Human Reproduction Vol.17, No.11 pp. 2904–2911, 2002

The impact of epididymal and accessory sex gland function on sperm motility

S.Elzanaty[1,4], J.Richthoff[2], J.Malm[3] and A.Giwercman[1]

[1]Scanian Andrology Centre, Fertility Centre, [2]Department of Urology and [3]Department of Clinical Chemistry, Malmö University Hospital, SE 205 02 Malmö, Sweden

[4]To whom correspondence should be addressed at: Scanian Andrology Centre, Fertility Centre, Malmö University Hospital, SE 205 02 Malmö, Sweden. E-mail: saad.elzanaty@kir.mas.lu.se

BACKGROUND: Little is known about the regulation of sperm motility, which is an important predictor of male fertility. However, both testicular and post-testicular factors may be involved, although the impact of the latter has been relatively poorly investigated. METHODS: In semen samples from 301 young men from the general Swedish population (mean ± SD age 18.2 ± 0.4 years), we assessed sperm motility by use of a manual method as well as computer-assisted sperm analysis (CASA) and correlated these values to seminal levels of neutral α-glucosidase (NAG), prostate-specific antigen (PSA), zinc and fructose. RESULTS: There were significant positive correlations between seminal levels of NAG, and PSA and CASA percentage motile sperm ($r = 0.158$, $P = 0.009$; $r = 0.155$, $P = 0.01$ respectively), and significant negative correlations with CASA percentage immotile sperm ($r = –0.206$, $P = 0.001$; $r = –0.157$, $P = 0.009$ respectively). In a multiple regression analysis it was found that, apart from sperm concentration, the level of PSA was the most significant and independent parameter in predicting percentage motile sperm ($\beta = 0.220$, $P = 0.037$). CONCLUSION: Our study demonstrated the regulatory effect of post-testicular glands on the motility of sperm. This is to our knowledge the first study showing a direct correlation between the seminal PSA levels and sperm motility in a group of men representing the general population. In future investigations and searches for specific treatment modalities in male infertility, more attention should be paid to the epididymis and accessory sex gland function.

Key words: accessory sex glands/epididymis/prostate-specific antigen/seminal plasma/sperm motility

Introduction

Sperm motility has been shown, in several studies, to be a good predictor of human male fertility *in vivo* and *in vitro* (Auger *et al*., 1994). Therefore, an insight into factors which may have an impact on the motility characteristics of sperm may give us a better understanding of the mechanisms behind male infertility and lead to the development of new, specific treatment modalities. However, our knowledge about the impact of testicular and post-testicular mechanisms on sperm motility is limited. It is well known that defects of spermatogenesis are not only associated with oligozoospermia but also with asthenozoospermia (Nistal and Paniagua, 1984; Okuyama *et al*., 1984; Viviani *et al*., 1991). On the other hand, it has also been shown that the epididymis and accessory sex glands also play a role for the functional status of the male gametes (Gonzales *et al*., 1992).

The epididymis has been shown to play an important role for sperm maturation including gaining the ability to be motile (Longo, 1987). Several biochemical markers of epididymal function can be detected in seminal fluid (Frenkle *et al*., 1974; Guerin *et al*., 1990), neutral α-glucosidase (NAG) being considered as one of the most useful from the clinical point of view (Roland *et al*., 1979; Kret and Milad, 1995). Although a number of studies have shown positive correlations between the seminal levels of NAG and sperm motility (Viljoen *et al*., 1990; Fourie *et al*., 1991), these results have been contradicted by other reports (Guerin *et al*., 1990; Krause and Bohring, 1999).

Even less knowledge exists regarding the impact of accessory sex glands on sperm motility characteristics. The gel-forming proteins—semenogelins—are mainly synthesized in the seminal vesicles (Lilja *et al*., 1989) and are believed to have an inhibitory effect on the ability of sperm to move (Robert and Gagnon, 1996). On the other hand, another vesical product, fructose, has been reported to be a source of energy for the motility of the gametes (Mann, 1964). Patel *et al*. demonstrated a positive correlation between seminal levels of fructose and percentage motile sperm (Patel *et al*., 1988), but other studies (Lewis-Jones, *et al*., 1996; Andrade-Rocha, 1999; Zöpfgen *et al*., 2000) did not find such a correlation.

Prostate-specific antigen (PSA) has been reported to be involved in degradation of semenogelins (Lilja *et al*., 1989;

Robert and Gagnon, 1996) and might therefore be expected to have a positive impact on sperm motility. Two studies (Ahlgren *et al*., 1995; Charlens *et al*., 1999) found that seminal PSA was low in patients with low sperm motility. Zinc, which to a high degree also originates from the prostate (Arver and Eliasson, 1982), also plays an important role in sperm function (Lewis-Jones *et al*., 1996), mainly via its effect on chromatin stability (Kvist *et al*., 1987). The results of reports on the association between seminal zinc levels and sperm motility have been conflicting, indicating a positive correlation (Fuse *et al*., 1999; Sin-Eng *et al*., 2000), a negative correlation (Carreras and Mendoza, 1990) or no association at all (Behne *et al*., 1988; Lin *et al*., 2000).

Some of the reasons behind this disagreement may be related to the selection of the study population and methodology for motility assessment. The majority of the studies are based on cohorts of infertile men in whom a mixture of different pathophysiologies may affect the motility of the sperm. Furthermore the traditional—laboratory technician-based—motility assessment is usually characterized by a significant inter- and intra-observer variation. The modern methods of computer-assisted sperm analysis (CASA) are giving a more subtle and more reproducible description of sperm motility (Larsen *et al*., 2000).

