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Sperm concentration in Latvian military conscripts as compared with other countries in the Nordic–Baltic area

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Summary

Recent studies of semen quality in men from the general population gave rise to the hypothesis of an East–West gradient in semen quality in the Nordic–Baltic area, with the highest sperm counts in Estonia, Lithuania and Finland, and the lowest in Denmark (30% difference in mean concentration). Genetic, lifestyle-related and environmental factors – alone or in combination – were suggested to be responsible for these differences. The aim of this study was to assess sperm concentration in men from the general population in Latvia and to investigate the impact of ethnic and lifestyle-related factors on this marker of male reproductive health. A total of 133 military conscripts from Latvia were investigated. We found that sperm counts among Latvian adolescents were at the same level (mean 74, median 63×10^6/mL) as those previously reported from Estonia, Lithuania and Finland. Sperm concentration was somewhat higher than in Sweden without reaching the level of statistical significance (mean difference 3×10^6/mL; 95% CI: −10, 16×10^6/mL), and statistically significantly higher that in Denmark (mean difference: 17×10^6/mL; 95% CI: 5, 2×10^6/mL). The study also revealed an impact of ethnic factors on sperm numbers. Sperm concentration was significantly higher in men with both parents born in Latvia ($77 \pm 60 \times 10^6$/mL), compared with men with both parents born outside Latvia ($55 \pm 45 \times 10^6$/mL, $p = 0.03$).

Keywords: environment, ethnic factors, lifestyle factors, semen quality

Introduction

It has been suggested that human semen quality has deteriorated significantly during the past few decades (Carlsen *et al.*, 1992; Auger *et al.*, 1995; Irvine, 1996), although this trend has not been apparent all over the world (Suominen & Vierula, 1993; Bujan *et al.*, 1996; Fisch *et al.*, 1996). The impairment of male reproductive function has been aetiologically linked to an increasing incidence of other abnormalities of the male reproductive system (Toppari *et al.*, 1996). It has been shown that the incidence of testicular germ cell cancer has increased two- to threefold in Europe and the United States during the last decades (Adami *et al.*, 1994; McKiernan *et al.*, 1999). An increasing incidence of cryptorchidism and hypospadias has also been suggested (reviewed in Giwercman *et al.*, 1993; Jensen *et al.*, 1995; Toppari *et al.*, 1996).

Apart from the secular trend, also geographical differences in the status of male reproductive function have been observed. In recent studies of semen quality of men from the general populations in Nordic–Baltic countries (Jorgensen *et al.*, 2002; Punab *et al.*, 2002; Richthoff *et al.*, 2002), surprisingly low sperm concentrations were found in Denmark (median 44×10^6/mL), somewhat higher in Sweden (median 52×10^6/mL), and significantly higher

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in men from Finland (median 61×10^6/mL), Lithuania (median 65×10^6/mL), and Estonia (median 62×10^6/mL). These findings led to the hypothesis of an East–West gradient in semen quality in the Nordic–Baltic area (Jorgensen *et al.*, 2002). The same East–West gradient was reported considering the incidence of testicular cancer: Finland and Estonia having the lowest risk of this malignancy, and the highest figures registered in Denmark and Norway. Also the incidence of cryptorchidism was recently reported to be much lower in Finland as compared with Denmark (Boisen *et al.*, 2004).

Consequently a common aetiology of the observed disorders of male reproductive function has been suggested. As a possible cause, exposure to environmentally derived compounds with hormone-like action, the so-called endocrine disrupters, has been proposed (Sharpe & Skakkebaek, 1993; Toppari *et al.*, 1996). The endocrine disrupters are thought to act in an oestrogen- or anti-androgen-like fashion, affecting the male gonad in early foetal life and causing the so-called testicular dysgenesis syndrome (Skakkebaek *et al.*, 2001).

However, also genetic as well as lifestyle related factors (e.g. smoking) might account for geographical differences in male reproductive function (Skakkebaek *et al.*, 2001; Bonde & Storgaard, 2002). An association of in utero exposure to maternal smoking with reduced semen quality and testis size in adulthood has been reported (Storgaard *et al.*, 2003; Jensen *et al.*, 2004). Mother's smoking during pregnancy has also been linked to the risk of testicular cancer among the male offspring (Pettersson *et al.*, 2004). The detrimental effects of exposure to smoking (Kunzle *et al.*, 2003) and to some other environmental and lifestyle-related factors during adulthood on semen quality have also been demonstrated (shortly reviewed in Bonde & Storgaard, 2002).

