG activity, however, remain speculative. Though an influence of testosterone cannot be ruled out as the neutral isoform of α-glucosidase is of epididymal origin and the epididymis is obligatory dependent on this male sex steroid (Orgebin-Crist, 1985; Cooper, 1990), decreased concentrations of the free testosterone will not only lead to morphological alterations of the epididymis but also to its dysfunction (Cooper, 1998). The assumption that the observed circ-

annual variation of seminal NAG activity is due to seasonal changes of epididymal function is supported by the observation that the seminal concentration of NAG (mU/mL) shows the same pattern of variation but not the ejaculate volume.

Seasonality of reproduction is well known among most animals in the animal kingdom, including mammals. The reproductive phase is restricted to specific times of the year in which reproductive success is most likely; that is, when environmental factors like food and water availability or ambient temperature are given and, thus, the conditions are optimum for survival of the offspring. Even though the human is considered as showing no seasonality of reproduction, epidemiological studies demonstrated circannual variations in the birth rates (Becker, 1981; Levine et al, 1990). Even after adjusting for confounding factors, fertilization rate, embryo quality, pregnancy rate, and birth rate success in in vitro fertilization show some seasonal variation (Stolwijk et al, 1994). Moreover, birth rates also seem to be dependent on the area where people live. For instance, while, in Northern Europe, its peak is in spring with the nadir in autumn, in the USA, most births occur in summer and the minimum is in spring (Lam and Miron, 1994).

Testosterone has repeatedly been shown to exhibit distinct seasonal variations (Abbaticchio et al, 1987; Reinberg et al, 1988; Dabbs, 1990). Our own data obtained in central Germany (not shown) show a maximum of the concentration of total testosterone in April/May and a minimum in autumn. On the other hand, the concentrations of the bioactive free testosterone decrease significantly while aging (Feldmann et al, 2002; Henkel et al, 2005), but no circannual changes could be found for seminal NAG activity. Alternatively, a direct influence of the photoperiod on epididymal function could be discussed, but such an effect has not been reported in the literature thus far. Methodological effects can be excluded because the technique is standardized and is performed under controlled temperature conditions. In addition, the maximum observed in spring does not support a methodological influence of room temperature on the assay, because the month with the highest temperatures is July.

With regard to sperm concentration and sperm motility and vitality, contradictory results have been published. While Mortimer et al (1983) and Ombelet et al (1996) could not find significant seasonal variations of these parameters, others clearly emphasize such an effect (Tjoa et al, 1982; Politoff et al, 1989; Saint Pol et al, 1989; Centola and Eberly, 1999; Andolz et al, 2001; Henkel et al, 2001). Seasonal variations in sperm concentration were clearly not attributable to differences in sexual abstinence (Gyllenborg et al, 1999). Moreover, there is also evidence of seasonal changes of functional sperm parameters, like acrosin activity (Krause and Krause, 2002) and chromatin condensation (Henkel et al, 2001), with maxima in springtime and winter, respectively. For chromatin condensation, inverse courses of variation on the Northern and Southern Hemispheres were observed (Henkel et al, 2001).

In conclusion, a significant seasonality of seminal NAG activity has been demonstrated in this report. Therefore, the predictive power of the test, which is routinely performed in many laboratories to distinguish between obstructive and testicular azoospermia, varies throughout the year. Because only a relatively small number of 470 patients in only 1 country have been examined thus far, the clinical consequences of this circannual variation cannot be estimated yet and more studies investigating this phenomenon should be performed. However, for the time being, clinicians should be aware of it when counseling patients.

Acknowledgment

The authors wish to express their appreciation to Sharon Henkel for her excellent linguistic review.

References

Abbaticchio G, de Fini M, Giagulli VA, Santoro G, Vendola G, Giorgino R. Circannual rhythms in reproductive functions of human males, correlations among hormones and hormone-dependent parameters. *Andrologia.* 1987;19:353–361.

Andolz P, Bielsa A, Andolz A. Circannual variation in human semen parameters. *Int J Androl.* 2001;24:266–271.

Becker S. Seasonality of fertility in Matlab, Bangladesh. *J Biosoc.* 1981; 13:97–105.

Ben Ali H, Guerin JF, Pinatel MC, Mathieu C, Boulieu D, Tritar B. Relationship between semen characteristics, alpha-glucosidase and the capacity of spermatozoa to bind to the human zona pellucida. *Int J Androl.* 1994;17:121–126.

Centola GM, Eberly S. Seasonal variations and age-related changes in human sperm count, motility, motion parameters, morphology, and white blood cell concentration. *Fertil Steril.* 1999;72:803–808.

Comhaire F, Mahmoud A, Schoonjans F, Kint J. Why do we continue determining α-glucosidase in human semen? *Andrologia.* 2002;34:8–10.

Cooper TG. In defence of a function for the human epididymis. *Fertil Steril.* 1990;54:965–975.

Cooper TG. Interactions between epididymal secretions and spermatozoa. *J Reprod Fert.* 1998; 53(suppl):119–136.

Cooper TG, Yeung CH, Nashan D, Jockenhövel F, Nieschlag E. Improvement in the assessment of human epididymal function by the use of inhibitors in the assay of alpha-glucosidase in seminal plasma. *Int J Androl.* 1990;13:297–305.

Dabbs JM Jr. Age and seasonal variation in serum testosterone concentration among men. *Chronobiol Int.* 1990;7:245–249.