We have, therefore, in a group of Swedish military conscripts, correlated the seminal levels of different markers of epididymal, prostatic and seminal vesicle function with sperm motility assessed by use of the manual method as well as CASA. The aim of this study was to evaluate the impact of the function of these three organs on sperm motility by focusing on a more homogeneous male population, thereby avoiding some of the methodological problems associated with the assessment of sperm motility.

Materials and methods

Subjects

Approximately 95% of all 18 year old Swedish men undergo a medical health examination prior to military service. Only men with serious chronic diseases are excluded from this conscript examination. Thus, the conscripts are representative of the general population of young Swedish males. A group of 2255 consecutive men living <60 km from the city of Malmö underwent the compulsory medical board examination between May and December 2000, and were asked to take part in this study, aiming to evaluate the reproductive function of young Swedish males from the general population. Of these, 305 (13.5%) agreed to participate in the study, which took place at Malmö University Hospital.

The volunteers were asked about their reproductive history including cryptorchidism and other diseases of the male reproductive organs. These data are reported in another publication (Richthoff *et al*., 2002). Following an andrological examination, the subjects were asked to deliver a semen sample in a separate room in the laboratory area. The volunteers were paid €55. The study was approved by the local ethics committee.

The mean ± SD age of the conscripts was 18.2 ± 0.4 years and their mean body mass index was 23 ± 3.1 kg/m^2. Two men did not deliver semen samples and an additional two men were azoospermic. Consequently, data from 301 men were used for the study.

Semen analysis

The semen samples were obtained by masturbation after 12–500 h of sexual abstinence (mean 83.4 h). After 30 min of liquefaction, 450 µl of the ejaculate was removed and mixed with 50 µl of bezamidine (0.1 mol/l) in order to stop the biochemical processes involved in liquefaction. The mixture was centrifuged for 20 min at 4500 *g*, and the seminal plasma was decanted and stored at –20°C until analysed for the levels of NAG, and the concentrations of PSA, zinc and fructose. The seminal volume in 10 of the 301 men was too low to perform biochemical analysis of seminal fluid. Therefore, biochemical markers were only assessed in 291 semen samples. After liquefaction, within 1 h of ejaculation, the ejaculates were analysed for the following characteristics: semen volume, sperm concentration, and sperm motility (defined as WHO motility grades A, B, C and D). All semen tests were performed according to published recommendations (World Health Organization, 1999). Thereafter computer-assisted analysis of sperm motility using the 'CRISMAS' system (Image House, Copenhagen, Denmark) was performed.

Biochemical analysis

Neutral α-glucosidase

Seminal plasma NAG was measured using a commercially available kit (Episcreen®; Fertipro, Gent, Belgium) according to the instructions given by the manufacturer. The test is based on the measurement of the intensity of a colour change evoked by the reaction between α-glucosidase and 0.125 of reagent 1 (0.09 % Na-azide) which was added to 0.125 ml of thawed seminal plasma. The mixture was mixed well by pipetting, one diagnostic tablet (*p*-nitophenyl-α-D-glucopyranoside) was then added, and the mixture was remixed and vortexed for 60 s and incubated for 4 h at 37°C. After incubation, 3 ml of reagent 2 (0.02 mol/l NaOH) was added to the solution, which was centrifuged for 6 min at 3000 *g*. The absorbance value, obtained by reading the supernatant against reagent 2 as a blank, was measured by use of a spectrophotometer at 405 nm. This value was plotted on a standard curve and the corresponding total α-glucosidase activity was read on the abscissa. The NAG level was estimated by the use of the corresponding table provided by the manufacturer.

Prostate-specific antigen

The concentration of PSA in seminal plasma was determined with the Prostatus™ kit (Wallac Oy, Finland). This is a Delfia™ method, i.e. an immunofluorometric method, using three monoclonal antibodies against PSA. The coefficient of variation was 12% for control samples with a mean PSA concentration of 660 mg/l.

Zinc

The concentration of zinc in seminal plasma was determined with a colorimetric method (Makino *et al*., 1982). The proteins in the sample were precipitated with trichloroacetic acid, the supernatant mixed with a water-soluble pyridylazo dye and the absorbance measured at 560 nm. The coefficient of variation was 7% for control samples with a mean zinc concentration of 2.0 mmol/l.

Fructose

The concentration of fructose in seminal plasma was determined with a spectrophotometric method, essentially as previously described (Wetterauer and Heite, 1976), run on a Beckman Synchron LX20 instrument. Proteins in the sample were precipitated with perchloric acid and the absorbance of the supernatant measured. After addition of phosphoglucose isomerase, resulting in conversion of fructose to glucose, the absorbance was measured again. The absorbance

difference corresponds to the concentration of fructose in the sample. The coefficient of variation was 5.0% for control samples with a mean fructose concentration of 12.7 mmol/l.

CASA sperm motility analysis

CASA sperm motility analysis was performed by the use of the CRISMAS system as previously described (Larsen *et al*., 2000). The system comprises a video camera connected to standard C-mount equipped microscope. A framegrabber installed in a standard PC operating under Windows NT converts the video signal obtained at 25 MHz to raw digital data. Images are available for random access in digital form for analysis and playback. For analysis, a 5 µl aliquot of the ejaculate was pipetted into a Makler counting chamber 10 µm deep, which was placed on a heated microscope stage (37°C). Video recordings were made from four different fields of the chamber using a ×20 magnification objective. CASA analysis was based upon capturing sequences of 64 frames per field and counting a minimum of 100 sperm.