The aim of this study was to investigate whether sperm concentration among Latvian adolescent males was at the same high level as reported from Estonia and Lithuania, a finding that might support the hypothesis of East–West gradient in the Nordic–Baltic area. Furthermore, we wished to assess the impact of lifestyle related factors on sperm number. Additionally, as Latvia is a multi-ethnic country, we also aimed to address the role of ethnic factors on the reproductive health of individuals exposed to the same environment. Such an approach was not exploited in previous similar studies in other countries.

Materials and methods

All young men at age of 19 years in Latvia are required to attend a military service board. The board makes a decision on the eligibility of the men for military service (students and men with serious chronic diseases are released from service). As all men must attend the board, whether or not eligible, they can be considered to represent the general population. All 1557 men attending the military service board in Riga from April 2001 until May 2002 were asked to participate. Among them, 133 (participation rate 8.7%) accepted to participate in the study.

The investigation of subjects was evenly distributed throughout the year. Thirty-six men were investigated during spring periods (March to May), 25 – during summer (June to August), 47 – during autumn (September to November), 25 – during winter (December to February). Sixty-four of investigated were students, 23 were studying and working in parallel, 46 were working. Of these seventeen had 12 classes of school education and 29 did have nine classes of school education. One hundred and twelve subjects came from the capital of Latvia Riga, 15 from smaller Latvian towns and six from rural areas. The mean ($\pm$SD) height of the subjects was 183 ± 6.5, median weight -71 ± 8.4. According to the data derived from the questionnaire, only one man reported previous difficulties in achieving pregnancy. Other men had no knowledge of their reproductive capability (no women had been impregnated from these men, and they have never tried to achieve pregnancy, according to the data from the questionnaires).

For comparison of different ethnic groups, the men were divided into three groups: both parents born in Latvia (group A, $n = 73$), one parent born outside Latvia (group B, $n = 35$), and both parents born outside Latvia (group C, $n = 18$). Men who were not been born in Latvia ($n = 3$) and men with parents born outside Latvia but within the Baltic region ($n = 4$) were excluded from this part of the study. Regions outside of Latvia from which the parents mostly originated included Russia ($n = 33$) and other East–Central Europe countries: Byelorussia ($n = 6$), Ukraine ($n = 9$), Uzbekistan ($n = 3$), Kazakhstan ($n = 1$), and Azerbaijan ($n = 1$).

Every participant filled in a questionnaire, underwent a physical examination (see Table 1) and delivered a semen sample for which he received 8 Euros.

Table 1. Background characteristics of Latvian, Swedish (Richthoff *et al.*, 2002) and Danish (Andersen *et al.*, 2000) military conscripts, based on combined data from the questionnaires and the physical examination

	Latvian men ($n = 133$)	Swedish men ($n = 248$)	Danish men ($n = 708$)
Abstinence time (h)			
Mean (SD)	168 (212)	84 (59)	83 (105)
Median (5–95% CI)	109 (61–277)	66 (37–168)	61 (14–168)
Testicular torsion (%)	0.8	1.2	0.9
Inguinal hernia (%)	1.5	2.8	3.8
Epididymitis (%)	0	0.4	0.1
Varicocele (%)	14	8	10

All subjects signed a written consent form and the local ethical committee approved the study.

Questionnaire

A standardized questionnaire used in the previous studies in the Nordic–Baltic area (Jorgensen *et al.*, 2002) was utilized. The questionnaire included information about previous or current diseases, birthplace of subject and parents and other factors that might influence their reproductive function (like history of cryptorchidism, smoking etc.).

Physical examination

All men underwent andrological examination, including scrotal palpation, which was performed by the same physician (J.E.). Testicular volume was assessed using the Prader orchidometer. Varicocele was detected by the palpation, and all stages of varicocele were considered as presence of this condition and allocated into one group.

Semen analysis

Each man provided a semen sample by masturbation in a room at the laboratory. The men were asked to adhere to 48–168 h of abstinence but in each case the actual abstinence period from the previous ejaculation time (in hours) was recorded. All semen samples were analysed by the same technician, according to published recommendations (World Health Organization, 1999). Samples were allowed to liquefy for 30 min. The sperm concentration was assessed using positive displacement pipettes and improved Neubauer haemocytometer. Sperm morphology was assessed after the Papanicolaou staining using World Health Organization (WHO) criteria.