Elzanaty S, Malm J, Giwercman A. Visco-elasticity of seminal fluid in relation to the epididymal and accessory sex gland function and its impact on sperm motility. *Int J Androl.* 2004;27:94–100.

Elzanaty S, Richthoff J, Malm J, Giwercman A. The impact of epididymal and accessory sex gland function on sperm motility. *Hum Reprod.* 2002;17:2904–2911.

Feldmann HA, Longcope C, Derby CA, Johannes CB, Araujo AB, Coviello AD, Bremner WJ, McKinlay JB. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. *J Clin Endocrinol Metab.* 2002;87:589–598.

Guerin JF, Ben Ali H, Rollet J, Souchier C, Czyba JC. α-Glucosidase as a specific epididymal enzyme marker: its validity for the etiologic diagnosis of azoospermia. *J Androl.* 1986;7:156–162.

Gyllenborg J, Skakkebaek NE, Nielsen NC, Keiding N, Giwercman A. Secular and seasonal changes in semen quality among young Danish men: a statistical analysis of semen samples from 1927 donor candidates during 1977–1995. *Int J Androl.* 1999;22:28–36.

Henkel R, Maaß G, Schuppe H-C, Jung A, Schubert J, Schill W-B. Molecular aspects of declining sperm motility in older men. *Fertil Steril.* 2005;84(5): in press.

Henkel R, Menkveld R, Kleinhappl M, Schill W-B. Seasonal changes in human sperm chromatin condensation. *J Assist Reprod Genet.* 2001; 18:371–377.

Krause W, Bohring C. Why do we determine α-glucosidase activity in human semen during infertility work-up? *Andrologia.* 1999;31:289–294.

Krause A, Krause W. Seasonal variations in human seminal parameters. *Eur J Obstet Reprod Biol.* 2002;101:175–178.

Lam DA, Miron JA. Global patterns of seasonal variation in human fertility. *Ann NY Acad Sci.* 1994;709:9–28.

Levine RJ, Mathew RM, Chenault BC, Brown MH, Hurtt ME, Bently KS, Mohr KL, Working PK. Differences in the quality of semen in outdoor workers during summer and winter. *N Engl J Med.* 1990;323: 12–16.

Mahmoud AM, Geslevich J, Kint J, Depuydt C, Huysse L, Zalata A, Comhaire FH. Seminal plasma α-glucosidase activity and male infertility. *Hum Reprod.* 1998;13:591–595.

Mortimer D, Templeton AA, Lenton EA, Coleman RA. Annual patterns of human sperm production and semen quality. *Arch Androl.* 1983; 10:1–5.

Ombelet W, Maes M, Vandeput H, Cox A, Janssen M, Pollet H, Fourie FL, Steeno O, Bosmans E. Chronobiological fluctuations in semen parameters with a constant abstinence period. *Arch Androl.* 1996;37: 91–96.

Orgebin-Crist MC. Epididymal maturation of spermatozoa. In: Saez JM, Forest MG, Dazord A, Bertrand J, eds. *Recent Progress in Cellular Endocrinology of the Testis.* Paris: INSERM; 1985:85.

Paquin R, Chapdelaine P, Dubé JY, Tremblay RR. Similar biochemical properties of human seminal plasma and epididymal alpha-1,4-glucosidase. *J Androl.* 1984;5:277–281.

Peña P, Risopatrón J, Villegas J, Miska W, Schill W-B, Sánchez R. Alpha-glucosidase in the human epididymis: topographic distribution and clinical application. *Andrologia.* 2004;36:315–320.

Politoff L, Birkhauser M, Almendral A, Zorn A. New data confirming a circannual rhythm in spermatogenesis. *Fertil Steril.* 1989;52:486–489.

Reinberg A, Smolensky MH, Hallek M, Smith KD, Steinberger E. Annual variation in semen characteristics and plasma hormone levels in men undergoing vasectomy. *Fertil Steril.* 1988;49:309–315.

Saint Pol P, Beuscart R, Leroy-Martin B, Hermand E, Jablonski W. Circannual rhythms of sperm parameters of fertile men. *Fertil Steril.* 1989; 51:1030–1033.

Spiessens C, D'Hooghe T, Wouters E, Meuleman C, Vanderschueren D. Alpha-glucosidase activity in seminal plasma: predictive value for outcome in intrauterine insemination and in vitro fertilization. *Fertil Steril.* 1998;69:735–739.

Stolwijk AM, Reuvers MJ, Hamilton CJ, Jongbloet PH, Hollanders JM, Zielhuis GA. Seasonality in the results of in-vitro fertilization. *Hum Reprod.* 1994;9:2300–2305.

Tjoa WS, Smolensky MH, His BP, Steinberger E, Smith KD. Circannual rhythm in human sperm count revealed by serially independent sampling. *Fertil Steril.* 1982;38:454–459.

Viljoen MH, Bornman MS, van der Merwe MP, du Plessis DJ. Alpha-glucosidase activity and sperm motility. *Andrologia.* 1990;22:205–208.

World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction.* Cambridge, United Kingdom; 1999.

Yeung CH, Cooper TG, Senge T. Histochemical localization and quantification of alpha-glucosidase in the epididymis of men and laboratory animals. *Biol Reprod.* 1990;42:669–676.

Zöpfgen A, Priem F, Sudhoff F, Jung K, Lenk S, Loening SA, Sinha P. Relationship between semen quality and the seminal plasma components carnitine, alpha-glucosidase, fructose, citrate and granulocyte elastase in infertile men compared with a normal population. *Hum Reprod.* 2000;15:840–845.