For each analysed sequence the following parameters were obtained: curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), amplitude of lateral head displacement (ALH), and linearity (LIN). These parameters were defined according to published guidelines (World Health Organization, 1999). The total population of sperm was divided into three categories: motile (VCL >25 µm/s), 'locally' motile (VCL = 5–25 µm/s), and immotile (VCL <5 µm/s). Sperm motility characteristics are only given for motile sperm since the percentage of these cells was previously found to be an independent predictor of male fertility (Larsen *et al*., 2000).

For technical reasons, samples from only 285 of the 291 ejaculates on which the biochemical analysis was performed were available for the CASA analysis of the main motility characteristics (percentage motile, locally motile, and immotile sperm), and from 270 subjects for the motility characteristics, VCL, VSL, VAP, ALH and LIN. However, there was no selection bias since the difference between the 21 ejaculates which were not fully analysed and the 270 which underwent the analysis, was not statistically significant for standard sperm characteristics, including motility and concentration.

Statistical methods

The statistical analysis was performed using the SPSS version 10.0 software (SPSS Inc., Chicago, IL, USA). Primarily, Pearson's coefficients were calculated in order to assess the bivariate correlations between the levels of biochemical seminal markers and sperm parameters as well as the length of the abstinence period. Prior to this, logarithmic transformation of the abstinence period and sperm concentration was undertaken in order to obtain a normal distribution of the data. For the remaining parameters no transformation was necessary. In addition, corresponding analyses were performed by use of the non-parametric Spearman's rank correlation test. As the two correlation tests gave identical results, and because when determining NAG the maximum activity that could be measured was 15 mU/ml, which means that samples with activity values >15 mU/ml were assigned a value of 16 mU/ml, hence possibly skewing the results, we decided to present only the results of the non-parametric bivariate analyses. After performing the bivariate analysis, the impact of seminal parameters found to have a significant correlation with sperm motility characteristics was tested in a multiple regression model with CASA percentage of motile sperm as the dependent variable. A correlation was considered statistically significant when $P < 0.05$.

Results

The descriptive statistics for the abstinence period, and semen parameters are presented in Table I.

Relationship between the levels of biochemical markers

As shown in Table II the levels of biochemical parameters in seminal fluid were closely correlated to each other. There were significantly positive correlations between the epididymal marker (NAG) and prostatic markers (PSA and zinc), and between PSA and zinc, and a significantly negative correlation between PSA and vesicle marker (fructose). Neither NAG nor zinc showed a correlation with fructose.

Relationship between biochemical markers and sperm motility

The levels of NAG and the concentrations of PSA correlated positively with CASA percentage motile sperm ($r = 0.158$, $P = 0.009$; $r = 0.155$, $P = 0.010$ respectively) (Figure 1), and negatively with CASA percentage immotile sperm ($r = –0.206$, $P = 0.001$; $r = –0.157$, $P = 0.009$ respectively). Concentration of zinc showed a significantly negative correlation with CASA percentage immotile sperm ($r = –0.138$, $P = 0.022$). For other combinations of levels of biochemical markers and sperm motility characteristics, no statistically significant correlations were found (Table III).

Relationship between sperm motility and other semen charactristics

Statistically significant positive correlations were found between sperm concentration and percentage of rapidly progressive sperm (grade A), percentage of total progressively motile sperm (A + B) and CASA percentage motile sperm, whereas the correlations between sperm concentration and percentage non-progressively motile sperm (C), immotile sperm (D), and CASA percentage immotile sperm were negative (Table IV). The length of abstinence period did not correlate with the percentage of CASA motile sperm or the percentages of categories A or A + B, as assessed manually (data not shown).

When the concentrations of sperm, the levels of NAG, and the concentrations of PSA and zinc were tested as independent variables in a linear multiple regression model, with CASA percentage motile sperm as the dependent variable, both the sperm concentration and PSA were found to be independent predictors of sperm motility ($\beta = 0.298$, $P < 0.001$; ($\beta = 0.220$, $P = 0.037$ respectively) (Table V).

Discussion

In a study of semen samples from 301 young men from the general Swedish population, we found correlations between the seminal levels of markers of epididymal (NAG), prostate (PSA, zinc) function and sperm motility characteristics. Thus, in bivariate analysis we found statistically significant positive correlation between CASA percentage motile sperm and seminal levels of NAG, as well as the concentrations of PSA. Correspondingly, there was a negative correlation with CASA percentage immotile sperm. Furthermore zinc levels showed

Table I. Descriptive statistics for semen variables in a group of 301 young men from the general Swedish population

Variables	*n*	Range	Median	Mean ± SD
Semen volume (ml)	301	0.3–8.4	32	3.2 ± 1.3
Sperm concentration (10^6/ml)	301	0.2–391	52	71.2 ± 66.0
Biochemical markers				
NAG (mU/ml)	291	1.0– >15	7	NA[a]
PSA (mg/l)	291	110–2211	638	701.0 ± 339.2
Zinc (mmol/l)	291	0.1–5.4	1.4	2.0 ± 1.0
Fructose (mmol/l)	291	1.0–44	15	15.0 ± 7.0
Manually assessed motility (%)				
Grade A	301	0–74	32	31.0 ± 18.0
Grade A + B	301	0–85	56	54.2 ± 16.2
Grade C	301	0–44	13	14.4 ± 8.0
Grade D	301	6–99	29	31.0 ± 13.1
CASA assessed motility				
Motile sperm (%)	285	0–100	51	52.0 ± 22.0
Locally motile sperm (%)	285	0–48	16	18.0 ± 9.4
Immotile sperm (%)	285	0–100	29	32.0 ± 22.1

[a]Not available because the concentrations >15 mU/ml were assigned the value of 16.
NAG = neutral α-glucosidase; PSA = prostate-specific antigen; CASA = computer-assisted semen analysis.