As the seminal volume in this study was not measured by weighing, as in the other countries, but by estimation from graduated Falcon tubes, our measurements of the seminal volume were not comparable (Jorgensen *et al.*, 1997). The seminal volumes and total sperm counts were therefore not used for the comparative analyses between Latvia and the other countries.

Background characteristics

The background characteristics of the Latvian population and the previously published data on Danish (Andersen *et al.*, 2000) and Swedish (Richthoff *et al.*, 2002) conscripts are given in Table 1. According to the questionnaire, four men did previously have asthma, five suffered of another chronic respiratory tract diseases (chronic rhinitis, bronchitis), one experienced cystitis, one has been surgically treated for hydronephrosis. Furthermore, one man reported an orchitis as a complication of parotitis at the age of 17. In another five cases of post-pubertal parotitis it was not complicated by an orchitis.

Statistics

The confidence intervals (CI) for the mean differences of sperm concentration between Latvia, Sweden and Denmark were calculated based on a normal distribution (Altman, 1991). In addition, to minimize the effect of length of abstinence period, the sperm concentration was evaluated separately in those subjects having at least 48 h of sexual abstinence prior to delivering the ejaculate, and compared with the data from the same subgroups of men available from Sweden (Richthoff *et al.*, 2002) and Denmark (Andersen *et al.*, 2000). Furthermore, in order to estimate the effect of the abstinence time on sperm concentration, men were divided into five groups: men with abstinence time <48 h ($n = 23$), 48–71 h ($n = 21$), 72–95 h ($n = 16$), 96–120 h ($n = 16$), and men with abstinence time >120 h ($n = 57$).

The influence of place of birth of parents (ethnic origin), smoking of subjects, smoking of mothers during pregnancy, smoking of parents during the childhood of subjects, usage of alcohol and premature birth on sperm concentration was analysed in general linear regression models, adjusted for the abstinence time. The effect of the ethnicity on sperm concentration was additionally adjusted for the smoking of the parents during the childhood of subjects, and vice versa, as these two factors showed the greatest impact on sperm concentration. All hypotheses testing were two-sided with a probability value of 0.05 deemed as significant.

For comparison of the incidence of self-reported cryptorchidism between Latvia, Sweden and Denmark, the Fisher's exact test was performed both for total numbers and for those treated for testicular maldescent. Analyses were conducted with SPSS 11.0 for Windows

Results

Semen quality parameters of Latvian men in comparison with data from Sweden and Denmark are shown in Table 2. Sperm concentrations of young Latvian men appear higher than those in Sweden and Denmark. However, the difference between Latvia and Sweden in mean sperm concentration was not statistically significant (mean difference: 3×10^6/mL; 95% CI: -10, 16×10^6/mL). The mean sperm concentration was significantly higher in Latvia

Table 2. Comparison of sperm concentration ($\times 10^6$/mL) for military conscripts from Latvia with previously published data from Sweden and Denmark (Andersen *et al.*, 2000; Richthoff *et al.*, 2002)

	Latvia	Sweden	Denmark
No. of men included	133	248	708
Mean (SD)	74 (58)	71 (67)	57 (57)
Median (5–95 percentiles)	63 (5.0–183.6)	52 (5.1–194.7)	41 (3.0–167.0)

compared with Denmark (mean difference: 17×10^6/mL; 95% CI: 3.2, 31×10^6/mL). The mean (±SD) seminal volume of Latvian men was 2.64 ± 1.5 mL, the mean (±SD) total sperm count −196 ± 183 $\times 10^6$/mL. The mean (±SD) proportion of morphologically normal spermatozoa was 13 ± 8%.

When divided into five intervals, an increasing duration of abstinence, up to the interval of 72–95 h, was associated with increasing sperm concentration ($p < 0.001$), where after no further effect of a longer abstinence period could be observed. In the group of subjects having an abstinence period ≥48 h, the median times of abstinence was 114 h (Table 3). For these subgroups sperm concentration in Latvia was significantly higher than in Denmark (mean difference: 17×10^6/mL; 95% CI: 4, 30×10^6/mL), but did not show any significant difference compared with Sweden (mean difference: 5×10^6/mL; 95% CI: −10, 2×20^6/mL). The median time of abstinence for Sweden and Denmark for these subgroups was 72 h in both countries.