Table II. Bivariate correlation coefficients (*r*) between levels of NAG, prostatic markers (PSA, zinc), and vesical marker (fructose) in 291 young men from the general Swedish population

	NAG (mU/ml)		PSA (mg/l)		Zinc (mmol/l)		Fructose (mmol/l)	
	r	P^a	*r*	P^a	*r*	P^a	*r*	P^a
NAG (mU/ml)	–	–	0.379	< 0.001	0.395	< 0.001	–0.020	0.731
PSA (mg/l)	0.379	< 0.001	–	–	0.813	< 0.001	–0.254	< 0.001
Zinc (mmol/l)	0.395	< 0.001	0.813	< 0.001	–	–	–0.089	0.130
Fructose (mmol/l)	–0.020	0.731	–0.254	< 0.001	–0.089	0.130	–	–

[a]$P < 0.05$ is considered as statistically significant.
NAG = neutral α-glucosidase; PSA = prostate-specific antigen.

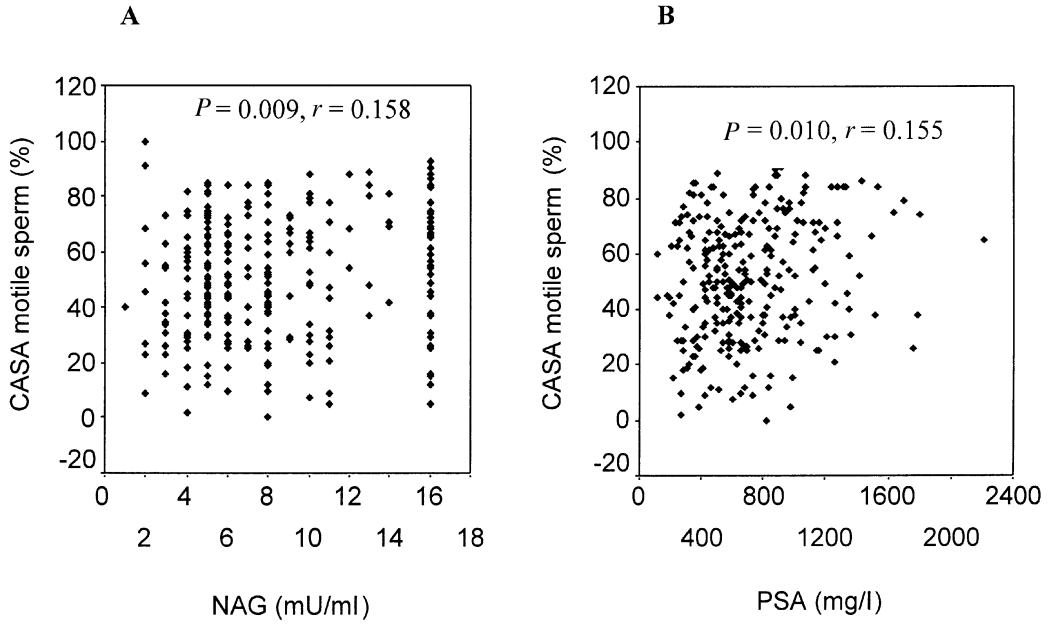

Figure 1. Correlation between concentrations of (**A**) neutral α-glucosidase (NAG, mU/ml), (**B**) prostate-specific antigen (PSA, mg/l), and computer-assisted sperm analysis (CASA) percentage motile sperm in 285 young men from the general Swedish population.

Table III. Bivariate correlation coefficients (*r*) between levels of the NAG, PSA, zinc and fructose, and sperm motility characteristics in 291 young men from the general Swedish population

	NAG (mU/ml)		PSA (mg/l)		Zinc (mmol/l)		Fructose (mmol/l)	
	r	P^a	*r*	P^a	*r*	P^a	*r*	P^a
Manually assessed motility (%)								
Grade A	0.066	0.264	0.082	0.164	0.032	0.587	–0.075	0.201
Grade A + B	0.107	0.069	0.097	0.097	0.077	0.192	–0.039	0.504
Grade C	–0.032	0.583	–0.073	0.212	–0.091	0.122	0.001	0.993
Grade D	–0.113	0.054	–0.053	0.370	–0.020	0.732	0.035	0.554
CASA-assessed motility								
Motile sperm (%)	0.158	0.009	0.155	0.010	0.104	0.085	–0.082	0.175
Locally motile sperm (%)	0.029	0.639	–0.022	0.720	–0.005	0.941	–0.100	0.099
Immotile sperm (%)	–0.206	0.001	–0.157	0.009	–0.138	0.022	0.084	0.168
Motile sperm VCL (μm/s)	0.010	0.874	0.034	0.591	–0.035	0.570	–0.080	0.198
Motile sperm VSL (μm/s)	0.056	0.373	0.023	0.716	–0.036	0.563	–0.080	0.198
Motile sperm VAP (μm/s)	0.037	0.548	0.022	0.723	–0.051	0.413	–0.089	0.152
Motile sperm ALH (μm)	–0.036	0.563	0.024	0.696	–0.033	0.595	–0.035	0.578
Motile sperm LIN	0.073	0.240	0.037	0.554	–0.003	0.964	–0.045	0.474