The mean sperm concentration was similar for the ethnic groups A and B: 77 ± 60 $\times 10^6$/mL and 79 ± 65 $\times 10^6$/mL ($p = 0.8$), respectively. Sperm concentration was higher in group A (77 ± 60 $\times 10^6$/mL), compared with group C (55 ± 45 $\times 10^6$/mL), but this difference was not statistically significant ($p = 0.2$). However, after adjusting for parents' smoking during childhood of subjects, which was shown to have a detrimental effect on sperm counts (Table 4), and for abstinence time, the difference between ethnic groups A and C became statistically significant ($p = 0.03$).

The proportion of men with self-reported cryptorchidism was significantly lower in Latvia as compared with Denmark (0.8% vs. 12.6%, $p < 0.001$) and even as compared with Sweden (2.8%), the latter difference not being statistically significant ($p = 0.09$). The same was true for men in whom cryptorchidism had been treated: 0.8% vs. 3.8% for Latvian and Danish men ($p = 0.05$), and 1.2% in Sweden ($p = 0.08$).

The impact of life style-related factors and premature birth on semen quality is shown in Table 4. Parents' smoking during the childhood of subjects seemed to be the only one statistically significant detrimental factor for the sperm concentration ($p = 0.01$). However, after adjusting for the abstinence time and the ethnicity, the difference between smoking and non-smoking groups was only borderline statistically significant ($p = 0.08$).

Table 3. Comparison of sperm concentration ($\times 10^6$/mL) for Latvian men with previously published data from Sweden (Richthoff *et al.*, 2002) and Denmark (Andersen *et al.*, 2000), restricted to subjects with an abstinence period ≥48 h

	Latvian men ($n = 100$)	Swedish men ($n = 223$)	Danish men ($n = 521$)
Mean (SD)	80 (61)	75 (70)	63 (57)
Median (5–95 percentiles)	69 (4.8–196)	55 (4.8–219)	45 (3.4–182)

Discussion

In this study we found that sperm concentration among Latvian military conscript is at the same high level as previously reported for corresponding groups of Estonian, Finnish and Lithuanian males, and significantly higher than in Denmark. Furthermore, men born by non-Latvian parents had significantly lower sperm concentration than those having both mother and father of Latvian origin. Apart from confirming the existence of Nordic–Baltic East–West gradient in sperm numbers, our study points to the possible impact of ethnic factors in relation to male reproductive function.

Table 4. The impact of different life style and premature birth on sperm concentration (adjusted for the abstinence time) in Latvian military conscripts

	Mean sperm concentration ($\times 10^6$/mL ± SD)		
Factor	Exposed to factor	Not exposed to factor	*p*-value
Subjects smoking	71 ± 56.2 ($n = 73$)	78 ± 61.5 ($n = 60$)	NS
Mother smoking during pregnancy	72 ± 52.8 ($n = 6$)	75 ± 60.8 ($n = 114$)	NS
At least one parent[a] smoking during subjects' childhood	66 ± 49.6 ($n = 79$)	88 ± 68.3 ($n = 53$)	NS[b]
Any usage of alcohol during the last week	71 ± 56.6 ($n = 61$)	78 ± 60.4 ($n = 72$)	NS
Premature birth	71 ± 50.8 ($n = 10$)	74 ± 59.6 ($n = 103$)	NS

[a]Number of men with both smoking parents was too small for meaningful comparison; [b]became not significant after has been adjusted for the ethnicity of the parents.
NS, not significant.

As only one man in this study mentioned previous difficulties in achieving pregnancy, and the remaining had no knowledge of their reproductive capability, the low participation rate should not imply any selection bias with respect to fertility. Therefore, we believe that the men delivering a semen sample were representative for the whole group of military conscripts and, therefore, for the general population of Latvian young men. The subjects were selected in a similar way and also age-matched to the men investigated in the other countries.

Because of the inter-laboratory variation in assessment of semen parameters we did not compare sperm motility and morphology but focused only on sperm concentration as the most objective sperm characteristic. Based on investigation of mailed frozen samples, an inter-laboratory coefficient of variation (CV) of 30% for determination of sperm concentration was previously reported (Neuwinger *et al.*, 1990). However, this figure is probably not valid for the CV between the laboratories participating in the Nordic–Baltic monitoring of semen quality. An external quality control study with participation of the laboratories in this region, based on used of mailed samples, has shown a month-to-month variation in the relative counting levels of the participating laboratories. The average CV over a period of 12 months was approximately only 10%, probably because all the participating laboratories performed the analysis according to the most recent WHO guidelines (Richthoff *et al.*, 2002). Furthermore, a 50% reduction of the CV was shown, when the sperm counting was made on fresh ejaculates, and not on mailed samples, which are generally of rather poor quality because of aggregation of the spermatozoa (Richthoff *et al.*, 2002). Additionally, within the framework of the EU project 'Envir.Reprod.Health', No QLK4–1999–01422 on monitoring male reproductive health, a Nordic–Baltic Quality Control workshop for semen quality assessment was organized in 2003. At the occasion, Latvian technician (the same who performed all semen analyses for the current study) was counting sperm concentration consistently lower than Danish and other Baltic technicians (Jorgensen *et al.*, unpublished data). Therefore, we believe that inter-laboratory variation is not the explanation of the 30% higher mean sperm counts found by us as compared with the Danish data.