[a]$P < 0.05$ is considered as statistically significant.
NAG = neutral α-glucosidase; PSA = prostate-specific antigen; VCL = curvilinear velocity; VSL = straight line velocity; VAP = average path velocity; ALH = amplitude of lateral head displacement; LIN = linearity.

Table IV. Bivariate correlation coefficients (*r*) between semen volume, sperm concentration and sperm motility characteristics in 291 young men from the general Swedish population

Variables	Semen volume (ml)		Sperm concentrations (10^6/ml)	
	r	*P*	*r*	*P*
Manually assessed motility (%)				
Grade A	0.049	0.395	0.318	< 0.001
Grade A + B	–0.022	0.706	0.261	< 0.001
Grade C	–0.003	0.956	–0.195	0.001
Grade D	–0.066	0.250	–0.326	< 0.001
CASA assessed motility (%)				
Motile sperm	0.078	0.191	0.391	< 0.001
Locally motile sperm	–0.024	0.684	0.115	0.053
Immotile sperm	–0.053	0.373	–0.449	< 0.001

[a]$P < 0.05$ is considered as statistically significant.
CASA = computer-assisted semen analysis.

Table V. Effect of sperm concentration, levels of NAG, PSA and zinc on CASA percentage motile sperm in 285 young men from the general Swedish population, obtained from multiple regression analysis

Variables	CASA percentage motile sperm	
	P^a	β
Sperm concentration (10^6/ml)	< 0.001	0.298
NAG (mU/ml)	0.773	0.020
PSA (mg/l)	0.037	0.220
Zinc (mmol/l)	0.130	–0.162

[a]$P < 0.05$ is considered as statistically significant.
NAG = neutral α-glucosidase; PSA = prostate-specific antigen; CASA = computer-assisted semen analysis.

a significant negative correlation with CASA percentage immotile sperm. In the multivariate linear regression analysis, apart from sperm concentration PSA was the only statistically significant predictor of the CASA percentage of motile sperm.

The physiological role of PSA in seminal fluid is largely unknown. This enzyme was shown to degrade rapidly semenogelins I and II into multiple fragments of low molecular mass. It has been suggested that this degradation considerably reduces the inhibitory effect of semenogelins on motility of sperm (Lilja *et al*., 1989; Robert and Gagnon, 1996). Previous studies based on materials from men with spinal cord injury (Charlens *et al*., 1999) and infertile men (Ahlgren *et al*., 1995) indicated low PSA levels in patients with reduced percentage of motile sperm. The present study is the first to indicate a direct correlation between PSA and motility of sperm in a group of men recruited from the general population and not due to infertility problems.

Some of the enzymes important for the function of sperm are zinc metallo-enzymes and can thus become dysfunctional when zinc is deficient. One of these, sorbitol dehydrogenase (SoDH), utilizes sorbitol to provide sperm with fructose for energy, so that SoDH activity is correlated to motility (Dhanad *et al*., 1981). Similarly lactate dehydrogenase-X, another zinc metallo-enzyme, was also reported to have some relationship with motile sperm (Eliasson and Virgi, 1985). The results of our study add to our understanding of the diverging results regarding the impact of zinc on sperm motility, which can be found in the literature (Fuse *et al*., 1999; Henkel *et al*., 1999; Sin-Eng *et al*., 2000; Wong *et al*., 2001; Eggert-Kruse *et al*., 2002). Thus, in a bivariate analysis there was a significantly negative correlation with percentage of immotile sperm and a positive correlation with percentage motile sperm, close to the level of statistical significance. However, these findings might be partly due

to the strong correlation between the seminal levels of zinc and PSA, also found by others (Ahlgren *et al*., 1995). In the multivariate analysis, when removing the effect of co-variation between the two markers, the effect of zinc on motility of sperm was negative, although not statistically significant. Robert *et al*. concluded that the concentration of free zinc in seminal plasma is likely to be an important factor in the processing of semenogelin after ejaculation (Robert *et al*., 1997). High concentrations of free zinc are likely to inhibit the PSA activity and thus degradation of the semenogelin, and lack of this metal may protect gel-forming proteins from proteolysis. These observations stress the need for a more holistic approach when evaluating the effect of the epididymis and accessory sex glands on sperm parameters.