The abstinence time was much longer in Latvia than in the other countries (Table 1). In the group of subjects with at least 48 h of abstinence, the median time period was 72 h in Denmark and 114 h in Latvia. The statistical model applied in this study, did not allow adjusting for the time of abstinence. However, in the Latvian material we did not find any changes in sperm concentration, where the length of the abstinence period exceeded the interval of 72–96 h. Therefore, although the impact of this important confounder cannot be totally excluded, we do not believe that the difference in the length of abstinence period can solely explain the difference in sperm concentration between Denmark and Latvia, reported by us.

We have not included the data on seminal volume (and, therefore, total sperm concentration) in comparative analyses as this parameter in our study was not measured by weighing, as in the other countries, but by estimation from graduated Falcon tubes, making the data incomparable. However, preliminary results of an ongoing study on semen quality of military conscripts indicate that when weighting of the ejaculates is applied, the seminal volume is at the same level in Latvia as in Estonia, Lithuania and Finland (Erenpreiss and Tsarev, personal communication).

The existence of East–West gradient in sperm counts, confirmed by us, was not because of the difference in abstinence period as a similar difference was seen for subjects with an abstinence time ≥48 h. Moreover, there was an inverse gradient (highest in Denmark and lowest in Latvia) for the proportion of men with self-reported cryptorchidism, which supports the hypothesis of a common cause of disorders of male reproductive health (Sharpe & Skakkebaek, 1993; Skakkebaek *et al.*, 2001).

As Baltic and Finnish men, compared with Danish and Norwegian men have different genetic backgrounds, it can be speculated whether the East–West gradient could be determined by ethnic factors. Finding of higher sperm counts in Southern Sweden as compared with Denmark (Richthoff *et al.*, 2002), indicate an effect of environmental or life style-related factors, as the genetic background of these two population is very similar. Latvians and Lithuanians are genetically different from the Finns, but sperm concentration in young men living in these countries seems to be at the same high level. On the other hand, finding of higher sperm concentration in Latvian men whose both parents are native, compared with those with both parents born outside of Latvia points to possible impact of genetic factors, as all of the men were born and raised in Latvia. The genetic differences between Latvians and Eastern/Central European nations like Russians have been shown based upon investigation of Y chromosome haplogroups and mitochondrial DNA (Rosser *et al.*, 2000; Krausz *et al.*, 2001; Tambets *et al.*, 2004). However, ethnic subgroups living in the same country might differ as considers lifestyle factors not analysed in this study, e.g. eating habits, although the observed ethnic difference became significant after accounting for parents' smoking during the childhood of subjects and the length of sexual abstinence.

It has been shown earlier that lifestyle and health factors can explain differences in male reproductive function (Bonde & Storgaard, 2002; Kunzle *et al.*, 2003). An association between in utero exposure to maternal smoking and reduced semen quality has been demonstrated (Storgaard *et al.*, 2003; Jensen *et al.*, 2004). The results of our study did not support these findings, probably because of the very small group of the mothers smoking during pregnancy ($n = 6$). Other factors, such as subjects' smoking, usage of alcohol, and premature birth showed a slight negative impact

on sperm numbers without reaching the level statistical significance. However, our analysis did have a relatively low statistical power and their impact on male reproductive function can not, therefore, be excluded.

In conclusion, our study showed the level of sperm concentration among young Latvian men to be similar to that in Estonia, Lithuania and Finland, somewhat higher than in Sweden and significantly higher than in Denmark. For further elucidation of the importance of genetic, environmental, health and lifestyle-related factors in relation to male reproductive function, a study with inclusion of higher numbers of men of different genetic background, and using an expanded questionnaire with more detailed information is undergoing in the Nordic–Baltic countries.

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