NAG is secreted by the epididymal epithelium, probably to provide optimal levels of energy for sperm maturation. The importance of the epididymal compartments in the maintenance of an NAG pool is well demonstrated by the observation that its exclusion by vas ligation leads to a drastic decrease of α-glucosidase (Roland *et al*., 1979). The results of our study are in accordance with other studies demonstrating positive correlation between NAG and motility of sperm (Fourie *et al*., 1991; Roland *et al*., 1979; Viljoen *et al*., 1990). It has been reported that a significantly lower number of sperm are bound to the zona pellucida in a zona-binding assay in samples with a low α-glucosidase activity when compared with samples with a high activity (Ben Ali *et al*., 1994). Moreover, it has recently been suggested that α-glucosidase activity could be used to predict the outcome of IVF (Spiessen *et al*., 1998). However, the clinical value of NAG analysis is still unclear. Thus, Krause and Bohring previously concluded that the determination of α-glucosidase activity does not give additional information of the fertility status exceeding that of other clinical investigations or parameters of semen analysis (Krause and Bohring, 1999). Furthermore, we found a significant association between NAG levels and sperm motility in the bivariate but not in the multivariate analysis.

Fructose has been reported to be a source of energy for the motility of the gametes (Mann, 1964). However, one might expect to find a negative correlation between the levels of fructose and motility of sperm, due to the sperm-immobilising effect of semenogelins (Robert and Gagnon, 1996; Robert and Gagnon, 1999), which are co-secreted by seminal vesicles. In accordance with the previous results (Lewis-Jones *et al*., 1996; Andrade-Rocha, 1999; Zöpfgen *et al*., 2000) our study did not demonstrate such association. Moreover, another group (Lay *et al*., 2001) found no significant difference between male fertility status and seminal plasma fructose level.

The bivariate correlations between the levels of biochemical markers and sperm motility characteristics were generally weak. However, sperm motility is dependent on a multitude of pre-testicular, testicular and post-testicular factors and it is hard to imagine a single factor as a strong predictor of the percentage of motile sperm. The motility of sperm has been reported to be one of the most important parameters in evaluating the fertilizing ability of ejaculated sperm both *in vivo* and *in vitro* (Gerris and Khan 1987; Auger *et al*., 1994; Amann 1995; Donnelly *et al*., 1998). Moreover, recent studies have indicated that sperm motility data obtained by CASA may also be predictive of fertility (Barratt *et al*., 1993; Yukihirano *et al*., 2001). In a study of Danish women planning their first pregnancy, performed using the same CASA (CRISMAS) system, the percentage of motile sperm, as assessed by CASA (but not manually) (Bonde *et al*., 1998; Larsen *et al*., 2000) was found to be an independent predictor of fecundability. Therefore, our data indicate that epididymal and prostatic function may play an important role for motility of sperm, and subsequently the fertility of an individual.

The design of the study did not allow us to draw conclusions that could be directly transferred to clinical practice. However, we obtained an insight into the biological mechanisms, which can help us in our understanding of the pathophysiology of male infertility, and be a starting point for designing more targeted studies of well-defined groups of patients.

The fact that we did not find any statistically significant correlation between the traditional sperm motility parameters and the levels of biochemical markers in seminal fluid may be due to a higher degree of intra- and inter-observer variation when sperm motility is assessed manually as compared with the CASA measurements (Krause and Viethen 1999).

Since the biochemical reactions taking place in the seminal fluid are not stopped before 30 min post ejaculation, our measurements may not exactly mirror the secretory activity of the epididymis and the accessory sex glands and thereby not give the full picture of the impact of these glands function on motility of sperm.

The participation rate in this study was only 13.5%, and one could ask whether this group of men was representative for the general population of young Swedish males. However, in a recent Danish study (Andersen *et al*., 2000) using the same approach with a similar overall number of participants, the proportion of men delivering a semen sample was considered representative of young males in the general population in terms of reproductive function. In that study, with a total participant rate of 80%, the conclusion was based on finding the same levels of reproductive hormones in those men delivering an ejaculate, as in subjects who were willing only to give a blood sample and not an ejaculate.

In conclusion, in a study of semen samples from 301 men from the general population, we found an indication of a positive impact of epididymal and prostate function on percentage of motile sperm. Since sperm motility is an important determinant of male fertility potential, more attention should be paid to the function of these organs in the future 'work-up' and in the search for therapeutic methods in male infertility.

Acknowledgements

We would like to thank Erna Stridsberg, Rita Hofvander and Dr Göran Svensson for their help in recruiting the participants in the study.

Anna Bremer, Camilla Anderberg and Katarina Jepson are thanked for their technical assistance. The study was financially supported by Swedish government founding for clinical research Crafoordska Fund, Ove Tulefjords Fund and Foundation for Urological research.

References

Ahlgren, G., Rannevik, G. and Lilja, H. (1995) Impaired secretory function of the prostate in men with oligo-asthenozoospermia. *J. Androl*., **16**, 491–498.

Amann, R.P. (1989) Can the fertility of a seminal sample be predicted accurately? *J. Androl*., **10**, 89–98.

Andersen, A.G., Jensen, T.K., Carlsen, E., Jørgensen, N., Anderssen, A.M., Krarup, T., Keiding, N. and Skakkebaek, N.E. (2000) High frequency of sub-optimal semen quality in an unselected population of young men. *Hum. Reprod*., **15**, 366–372.

Andrade-Rocha, F.T. (1999) Seminal fructose levels in male infertility: relationship with sperm characteristics. *Int. Urol. Nephrol*., **31**, 107–111.

Arver, S. and Eliasson, R. (1982) Zinc and zinc ligands in human seminal plasma. II. Contribution by ligands of different origin to the zinc binding properties of human seminal plasma. *Acta Physiol. Scand*., **115**, 217–24.

Auger, J., Serres, C., Wolf, J.P. and Jouannet, P. (1994) sperm motility and fertilization. *Contracept. Fertil. Sex*., **22**, 314–8.

Barratt, C.L.R., Tomlinson, M.J. and Cook, I.D. (1993) Prognostic significance of computerized motility analysis for *in vivo* fertility. *Fertil. Steril*., **60**, 520–525.

Behne, D., Gessner, H., Wolters, G. and Brotherton, J. (1988) Selenium, rubidium and zinc in human semen and semen fractions. *Int. J. Androl*., **11**, 415–423.

Ben Ali, H., Guerin, J.P., Pinatel, M.C., Mathieu, C., Boulieu, D. and Tritar, B. (1994) Relationship between semen characteristics, α-glucosidase and the capacity of spermatozoa to bind to the human zona pellucida. *Int. J. Androl*., **17**, 121–126.

Bonde, J.P., Ernst, E., Jensen, T.K., Hjollund, N.H., Kolstad, H., Henriksen, T.B., Scheike, T., Giwercman, A., Olsen, J. and Skakkebaek, N.E. (1998) Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. *Lancet*, **10**, 1172–1177.

Carreras, A. and Mendoza, C. (1990) Zinc levels in seminal plasma of fertile and infertile men. *Andrologia*, **22**, 279–283.

Charlens, M.L., Teodoro, C.A., Tang, J.W., Harry, G.R., Sean, M.F. and Nancy, L.B. (1999) Serum and semen prostate specific antigen concentrations are different in young spinal cord injured men compared to normal controls. *J. Urol*., **162**, 89–91.

Dhanad, O.P., Rao, B.R. and Razdan, M.N. (1981) Sorbitol dehydrogenase and hyaluronidase activity in buffalo semen. *Ind. J. Exp. Biol*., **19**, 286–289.

Donnelly, E.T., Lewis, S.E., McNally, J.A. and Thompson, W. (1998) In vitro fertilization and pregnancy rates: the influence of sperm motility and morphology on IVF outcome. *Fertil. Steril*., **70**, 305–314.

Eggert-Kruse, W., Zwick, E.M., Batschulat, K., Rohr, G., Armbruster, P.E., Petzoldt, D. and Dstrowitzki, T. (2002) Are zinc levels in seminal plasma associated with seminal leukocytes and other determinants of semen quality? *Fertil. Steril*., **77**, 260–269.

Eliasson, R. and Virji, N. (1985) LDH-C4 in human seminal plasma and its relationship to testicular function. 11. Clinical aspects. *Int. J. Androl*., **8**, 201–203.

Fourie, M.H., Du Toit, D., Bornman, M.S., Van Der Merwe, M.P. and Du Plessis, D.J. (1991) α-Glucosidase, sperm ATP concentrations, and epididymal function. *Arch. Androl*., **26**, 139–141.

Frenkle, G., Peterson, R.N., Davis, J.E. and Freund, M. (1974) Glyceryl phosphocholine and carnitine in normal human semen and in post vasectomy semen differences in concentration. *Fertil. Steril*., **25**, 84–87.

Fuse, H., Kazama, T., Ohta, S. and Fujiuchi, Y. (1999) Relationship between zinc concentrations in seminal plasma and various sperm parameters. *Int. Urol. Nephrol*., **31**, 401–408.

Gerris, J. and Khan, I. (1987) Correlation between in vitro fertilization and human sperm density and motility. *J. Androl*., **8**, 48–54.

Gonzales, G.F., Kortebani, G. and Mazzolli, A.B. (1992) Leukocytospermia and function of the seminal vesicles on seminal quality. *Fertil. Steril*., **57**, 1058–1065.

Guerin, J.F., Ben Ali, H., Cottinet, D. and Rollet, J. (1990) Seminal alpha-glycosidase activity as a marker of epididymal pathology in normozoospermic men consulting for infertility. *J. Androl*., **11**, 240–245.

Henkel, R., Bittner, J., Weber, R., Huther, F. and Miska, W. (1999) Relevance of zinc in human sperm flagella and its relation to motility. *Fertil. Steril*., **71**, 1138–1143.

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

Krause W. and Viethen, G. (1999) Quality assessment of computer-assisted semen analysis (CASA) in the andrology laboratory. *Andrologia*, **31**, 125–129.

Kret, B. and Milad, M. (1995) New discriminatory level for glucosidase activity to diagnose epididymal obstruction or dysfunction. *Arch. Androl*., **35**, 29–33.

Kvist, U., Björndhal, L. and Kjellberg, L. (1987) Sperm nuclear zinc, chromatin stability, and male fertility. *Scann. Microsc*., **1**, 1241–1247.

Larsen, L., Scheike, T., Jensen, T.K., Bonde, J.P., Ernst, E., Hjollund, N.H., Zhou, Y., Skakkebæk, N.E. and Giwercman, A. (2000) Computer-assisted semen analysis parameters as predictors for fertility of men from the general population. *Hum. Reprod*., **15**, 1562–1567.

Lay, M.F., Richardson, M.E., Boone, W.R., Bodine, A.B. and Thurston, R.J. (2001) Seminal plasma and IVF potential. Biochemical constituents of seminal plasma of males from in vitro fertilization couples. *J. Assist. Reprod. Genet*., **18**, 144–150.

Lewis-Jones, D.I., Aird, I.A., Biljan, M.M. and Kingsland, C.R. (1996) Effects of sperm activity on zinc and fructose concentrations in seminal plasma. *Hum. Reprod*., **11**, 2465–2467.

Lilja, H., Abrahamsson, P.A. and Lundwall, A. (1989) Semenogelin, the predominant protein in human semen. Primary structure and identification of closely related proteins in the male accessory sex glands and on the spermatozoa. *J. Biol. Chem*., **25**, 1894–900.

Lin, Y.C., Chang, T.C., Tseng, Y.J., Lin, Y.L., Huang, F.J., Kung, F.T. and Chang, S.Y. (2000) Seminal plasma zinc levels and sperm motion characteristics in infertile samples. *Chang. Gung. Med. J.*, **23**, 260–266.

Longo, F.J. (1987) Sperm activation. In Longo, F.J. (ed.), *Fertilization*. Chapman and Hall, London, p. 5.

Makino, T., Saito, M., Horiguchi, D. and Kina, K. (1982) A highly sensitive colorimetric determination of serum zinc using water-soluble pyridylazo dye. *Clin. Chim. Acta*, **120**, 127–135.

Mann, T. (1964) Fructose, polyols, and organic acids. In Nam, T. (ed.), *The Biochemistry of Semen and of the Male Reproductive Tract*. Methuen, London, pp. 237–264.

Nistal, M. and Paniagua, R. (1984) Infertility in adult males with retractile testes. *Fertil. Steril*., **41**, 395–403.

Okuyama, A., Namiki, M., Aono, T., Matsumoto, K., Utsunomiya, M., Itoh, H., Yoshioka, T., Itatani, H. and Sonoda, T. (1984) Improvement of spermatogenesis by hCG and hMG treatment in pubertal boys with history of orchiopexy at early childhood. *Arch. Androl*., **12**, 29–33.

Patel, S.M., Skandhan, K.P. and Mehta, Y.B. (1988) Seminal plasma fructose and glucose in normal and pathological conditions. *Acta Eur. Fertil.*, **19**, 329–332.

Richthoff, J., Rylander, L., Hagmar, L., Malm, J. and Giwercman, A. (2002) Higher sperm counts in southern Sweden compared with Denmark. *Hum. Reprod*., **17**, 2468–2473.

Robert, M. and Gagnon, C. (1996) Purification and characterization of the active precursor of a human sperm motility inhibitor secreted by the seminal vesicles: identity with semenogelin. *Biol. Reprod*., **55**, 813–821.

Robert, M. and Gagnon, C. (1999) Semenolegin I: a coagulum forming, multifunctional seminal vesicle protein. *Cell. Mol. Life Sci*., **55**, 944–60.

Robert, M., Gibbs, B.F., Jacobson, E. and Gagnon, C. (1997) Characterization of prostate-specific antigen proteolytic activity on its major physiological substrate, the sperm motility inhibitor precursor/semenogelin 1. *Biochemistry*, **36**, 3811–3819.

Roland, R.T., Pierre, C. and Jean, M. (1979) α-1–4-glucosidase activity in human semen: variations with number and motility of spermatozoa. *Fertil. Steril*., **31**, 592–593.

Sin- Eng, C., Choon-Nam, O., Lay-Ha, C., Lee-Mee, H. and Sun-Kuie, T. (2000) Comparison of zinc concentrations in blood and seminal plasma and the various sperm parameters between fertile and infertile men. *J. Androl*., **21**, 53–57.

Spiessen, C., Thomas, D., Wouters, E., Meuleman, C. and Vanderscueren, D. (1998) α-Glucosidase activity in seminal plasma: predictive value for outcome in intrauterine insemination and in vitro fertilization. *Fertil. Steril.*, **69**, 735–739.

Viljoen, M.H., Bornman, M.S., Van Der Merwe, M.P. and Du Plessis, D.J. (1990) Alpha-glucosidase activity and sperm motility. *Andrologia*, **22**, 205–208.

Viviani, S., Ragni, G., Santoro, A., Perotti, L., Caccamo, E., Negretti, E., Valagussa, P. and Bonadonna, G. (1991) Testicular dysfunction in Hodgkin's disease before and after treatment. *Eur. J. Cancer*, **27**, 1389–1392.

Wetterauer, U. and Heite, H-J. (1976) Eine empfehlenswerte Methode zur gleichzeitigen enzymatischen Bestimmung von Citrat und Fruktose im Seminalplasma. *Akt Dermatol.*, **2**, 239–248.

Wong, W.Y., Flik, G., Groenen, P.M., Swinkels, D.W., Thomas, C.M., Copius-Peereboom, J.H., Merkus, J.H. and Steegers-Theunissen, R.P. (2001) The impact of calcium, magnesium, zinc, and copper in blood and seminal plasma on semen parameters in men. *Reprod. Toxicol.*, **15**, 131–136.

World Health Organization (1999) *WHO Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction*, 4th edn. Cambridge University Press, Cambridge.

Yukihirano, H., Hiroaki, S., Hiromi, O., Tatsuya, S., Satoru, T., Chieko, Y., Hiromichi, T. and Ikuo, S. (2001) Relationships between sperm motility characteristics assessed by the computer-aided sperm analysis (CASA) and fertilization rates *in vitro*. *J. Assist. Reprod. Genet.*, **18**, 213–218.

Zöpfgen, A., Priem, F., Sudhoff, F., Jung, K., Lenk, S., Loening, S.A. and Sinha, P. (2000) 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.*, **15**, 840–845.

Submitted on May 7, 2002; accepted on August 2, 2002